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for better
tomorrow

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Plenary Session

Carbon Matters: Reclaiming Organic Carbon for a Resilient Future

Roberta Farina

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Summary

Soil Organic Carbon (SOC) is a foundational element of terrestrial ecosystems, playing a critical role in maintaining soil fertility, supporting biodiversity, and enhancing the resilience of agricultural systems to climate change. As the principal component of soil organic matter, SOC regulates key physical, chemical, and biological processes, including nutrient cycling, water retention, and aggregate stability. Despite its importance, SOC is being depleted at alarming rates due to intensive agriculture, land degradation, and climate-related pressures. This paper examines the vital functions of SOC in agroecosystems, its role in climate regulation, and the socio-ecological implications of its loss. It explores the concept of carbon farming as a strategy for enhancing SOC stocks, highlighting both legislative frameworks and technical practices such as reduced tillage, cover cropping, organic amendments, and agroforestry. Special attention is given to the potential and limitations of carbon farming in the context of European policy and global climate goals. By synthesizing current scientific understanding and policy directions, the paper underscores the urgent need to reclaim and protect SOC as a cornerstone of sustainable agriculture, climate mitigation, and resilient rural development.

Key words: soil organic carbon, climate change mitigation, carbon farming

Introduction

In the age of climate change and environmental degradation, one of our most important allies is hidden beneath our feet: soil organic carbon (SOC). SOC, the major component of soil organic matter (SOM), is the cornerstone of ecosystems. It nurtures microbial activity, stores nutrients, retains water, and binds soil particles—making it indispensable for agriculture, biodiversity, and climate resilience. In general, SOC content is positively correlated with a fertile soil status and plays a mostly beneficial role in determining the physical, chemical and biological qualities of a soil, the ecosystem functioning, and the magnitude of the different processes (Francaviglia et al., 2018).

At global level, soils contain about three times the amount of C stored in plant biomass and twice the amount of that present in the atmosphere (IPCC 2000), amounting to about 1,500 Pg (1Pg=1Gt=10¹⁵g) to 1-m depth (Batjes, 1996). The annual fluxes of CO₂ from atmosphere to land (Net Primary Productivity [NPP] through photosynthesis) and land to atmosphere (respiration and fire) are each of the order of 60 Pg C y⁻¹ (IPCC 2000).

Yet, despite its fundamental role, SOC is being lost at an alarming rate. Decades of intensive agriculture, land degradation, deforestation, and urban sprawl have led to the depletion of soil organic matter in many regions. To date, soils have lost between 40 and 90 Pg C due to cultivation and disturbance (Smith et al., 2007). In addition, Naipal et al. (2018) estimate that over the period between 1850–2005 soil erosion was responsible to a SOC removal flux of 74±18 Pg C, occurring in the majority (>80%) on agricultural land and grassland.

These SOC losses not only reduce soil fertility and productivity but also contributes significantly to atmospheric CO₂ levels, thereby exacerbating global warming. A release of soil organic carbon by just 10%, would be equivalent to all the anthropogenic CO₂ emitted over 30 years (Kirschbaum, 2000)

Soil carbon loss is a silent crisis. It rarely dominates headlines, yet it underpins many of today's pressing global challenges, including declining crop yields, desertification, water scarcity, and ecosystem instability.

This paper examines the critical role of SOC in agroecosystems and explores strategies for its reclamation and enhancement through sustainable agricultural and agroforestry practices.

The role of soil organic carbon in agroecosystems

SOC plays a pivotal role in sustaining agroecosystem health by influencing the physical, chemical, and biological properties of soils. It is increasingly recognized as a key lever for improving agricultural productivity, mitigating climate change, and ensuring long-term environmental sustainability (Hoffland et al., 2020)

SOC serves as a major reservoir of essential nutrients such as nitrogen (N), phosphorus (P), and sulfur (S), which are released gradually through microbial mineralization, thereby supporting long-term soil fertility and crop productivity. Numerous studies have demonstrated positive correlations between SOC levels and yields across diverse agroecological zones (Lal, 2004; Oldfield et al., 2019). In addition, SOC enhances the soil's cation exchange capacity (CEC), improving its ability to retain and supply nutrients to plant roots (Kaiser et al., 2008).

In hydrological terms, SOC enhances soil water retention, particularly by increasing field capacity more than the permanent wilting point. This effect varies depending on soil texture and initial SOM content (Lal, 2020) .

From a physical perspective, SOC supports the formation and stabilization of soil aggregates, improving porosity, reducing bulk density, and enhancing infiltration rates. These effects, in turn, increase the soil's ability to retain moisture and resist erosion, especially under conditions of drought or intense rainfall (Bronick & Lal, 2005).

SOC also plays an increasingly prominent role in climate regulation. Soils represent one of the largest terrestrial carbon reservoirs, and practices that increase SOC—such as reduced tillage, organic amendments, cover cropping, and agroforestry—are considered effective pathways for carbon sequestration (Paustian et al., 2016). In addition to carbon sequestration, SOC-rich soils tend to have lower emissions of non-CO₂ greenhouse gases, particularly nitrous oxide (N₂O), which is a potent contributor to global warming (Six & Paustian., 2004).

Biologically, SOC is the primary energy source for heterotrophic microorganisms, facilitating decomposition and nutrient cycling. Elevated SOC levels are associated with higher microbial biomass, enhanced enzymatic activity, and greater functional diversity—all of which improve nutrient availability and plant health (Lehmann & Kleber, 2015). This is particularly relevant in Mediterranean and other climate-vulnerable regions where land degradation and climate variability present mounting challenges (Farina et al., 2024).

In summary, SOC underpins the functionality of agroecosystems across multiple dimensions. Its restoration and conservation are central to sustainable agriculture, food security, and climate resilience.

Soil Organic Carbon and the Resilience of Agricultural Systems

The resilience of social-ecological systems, including agroecosystems, refers to their inherent capacity to sustain a desirable provision of ecosystem services under external disturbances, including both anthropogenic pressures and environmental variability (Brand & Jax, 2007). Central to this resilience is the system's ability to avoid crossing critical ecological thresholds that could lead to irreversible shifts to less productive states.

Among the key biophysical variables that influence resilience, SOC plays a particularly crucial role. Notably, SOC changes slowly relative to other soil parameters, which means that it reflects both long-term management history and future trajectories (Freibauer et al., 2004).

The inherently slow turnover of SOM—and by extension SOC—is further complicated by the dynamic nature of agricultural management. Practices such as tillage intensity, cropping systems, and organic input regimes are often altered before SOC reaches a new equilibrium, resulting in soils that are frequently in a state of non-equilibrium (Johnston et al., 2009).

From a resilience perspective, SOC supports agroecosystems by enhancing structural integrity, water retention, and nutrient cycling. Improved aggregate stability fosters deeper root penetration, reduces erosion risk, and increases tolerance to water stress (Kay, 2018).

Biologically, SOC supports microbial networks essential for nutrient mineralization, organic matter decomposition, and disease suppression (Leinweber et al., 2008, Bhattacharyya et al., 2020). A diverse microbial community improves the system's capacity to adapt to biotic stressors and environmental changes.

Chemically, SOC buffers soils against pH fluctuations and nutrient imbalances, thereby supporting plant health under a range of climatic and edaphic condition (Krull et al., 2004)

Economically, farms with SOC-rich soils often exhibit lower reliance on external inputs—such as fertilizers and irrigation—due to improved nutrient and water use efficiency. This enhances the economic viability and sustainability of farming systems, especially for smallholders and resource-limited producers. Stabilized yields further contribute to food security and livelihood resilience.

Taken together, SOC is a key asset for agroecosystem resilience. Its conservation should be a cornerstone of agricultural adaptation strategies, land restoration frameworks, and climate-resilient development planning

Carbon farming

a) Legislative aspects

Carbon farming encompasses land management practices that either sequester carbon in biomass and soils or reduce greenhouse gas (GHG) emissions. Recognized as a nature-based solution, it aligns with the EU's long-term objective of climate neutrality by 2050, as outlined in the European Green Deal (European Commission, 2019a), and is reinforced by sectoral strategies such as the Farm to Fork and Biodiversity Strategies (European Commission, 2020a and 2020b).

The reformed Common Agricultural Policy (CAP) 2023–2027 promotes carbon farming through eco-schemes that incentivize sustainable practices. However, these payments are practice-based and do not require quantifiable evidence of SOC gains (Regulation (EU) 2021/2115).

The proposed Carbon Removal Certification Framework (CRCF) (European Commission, 2022) represents a more rigorous development. It aims to standardize certification of carbon removals—agricultural and otherwise—based on quality criteria such as additionality, permanence, and environmental integrity. This framework seeks to harmonize existing voluntary carbon markets and potentially prepare the ground for regulated carbon credit trading involving soils.

Further, the amended LULUCF Regulation [Regulation (EU) 2018/841; 2023/839] establishes binding targets for net carbon removals, explicitly incorporating agricultural soils for the first time. This inclusion strengthens the alignment between agricultural policy and climate mitigation goals under the broader Effort Sharing Regulation.

Nonetheless, challenges persist. Soil carbon sequestration is reversible and context-dependent, and its quantification remains complex. The EU's phased strategy—early incentives through CAP and more stringent certification via CRCF—represents a pragmatic pathway toward mainstreaming carbon farming while maintaining scientific rigour and transparency.

b) Technical aspects

Carbon farming encompasses a suite of land management practices aimed at enhancing the sequestration of atmospheric carbon dioxide (CO₂) in soils and biomass, as well as reducing emissions of greenhouse gases (GHGs) such as methane (CH₄) and nitrous oxide (N₂O). It is founded on two principal mechanisms: (i) increasing the stock of soil organic carbon (SOC), and (ii) minimizing emissions associated with conventional agricultural activities.

Essentially, carbon farming enhances the natural processes of photosynthesis and organic matter cycling. Leverages natural processes—particularly photosynthesis and organic matter cycling—to enhance carbon inputs into soils. Plants absorb CO₂ and transfer part of the fixed carbon into the soil through root exudates and residues. With appropriate management, a fraction of this organic carbon can become stabilized in the soil matrix.

Common techniques include conservation tillage or no-till systems, which minimize soil disturbance and slow the decomposition of organic matter (Six et al., 2000, Farina 2024). Cover crops, including inter-row vegetation in woody systems, enhance carbon inputs, reduce erosion, and support nutrient cycling (Farina et al., 2024, Garcia-Ruiz et al., 2013).

Agroforestry systems integrate woody perennials into agricultural landscapes, offering carbon sequestration benefits both above and below ground. These systems also promote biodiversity and generate multiple ecosystem services (Burgess, et al., 2022).

Organic amendments such as compost, manure, and biochar provide direct carbon inputs while enhancing microbial activity and nutrient availability. Biochar, in particular, contains recalcitrant carbon forms that resist decomposition, making it a promising tool for long-term sequestration (Lorenz & Lal, 2014).

In grazing systems, rotational grazing and managed stocking densities support perennial root growth, which contributes to below-ground carbon deposition (Sanderman et al., 2015). In some cases, wetland restoration or peatland re-wetting can drastically reduce emissions from oxidized organic soils (Hemes et al., 2019).

Accurate carbon accounting is essential for classification as a carbon farming practice. Quantification methods include direct soil sampling, modeling tools, and emerging technologies such as remote sensing and proximal sensors. However, these require further validation, particularly to ensure cost-effectiveness and accuracy at scale (Petropoulos et al., 2025).

Establishing credible baselines and ensuring additionality remain critical, as does ensuring permanence. Unlike geological carbon storage, biological sequestration is reversible and must be safeguarded by long-term commitments and adaptive risk management.

In conclusion, while carbon farming is both agronomically sound and environmentally beneficial, its effectiveness in delivering climate mitigation depends on robust implementation, site-specific adaptation, and transparent MRV systems.

Conclusions

Healthy soils rich in organic carbon are the foundation of resilient agroecosystems. They buffer against climatic extremes, enhance biological diversity, and sustain productivity. Moreover, they represent a vital lever for climate mitigation through carbon sequestration.

Reclaiming SOC is not merely an environmental priority but a socio-economic imperative. It bridges ecological integrity with agricultural viability and rural livelihoods. As such, restoring SOC should be integral to climate adaptation, sustainable food systems, and land stewardship agendas.

References

- Batjes, N. H. (1996). Total carbon and nitrogen in the soils of the world. *European journal of soil science*, 47(2): 151-163.
- Bhattacharyya, R., Rabbi, S. M., Zhang, Y., Young, I. M., Jones, A. R., Dennis, P. G., Menzies, N.W., Kopittke, P.M., Dalal, R. C. (2021). Soil organic carbon is significantly associated with the pore geometry, microbial diversity and enzyme activity of the macro-aggregates under different land uses. *Science of the Total Environment*, 778, 146286. <https://doi.org/10.1016/j.scitotenv.2021.146286>
- Brand, F. S., & Jax, K. (2007). Focusing the meaning (s) of resilience: resilience as a descriptive concept and a boundary object. *Ecology and society*, 12(1).
- Bronick, C. J., & Lal, R. (2005). Soil structure and management: a review. *Geoderma*, 124(1–2): 3–22.
- Burgess P., Graves A. (2022). The potential contribution of agroforestry to net zero objectives. Report for the Woodland Trust. Cranfield University; 2022 Nov. Available: <https://dspace.lib.cranfield.ac.uk/handle/1826/18664>
- European Commission (2019a). The European Green Deal. COM(2019) 640 final. Brussels.
- European Commission (2020a). A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system. COM(2020) 381 final. Brussels.
- European Commission (2020b). EU Biodiversity Strategy for 2030: Bringing nature back into our lives. COM(2020) 380 final. Brussels.
- European Commission (2021). EU Soil Strategy for 2030: Reaping the benefits of healthy soils for people, food, nature and climate. COM(2021) 699 final.
- European Commission (2022). Proposal for a Regulation establishing a Union certification framework for carbon removals. COM(2022) 672 final.
- Farina, R., Piccini, C., Di Bene, C., Fornasier, F., Francaviglia, R., Pennelli, B., Vanino, S., Russo, M., Cerasuolo, M., Troccoli, A. (2024). No tillage and leguminous cover crop improve soil quality in a typical rainfed Mediterranean system. *Canadian Journal of Soil Science*, 104(3): 257-272.
- Francaviglia, R., Ledda, L., Farina, R. (2018). Organic carbon and ecosystem services in agricultural soils of the Mediterranean Basin. *Sustainable Agriculture Reviews 28: Ecology for Agriculture*, 183-210.
- Freibauer, A., Rounsevell, M. D., Smith, P., & Verhagen, J. (2004). Carbon sequestration in the agricultural soils of Europe. *Geoderma*, 122(1): 1-23.
- Hemes, K. S., Chamberlain, S. D., Eichelmann, E., Anthony, T., Valach, A., Kasak, K., Szutu, D., Verfaillie, J., Silver, W.L., Baldocchi, D. D. (2019). Assessing the carbon and climate benefit of restoring degraded agricultural peat soils to managed wetlands. *Agricultural and Forest Meteorology*, 268: 202-214.
- Hoffland, E., Kuyper, T. W., Comans, R. N., Creamer, R. E. (2020). Eco-functionality of organic matter in soils. *Plant and Soil*, 455: 1-22.
- Johnston, A. E., Poulton, P. R., & Coleman, K. (2009). Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. *Advances in agronomy*, 101, 1-57.
- Kaiser, M., Ellerbrock, R.H., Gerke, H.H., 2008. Cation Exchange Capacity and Composition of soluble Soil Organic Matter Fractions. *Soil Science Society of America Journal*, 72, 5: 1278-1285
- Kay, B. D. (2018). Soil structure and organic carbon: a review. *Soil processes and the carbon cycle*, 169-197.
- Kirschbaum, M. U. (2000). Will changes in soil organic carbon act as a positive or negative feedback on global warming?. *Biogeochemistry*, 48: 21-51.

- Krull, E. S., Skjemstad, J. O., & Baldock, J. A. (2004). Functions of soil organic matter and the effect on soil properties (p. 129). Canberra: Cooperative Research Centre for Greenhouse Accounting.
- IPCC, 2000 – Robert T. Watson, Ian R. Noble, Bert Bolin, N. H. Ravindranath, David J. Verardo and David J. Dokken (Eds.) Cambridge University Press, UK. pp 375 Available from Cambridge University Press, The Edinburgh Building Shaftesbury Road, Cambridge CB2 2RU ENGLAND
- Garcia-Ruiz, J. M., Nadal-Romero, E., Lana-Renault, N., & Beguería, S. (2013). Erosion in Mediterranean landscapes: Changes and future challenges. *Geomorphology*, 198: 20-36.
- Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science*, 304(5677): 1623–1627.
- Lal, R. (2020). Soil organic matter and water retention. *Agronomy Journal*, 112(5), 3265-3277.
- Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, 528(7580): 60–68.
- Leinweber, P., Jandl, G., Baum, C., Eckhardt, K. U., & Kandeler, E. (2008). Stability and composition of soil organic matter control respiration and soil enzyme activities. *Soil Biology and Biochemistry*, 40(6): 1496-1505.
- Lorenz, K., & Lal, R. (2014). Biochar application to soil for climate change mitigation by soil organic carbon sequestration. *Journal of plant nutrition and soil science*, 177(5): 651-670.
- Naipal, V., Ciais, P., Wang, Y., Lauerwald, R., Guenet, B., Van Oost, K. (2018). Global soil organic carbon removal by water erosion under climate change and land use change during AD 1850–2005. *Biogeosciences*, 15(14): 4459-4480.
- Oldfield, E. E., Bradford, M. A., & Wood, S. A. (2019). Global meta-analysis of the relationship between soil organic matter and crop yields. *Soil*, 5(1): 15-32.
- Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., Smith, P. (2016). Climate-smart soils. *Nature*, 532(7597): 49-57.
- Petropoulos, T., Benos, L., Busato, P., Kyriakarakos, G., Kateris, D., Aidonis, D., & Bochtis, D. (2025). Soil Organic Carbon Assessment for Carbon Farming: A Review. *Agriculture*, 15(5): 567.
- Regulation (EU) 2021/2115. Establishing rules on support for strategic plans to be drawn up by Member States under the CAP.
- Regulation (EU) 2018/841. LULUCF Regulation, as amended by Regulation (EU) 2023/839.
- Regulation (EU) 2018/842. Effort Sharing Regulation.
- Sanderman, J., Reseigh, J., Wurst, M., Young, M. A., & Austin, J. (2015). Impacts of rotational grazing on soil carbon in native grass-based pastures in southern Australia. *PLoS One*, 10(8): e0136157.
- Six, J., Paustian, K. (2014). Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry*, 68: A4-A9.
- Six, J. A. E. T., Elliott, E. T., & Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, 32(14): 2099-2103.
- Smith, P., Falloon, P., Franko, U., Körschens, M., Lal, R., Paustian, K., Romanenkov, V., Shevtsova L., Smith, J. (2007). Greenhouse gas mitigation potential in agricultural soils. *Terrestrial ecosystems in a changing world*: 227-235.

Integrating digital phenotyping and genomics: Unraveling cattle behavior through sensors and genomic data

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Summary

This paper reviews the integration of digital phenotyping and genomics in dairy cattle farming, focusing on sensors such as automated milking systems (AMS), collars, and pedometers for behavioral and physiological monitoring. Sensor-based traits, including milking behavior, rumination, activity, and feeding patterns, exhibit moderate heritability (0.09–0.42), highlighting their suitability for selective breeding. Incorporating these real-time data into breeding programs could significantly enhance cattle welfare, health monitoring, and overall farm productivity.

Key words: selection, breeding program, genomics, phenotyping, sensors, precision livestock farming

Introduction

The global livestock industry is currently undergoing a significant transformation, driven primarily by the widespread adoption of Precision Livestock Farming (PLF) and, in particular, new digital phenotyping techniques (Brito et al., 2020; Kleen and Guatteo, 2023; Koltes et al., 2019; Menezes et al., 2024). This transformation is particularly relevant nowadays, while addressing concerns about global population growth. According to United Nations projections, the world's population is expected to reach 10 billion by 2050 (UN report, 2017). To adequately feed this expanding population, agricultural productivity must increase by approximately 70% (Fraser & Campbell, 2019). Digital phenotyping, which employs sensors and wearable technologies analysed using advanced artificial intelligence (AI) algorithms - enables comprehensive monitoring of cattle behaviour and physiological conditions. Such advancements represent a new shift in livestock management practices (Pedrosa et al., 2024; Neethirajan, 2023; Menezes et al., 2024; Mayberry et al., 2023) and offer a promising strategy for meeting the increasing demand for agricultural productivity. Sensor technologies currently employed in cattle farming, including accelerometer-based collars, pedometers, and automated feeding and milking systems, enable continuous, non-invasive monitoring of animal behavior (Lamanna et al., 2025). These sensors provide real-time data on locomotion patterns, rumination, feeding behavior, and social interactions, significantly improving the accuracy of predicting estrus or health conditions such as lameness or metabolic disorders, and typically those are incorporated in some of the farm management solutions. Another highly promising

technology that will play important role in animal production is video surveillance. Video surveillance with regular 2D cameras, depth cameras, or even 3D vision can be used to automatically observe cow behavior and body posture without attaching devices to the animal (Menezes et al., 2024). Computer vision algorithms can analyze video feeds to identify individual animals and monitor behaviors such as feeding, lying, or social interactions. Thermal infrared cameras provide a heat map of the animal, enabling remote measurement of body surface temperatures. This helps detect signs of fever or inflammation early, for instance, a thermal camera can highlight a hoof with higher temperature due to hoof infection. Besides, environmental sensors placed around barns track temperature, humidity, and gas levels to assess the micro-climate cattle experience (Lamanna et al., 2025). Monitoring barn temperature-humidity index in real-time helps manage heat stress, which can impact feed intake and fertility. However, in the context of animal breeding and selection, where traits with economic value are main targets, all this information obtained from various sensor technologies was usually neglected. Besides, this data is highly complex, incompatible between different countries, farms etc. Despite the significant technological advances, breeding programs predominantly rely on static variables such as milk yield, fat and protein content, and somatic cell count. Although genomic selection has notably increased genetic progress by integrating DNA information with phenotypic records, incorporating real-time data into breeding schemes presents both significant opportunities and challenges. These include handling vast volumes of data, standardizing phenotypic definitions across different technologies, and accurately estimating genetic parameters for novel sensor-derived traits. Besides, the integration of these technologies could also improve animal welfare mainly as these approaches are safe and non-invasive, which is additional benefit in future farm management practices (Brito et al., 2020). The objective of this review is to summarize the current knowledge on PLF technologies in cattle breeding sector and discuss their potential integration with genomic selection in future breeding strategies. Overcoming technical, economic, and practical barriers will be essential to fully capitalize on these technological advances, ultimately shaping the future of dairy cattle sector (Pedrosa et al., 2024; Brito et al., 2020).

Early scientific studies

One of the first scientific reports of using sensors for measuring activity on dairy cows dates back to the 1950s. Farris (1954) strapped a pedometer (a flat wooden block with a metal cover and leather leg strap) to cows' hind legs to record steps, demonstrating that cows exhibit increased walking activity during estrus. In Farris's study on 13 Guernsey cows, most animals showed a marked rise in step-count for 24–48 hours around estrus. This was the earliest evidence that measuring a cow's physical activity could help indicate heat (estrus). Two decades later, Kiddy (1977) published a study in *Journal of Dairy Science* using modern pedometers on 40 cows to quantify activity changes at estrus. The study confirmed that cows were 2.8–4 times more active during estrus compared to other periods, and suggested that pedometer-recorded activity peaks could be used to decide optimal timing for insemination (Kiddy, 1977; Eckelkamp, 2019). This 1977 study was the first to explicitly propose pedometer monitoring as a practical aid for estrus detection on dairy farms. Subsequent research in the 1980s further confirmed correlation between activity levels and estrus (Lewis and Newman, 1984) while also expanding the use of pedometers beyond fertility. In later years, pedometers were additionally tested for detecting health issues such as early lameness by analysing drops in daily step counts (Mazrier et al., 2006). Wearable neck sensors or collars for monitoring cattle behavior or physiology also began to appear in the literature by the 1980s. An early example is the work of Luginbuhl et al. (1987), who developed an electronic halter collar with a pressure sensor to monitor chewing cycles in stall-fed cows. Their system used a flexible noseband with embedded electrodes that detected jaw movements by changes in electrical

resistance as the cow chewed. This allowed automatic recording of eating and rumination behavior, demonstrating one of the first collar-based sensors for dairy cattle physiology. Around the same time, researchers also experimented with acoustic and motion sensors on collars to record grazing or ruminating activity (e.g. a microphone on the collar to capture cud-chewing sounds) (Hoffmann et al., 2024). These 1980s studies proved that collar-mounted sensors (whether pressure transducers or microphones) could reliably track bovine behaviors like feeding and rumination. By the 1990s, accelerometers and other electronics became smaller, leading to trials in which motion sensors were placed on collars to detect various cattle behaviors. Nevertheless, the earliest scientific applications of collars in dairy cattle were focused on monitoring ingestive behavior and activity, laying the groundwork for today's multi-sensor smart collars.

First commercial uses

The transition of pedometer technology from research to farms occurred by the late 1970s and early 1980s. In Israel, the Afikim (Afimilk) company developed the first commercial heat/estrus detection pedometer for dairy cows around the early 1980s. This electronic pedometer, attached to a cow's leg, counted steps and interfaced with herd management software, alerting farmers to increased activity associated with estrus. By the mid-1980s, such pedometer systems were being adopted in dairy herds for automated estrus detection. For instance, early on-farm trials showed pedometer-based systems could raise heat detection rates above traditional visual observation levels (Senger, 1994). Throughout the 1990s, similar on farms systems became more widespread globally, and companies like Nedap and DeLaval integrated activity meters into their milking and herd management systems. DeLaval's ALPRO system (1990s) could read leg-mounted activity counters via radio every hour, and report cows with activity spikes for breeding (Brehme et al., 2006). Thus, by the late 20th century, pedometers became an established precision dairy tool for estrus detection on commercial farms. In the 2000s, farmers and vets also began using pedometer data for secondary applications such as identifying lame cows that walk less (Mazrier et al., 2006), but estrus detection was the initial driving force of on-farm pedometer adoption. Widespread on-farm use of wearable neck sensors took hold slightly later, as the technology matured in the 2000s. One of the first commercial products was the SCR Heatime, introduced by SCR company (also from Israel). SCR engineers developed their first cow collar tags in 1998, and by 2006 began mass-marketing these collars globally. The SCR collar (often called a "tag") contained motion sensors and later combined with microphone. It could transmit data wirelessly to detect heats via increased activity and (in newer versions) monitor rumination via chewing sounds (Bar and Solomon, 2010). After its 2006 launch, SCR's Heatime quickly became one of the most popular commercial heat detection solutions worldwide (Press release, 2014). Farmers, from New Zealand pasture operations to large-scale US dairies, adopted it to continuously track each cow's activity and health. Around the same time, other companies launched similar smart collars, Allflex and Afimilk (in the 2010s) released collar devices that monitor rumination, feeding, and activity. By 2010, many progressive dairies were using collars to not only detect estrus but also to catch early signs of illness or stress, since rumination minutes and activity patterns often change when a cow is sick or heat-stressed. It's worth noting that an even earlier use of collars in dairy farming was electronic identification (RFID) neck collars in the 1980s, which allowed automated feeding and milking systems to recognize individual cows (Prasad, 2023). However, those served primarily as ID tags. The first collars with true behavioral/physiological sensors became commercially viable in the early 21st century, culminating in today's smart collars that are an integral part of precision dairy management (Lamanna et al., 2025). Overall, pedometers have been used in dairy cattle research since the 1950's and saw farm adoption by the 1980s mainly for estrus detection, while sensor collars emerged in research by the late 1980s and reached

commercial farms by the 2000s, expanding monitoring to rumination, feeding, and health. These technologies, initially tested in labs and universities are now common on modern dairy operations, where they continue to evolve as invaluable tools for monitoring cow behavior and physiology. If the main target is to include activity data in breeding programs with aim to genetically select cattle with more desirable traits, the following step is to carefully design these new phenotypes and to develop their standards.

Current breeding programs with classical phenotypic and genomic data

Traditional animal breeding programs have usually focused on a set of static performance traits that are recorded intermittently. In dairy cattle, for example, national selection indices (such as the Lifetime Net Merit in the U.S. or the TPI) heavily weight milk production (milk yield and fat/protein content in standardized 305-day lactations), along with traits like fertility (e.g. calving interval or conception rate), longevity, and udder health (often indicated by somatic cell count) (Wiggans and Carrillo, 2022). These phenotypes are typically measured during routine testing or evaluations, for instance, milk yield and components are measured monthly or bimonthly on dairy farms, and somatic cell count is checked in milk samples as a mastitis indicator. In beef cattle, important recorded traits include weaning and yearling weights, feedlot gain, carcass quality measures, and calving ease. Historically, such data were collected manually or via periodic weigh-ins and then used in genetic evaluations. Additionally, traditional selection relied on pedigree-based estimated breeding values (EBVs), and the rate of genetic progress was constrained by the accuracy and availability of these phenotypic records. Traits like milk yield are moderately heritable and could be improved steadily, but many health or fertility traits are lowly heritable and hard to measure, thus received less direct selection emphasis. Because observations were not continuous, important aspects of animal performance, like how an animal behaves or copes day-to-day remained unrecorded in breeding programs (Neethirajan and Kemp, 2021). This meant that breeding decisions were based on a snapshot of an animal's performance (e.g. an average lactation yield or a single classification score for conformation) rather than the full spectrum of its behavioral or physiological profile.

The adoption of genomic selection in the past 15 years has dramatically enhanced breeding programs by integrating DNA information with phenotypic data. Genomic selection uses dense genetic maps of single nucleotide polymorphisms (SNPs), usually ~50k markers across the genome to predict an animal's genetic merit, allowing selection decisions to be made early in life with improved accuracy (Brito et al., 2020). In dairy cattle, genomic evaluations were widely implemented around 2008–2010, and since then the rate of genetic gain has roughly doubled (Wiggans and Carrillo, 2022). For example, the U.S. Holstein breeding program saw annual gain in net merit (a composite index in economic \$) increase from about \$40 before genomics to about \$85 after genomics was introduced (Wiggans and Carrillo, 2022). This jump is attributed to the ability to select young bulls and heifers based on genomic breeding values that have accuracy comparable to having several lactations of progeny data. The impact on genetic progress has been profound: shorter generation intervals and more accurate selection mean faster improvement in milk yield, feed efficiency, and even harder-to-improve traits like fertility. Moreover, genomic selection has facilitated the inclusion of new traits into breeding objectives. Because thousands of animals are now genotyped and their DNA was used to predict traits, breeders have started to incorporate traits that were previously ignored due to limited data. For instance, many dairy populations now evaluate traits such as heifer fertility, calving ease, stillbirth rate, and even health disorders (e.g. mastitis, lameness, metritis) using producer-recorded event data combined with genomics. The U.S. dairy evaluation now formally includes over 50 traits, having added fertility traits (age at first calving), longevity (cow livability), calving traits, six common health disorders, feed efficiency (residual feed intake), and even

gestation length in recent years. Genomic selection enabled these additions by providing the statistical power to estimate breeding values even for traits with sparse or binary phenotypes, as long as a reference population with phenotypes and genotypes exists. The net result is that current breeding programs are increasingly multi-trait, aiming for balanced improvement. For example, Nordic dairy breeding programs include an index of health and fertility traits alongside production, and beef breeding programs consider temperament scores or feed intake measures if available. However, even with these expansions, the phenotypic data used in genetic evaluations today are still largely intermittent, manually collected measures (like veterinary recorded disease events or monthly production) rather than continuous behavioral or physiological metrics. Integrating rich activity data (behavioral and physiological) from sensors into existing breeding programs poses several challenges. One major hurdle is data standardization and management. Traditional breeding databases handle relatively low-frequency records (e.g. one lactation yield per cow per year, or a few disease events per cow) and are not designed for streaming data. In contrast, a single sensor-equipped cow could generate thousands of data points per day (activity counts, position coordinates, etc.), creating a massive amount of information unused in current genetic evaluations (Koltes et al., 2019). Breeding organizations would need new infrastructure to store, curate, and preprocess this big data so that it can be distilled into meaningful phenotype or trait values (for example, summarizing a cow's daily step count into a monthly "activity level" trait). Therefore, this explained challenge refers to phenotype definition - translating raw sensor readings into reproducible phenotypic traits suitable for selection. While it is straightforward to define traditional traits (like 305-day milk yield), it is less obvious how to define a "behavioral trait" from continuous sensor data. Researchers must decide whether the "average daily lying time" or lying bout are the relevant heritable metrics to use for genetic analysis. This requires extensive validation to ensure these derived traits are biologically meaningful and have sufficient genetic variation. Early attempts have shown that many sensor-based measures do have genetic variability, but reaching consensus on trait definitions is ongoing. Another challenge is data compatibility and scale across farms. Different farms may use different brands of sensors with different calibrations or have different management that influences behavior data. For breeding use, data from many herds must be comparable, which might require standardizing outputs from various devices or developing robust genotype-by-environment models. Another major challenge is standardization. Furthermore, not all farms currently use sensors, potentially biasing which animals have rich phenotype data. Often these are elite herds or research farms that invest in advanced monitoring, which could lead to a biased sample if used directly in breeding value estimation. Breeding programs must be cautious in integrating such data or find ways to encourage broader farmer participation in data collection. There are also concerns about the heritability and economic value of these novel phenotypes. Breeders are accustomed to traits like milk yield (highly heritable and directly tied to income) or SCC (moderate heritability, tied to mastitis costs). But for a trait like "daily step count variability" or "rumination time", the heritability might be low or unknown, and the economic value is indirect. It remains challenging to convince industry stakeholders to include a new trait in a selection index unless its genetic parameters and impact on profitability are well understood (Behren et al. 2023). Lastly, technical and logistical issues such as handling massive datasets and computational load must be resolved. National genetic evaluations are already computationally intensive with millions of animals; adding high-frequency phenotypes could require new statistical models (e.g. random regression models to handle longitudinal data) and greater computing resources. In summary, while current breeding programs have successfully incorporated genomic data and expanded trait goals, they are only beginning to scratch the surface of using real-time behavioral and physiological data. Bridging this gap will require addressing data pipeline issues and demonstrating that these new phenotypes can be reliably

measured at scale and will improve selection decisions. Table 1 provides examples of heritability estimates reported for sensor-based traits in cattle.

Table 1. Heritability of activity-related traits in dairy cattle (wearable sensors and automated milking system)

Rumination (min/day)	time	Collar (chewing sensor)	0.34	0.05	Moretti et al., (2018)
Lying time (hours/day)		Leg/Collar accelerometer	0.37	0.07	Nascimento et al., (2024)
Step count (steps/day)		Pedometer (activity tag)	0.19	0.06	Nascimento et al., (2024)
Feeding time (min/day)		Collar (eating time sensor)	0.23	0.03	Cavani et al., (2022)
Activity time (min/day active)		Collar (motion/active time)	0.14	0.02	Lemal et al., (2024)
Milking (milkings/day)	frequency	AMS ¹ robot logs (voluntary)	0.23	0.04	Aerts et al., (2021)
Milking speed (kg/min)		AMS (milk flow rate)	0.42	0.07	Aerts et al., (2021)
Milking (min/visit)	duration	AMS stall time per milking	0.19	0.07	Santos et al., (2018)
Milking (count/day)	refusals	AMS (robotic refusal events)	0.09	0.01	Pedrosa et al., (2023)
Milking interval (hours)		AMS (time between milkings)	0.07	0.03	Santos et al., (2018)

¹automated milking system

Table 1: Examples of heritability estimates for novel sensor-derived or activity traits in cattle. (SE = standard error of heritability estimate)

Future breeding programs with activity and genomic data

The integration of real-time data holds great promise for enhancing the accuracy and scope of selection in dairy cattle breeding. One clear advantage is the ability to capture traits that were previously unmeasured or to measure known traits with much higher frequency and precision. By continuously monitoring animals, breeders can obtain a far more detailed picture of each animal's phenotype, sometimes called a "phenomic profile" or digital fingerprint (Neethirajan and Kemp, 2021). This can improve selection in several ways. First, it can increase selection accuracy for existing traits. For example, instead of relying on a single 305-day milk yield, a model could use daily yield curves or deviations as indicators of health and efficiency. High-frequency data tend to reduce noise through averaging or enable detection of anomalies (like drops due to illness), leading to more precise estimates of an animal's true genetic merit for production or health. Second, real-time data enable selection for new traits related to animal resilience, efficiency, and welfare. Breeding programs could rank animals not just for how much milk they produce, but how they produce it, for instance, cows that maintain steady feed intake and milk output even during heat stress or after a social regrouping (indicator of resilience). Continuous monitoring makes it possible to quantify such resilience traits: one could measure how quickly a cow's rumination drops and recovers around a calving event or during a heatwave, and treat the recovery rate as a heritable trait. Similarly, detailed behavioral data can define temperament or welfare traits. Instead of simple chute score temperament assessments, one could use sensor data to derive a "calmness index" based on how a cow's

heart rate or movement spikes in response to handling. These nuanced traits could then be included in breeding objectives to improve robustness and welfare. Researchers are already exploring genetic parameters for traits like feeding behavior, activity levels, and milking behavior that come from automated systems, as a precursor to including them in evaluations (Behren et al., 2023). The continuous nature of sensor data also allows early-life phenotyping of young animals, which can aid selection. For instance, in heifer calves, we could track activity and growth with sensors to identify those with superior efficiency or healthier behavior patterns before they even enter the milking herd, and use that information in selection or culling decisions. Overall, future breeding programs that harness real-time data can shift from improving solely output traits (milk, meat) to improving process and fitness traits (how the animal achieves that output), and ultimately breeding cattle that are not only productive but also healthy, behaviorally well-adjusted, and environmentally resilient. A crucial step toward this future is determining the genetic parameters (heritability, genetic correlations) of novel sensor-derived traits. Early studies in this area are promising, indicating that many behavior and physiological metrics do have a genetic basis (Table 1). Genetic analysis of Brown Swiss dairy cows reported heritabilities of ~ 0.38 for general temperament and ~ 0.12 for aggression toward herd mates (Kramer et al., 2013). Those values suggest that selection could alter these behaviors over generations. Similarly, data from automatic milking systems (AMS) has revealed moderate heritabilities for milking behavior traits. Traits like milking speed, refusals, or the time taken for a cow to attach to the milking unit show heritabilities on the order of 0.2–0.5 in various studies (Behren et al., 2023). One study found that the time a cow spends in the milking stall getting the milking unit attached (an indicator of cooperation with the robot) had a heritability ~ 0.26 (Piwcynski et al., 2021). This indicates substantial genetic variation in how cows behave in robotic milking setups. Genetic correlations from those studies also seem promising, for instance, calmer cows that don't kick off the milking unit tend to have higher milking efficiency (genetic correlation around -0.5 between kicking behavior and milk yield per robot visit) (Behren et al., 2023). This means selecting for good robot behavior could indirectly improve production efficiency. Feeding behavior traits measured by sensors show moderate genetic variation as well. In beef cattle, using electronic feeders, researchers have documented heritabilities of ~ 0.28 for daily feeding duration and ~ 0.38 for daily feeding frequency (Brito et al., 2020). These are comparable to heritabilities of traditional growth traits, implying that if feed intake data are collected, one can select for more feed-efficient feeding patterns. Traditional feed efficiency (residual feed intake) is expensive to measure, but with automated systems the industry can gather large-scale data and exploit those moderate heritabilities to breed more feed-efficient animals. Another novel phenotype is health-related sensor traits; for example, automated milking systems can measure electrical conductivity of milk (for mastitis detection) or body weight daily. The heritability of changes in milk conductivity as an indicator of mastitis has been reported in range of 0.23 – 0.53 (Santos et al., 2018; Wethal et al., 2020), therefore, this trait can be managed with genomic selection if the trait is economically important. From the physiological perspective, traits like body temperature or respiration rate measured by sensors tend to have low heritability on the observed scale as one study found a heritability of 0.04 for respiration rate in heat-stressed cows (Luo et al., 2022). This low value suggests that while genetics plays a role, these values are heavily influenced by environment and short-term factors. Nonetheless, even lowly heritable traits might be useful as part of an index if they correlate genetically with important outcomes (for instance, a cow's ability to keep a lower respiration rate under heat stress might genetically correlate with heat tolerance in milk production). As more data accumulates, we expect more reliable estimates of heritability for traits like daily activity level, lying time, rumination time, or vocalization rate. Many of these are likely polygenic, but with enough data and genomic tools, breeding values can be predicted.

The emerging consensus is that most sensor-derived behavioral and welfare traits are at least slightly heritable and thus selective breeding can improve them (Kaur et al., 2023).

Conclusion

The future prospects of digital phenotyping in cattle breeding are extremely promising. We are moving towards an era where an animal's genetic merit may be informed by thousands of data points capturing its behaviour, physiology, and performance under various conditions, marking a significant departure from the sparse and static records of the past. Such “deep phenotyping”, when combined with genomic information, can substantially increase the accuracy of selection and enable genetic progress in traits that were previously elusive. This will contribute to breeding cattle that are not just high-producing, but also healthier and more sustainable. The new approach is creating a precision breeding program that mirrors precision farming: making finely tuned decisions for each breeding animal based on rich, real-time data. To reach this goal, ongoing research and development are needed. Key areas for advancement include improving sensor hardware, developing better algorithms to translate raw sensor data into standardized traits, and updating genetic evaluation models to handle new data types.

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References

- Aerts, J., Piwczyński, D., Ghiasi, H., Sitkowska, B., Kolenda, M., & Önder, H. (2021). Genetic parameters estimation of milking traits in Polish Holstein-Friesians based on automatic milking system data. *Animals*, 11(7): 1943.
- Bar, D., & Solomon, R. (2010). Rumination collars: What can they tell us? In *Proceedings of the First North American Conference on Precision Dairy Management* (pp. 214–216). Toronto, Canada.
- Behren, L. E., König, S., & May, K. (2023). Genomic selection for dairy cattle behavior considering novel traits in a changing technical production environment. *Genes*, 14(10): 1933.
- Brito, L. F., Oliveira, H. R., McConn, B. R., & Schinckel, A. P. (2020). Large-scale phenotyping of livestock welfare in commercial production systems: A new frontier in animal breeding. *Frontiers in Genetics*, 11: 793.
- Cavani, L., Brown, W. E., Parker Gaddis, K. L., Tempelman, R. J., VandeHaar, M. J., White, H. M., Peñagaricano, F., & Weigel, K. A. (2022). Estimates of genetic parameters for feeding behavior traits and their associations with feed efficiency in Holstein cows. *Journal of Dairy Science*, 105(9): 7564–7574.
- Eckelkamp, E. A. (2019). Invited review: Current state of wearable precision dairy technologies in disease detection. *Applied Animal Science*, 35(3): 209–220.
- Farris, E. J. (1954). Activity of dairy cows during estrus. *Journal of the American Veterinary Medical Association*, 125: 117–120.
- Fraser, E. D. G., & Campbell, M. (2019). Agriculture 5.0: Reconciling production with planetary health. *One Earth*, 1(3): 276–279.
- Gur, P. (n.d.). Cow Welfare – the Afimilk way. Afimilk Ltd. (Industry article noting Afikim's introduction of the first electronic milk meter and heat-detection pedometer)
- Hoffmann, G., Strutzke, S., Fiske, D., Heinicke, J., & Mylostyvyi, R. (2024). A new approach to recording rumination behavior in dairy cows. *Sensors*, 24(17): 5521.
- Kaur, A., Cavani, L., Parker Gaddis, K. L., Tempelman, R. J., VandeHaar, M. J., White, H. M., Peñagaricano, F., & Weigel, K. A. (2023). Genetic relationships between behavioral traits

- and feed efficiency traits in lactating Holstein cows. *Journal of Dairy Science*, 106(10): 8141–8149.
- Kiddy, C. A. (1977). Variation in physical activity as an indication of estrus in dairy cows. *Journal of Dairy Science*, 60(2): 235–243.
- Kleen, J. L., Guatteo, R. (2023). Precision Livestock Farming: What does it contain and what are the perspectives? *Animals*, 13(5): 779.
- Koltes, J. E., Cole, J. B., Clemmens, R., Dilger, R. N., Kramer, L. M., Lunney, J. K., McCue, M. E., McKay, S. D., Mateescu, R. G., Murdoch, B. M., Reuter, R., Rexroad, C. E., Rosa, G. J. M., Serão, N. V. L., White, S. N., Woodward-Greene, M. J., Worku, M., Zhang, H., & Reecy, J. M. (2019). A vision for development and utilization of high-throughput phenotyping and big data analytics in livestock. *Frontiers in Genetics*, 10: 1197.
- Kramer, M., Erbe, M., Bapst, B., Bieber, A., & Simianer, H. (2013). Estimation of genetic parameters for novel functional traits in Brown Swiss cattle. *Journal of Dairy Science*, 96(9): 5954–5964.
- Lamanna, M., Bovo, M., & Cavallini, D. (2025). Wearable collar technologies for dairy cows: A systematized review of current applications and future innovations in precision livestock farming. *Animals*, 15(3): 458. <https://doi.org/10.3390/ani15030458>
- Lemal, P., Tran, M.-N., Atashi, H., Schroyen, M., & Gengler, N. (2024). Adding behavior traits to select for heat tolerance in dairy cattle. *JDS Communications*, 5: 368–373. DOI: 10.3168/jdsc.2023-0421
- Lewis, G. S., & Newman, S. K. (1984). Changes throughout estrous cycles of variables that might indicate estrus in dairy cows. *Journal of Dairy Science*, 67(1): 146–152.
- Luginbuhl, J. M., Pond, K. R., Russ, J. C., & Burns, J. C. (1987). A simple electronic device and computer interface system for monitoring chewing behavior of stall-fed ruminant animals. *Journal of Dairy Science*, 70(6): 1307–1312.
- Luo, H., Hu, L., Brito, L. F., Dou, J., Sammad, A., Chang, Y., Ma, L., Guo, G., Liu, L., Zhai, L., Xu, Q., & Wang, Y. (2022). Weighted single-step GWAS and RNA sequencing reveals key candidate genes associated with physiological indicators of heat stress in Holstein cattle. *Journal of Animal Science and Biotechnology*, 13: 88.
- Mayberry, D. E., Ash, A., Prestwidge, D., Godde, C., Harrison, M. T., & Eckard, R. J. (2023). Opportunities and challenges for integrating precision livestock technologies into dairy cattle breeding programmes. *Animal Production Science*, 63(6): 578–586.
- Mazrier, H., Tal, S., Aizinbud, E., & Bargai, U. (2006). A field investigation of the use of the pedometer for the early detection of lameness in cattle. *The Canadian Veterinary Journal*, 47(9): 883–886.
- Menezes, G. L., Pontes Ferreira, R. E., Chinelato, L. S., et al. (2024). Artificial intelligence for livestock: a narrative review of the applications of computer vision systems and large language models for animal farming. *Animal Frontiers*, 14(6): 36–47.
- Moretti, R., de Rezende, M. P. G., Biffani, S., & Bozzi, R. (2018). Heritability and genetic correlations between rumination time and production traits in Holstein dairy cows during different lactation phases. *Journal of Animal Breeding and Genetics*, 135(4): 293–299.
- Nascimento, B. M., Cavani, L., Caputo, M. J., Marinho, M. N., Borchers, M. R., Wallace, R. L., Santos, J. E. P., White, H. M., Peñagaricano, F., & Weigel, K. A. (2024). Genetic relationships between behavioral traits and feed efficiency traits in lactating Holstein cows. *Journal of Dairy Science*, 107(10): 8141–8149.
- Neethirajan, S. (2023). Digital phenotyping: A game changer for the broiler industry. *Animals*, 13(16): 2585.
- Neethirajan, S., & Kemp, B. (2021). Digital phenotyping in livestock farming. *Animals*, 11(7): 2009.

- Pedrosa, V. B., Boerman, J. P., Gloria, L. S., Chen, S.-Y., Montes, M. E., Doucette, J. S., & Brito, L. F. (2023). Genomic-based genetic parameters for milkability traits derived from automatic milking systems in North American Holstein cattle. *Journal of Dairy Science*, 106(4): 2613–2629.
- Pedrosa, V. B., Chen, S. Y., Gloria, L. S., Doucette, J. S., & Silva, M. A. (2024). Machine learning methods for genomic prediction of cow behavioral traits measured by automatic milking systems in North American Holstein cattle. *Journal of Dairy Science*.
- Piwczyński, D., Kolenda, M., Sitkowska, B., & Aerts, J. (2021). Genetic parameters of workability traits in the population of Polish Holstein-Friesian cows based on conventional and genomic data. *Animals*, 11(8), 2443.
- Prasad, S. (2023). RFID technology and its application in milk production management. *AgriGate – An International Multidisciplinary e-Magazine*, 2(8): 56–62.
- Santos, L. V., Brügemann, K., Ebinghaus, A., & König, S. (2018). Genetic parameters for longitudinal behavior and health indicator traits generated in automatic milking systems. *Archives Animal Breeding*, 61: 161–171.
- Seidel, A., Krattenmacher, N., & Thaller, G. (2020). Dealing with complexity of new phenotypes in modern dairy cattle breeding. *Animal Frontiers*, 10(2): 23–29.
- Senger, P. L. (1994). The estrus detection problem: New concepts, technologies, and possibilities. *Journal of Dairy Science*, 77(9): 2745–2753.
- Shamah, D. (2014, December 12). Agritech firm SCR acquired for \$250 million. *The Times of Israel*. (Reports SCR's first collar tags in 1998 and Heatime collar system launch in 2006)
- UN: World Population Prospects: The 2017 Revision.
- Upadhyay, V. R., Ramesh, V., Kumar, H., & Singh, S. K. (2024). Phenomics in livestock research: Bottlenecks and promises of digital phenotyping and other quantification techniques on a global scale. *OMICS: A Journal of Integrative Biology*.
- Wethal, K. B., Ødegård, J., Svendsen, M., & Heringstad, B. (2020). Genetic analysis of mastitis-related traits in Norwegian Red cows. *Journal of Dairy Science*, 103(8): 7188–7198.
- Wiggans, G. R., & Carrillo, J. A. (2022). Genomic selection in United States dairy cattle. *Frontiers in Genetics*, 13, 994466.

Session 1

Plant and Food Production

ORIGINAL SCIENTIFIC PAPER

The impact of foliar application of inactive yeasts on the grape chemical composition of 'Babic crni' grape variety

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Summary

Yeasts extracts can change the chemical composition of the treated berries and are considered biological elictors.

Aim of this study was to examine the impact of foliar application of inactive yeasts of the species *Saccharomyces cerevisiae* on the grape chemical composition of the indigenous Croatian grapevine variety 'Babić crni'.

Significant differences were found between treated and control samples total flavonols, total anthoyanins and total stilbens. Yeast treatment did not affect titratable acidity, pH, organic acid content, and sugar content in must.

The study showed that treatment with inactive yeasts "LalVigne® MATURE" can significantly affect the properties of the 'Babic crni' grape variety, thus suggesting potential for improving wine quality.

Key words: grapevine, polyphenols, 'Babic crni', inactive yeasts

Introduction

Climate change could have serious consequences and cause major economic damage in the vine and wine sector due to a strong vulnerability of grapevine (Ollat et al., 2017). Climate is a limiting factor determining phenology, vegetative growth, physiological development, fruit production and, therefore, the quality of grapes and wines (Van Leeuwen et al., 2004; Keller et al., 2010). Extreme heat stress during the maturation period abruptly slows down the metabolism of the vine. This may result in increased sugar levels and reduced acidity, potentially increasing the risk of wine deterioration (De Orduna, 2015), leading to a decrease in production and quality. Due to the increasing number of exceptionally warm years, forecasts of future climate scenarios indicate that areas suitable for vine cultivation will move towards northern areas, while southern regions become too hot and therefore no longer suitable for cultivation of certain varieties (Lazoglou et al., 2018). Polyphenols are secondary plant metabolites and significant organic compounds present in grapes, must and wine. Their importance in grapes stems mainly from the influence on the quality of wines, as they play a key role in shaping the colour, taste, astringency, bitterness and the potential for ageing wines (Niculescu et al., 2017). Polyphenols are generally defined as compounds containing multiple aromatic rings, each with one or more hydroxyl groups. Phenols represent a class of compounds containing an aromatic ring, regardless of the number of hydroxyl groups associated with it (Quideau et al., 2011). Polyphenols are divided into two main groups, namely flavonoids and non-flavonoids (phenolic acids) (En-Qin et al., 2010). Representatives of main groups of polyphenols in grapes include flavanoids and stilbens. Flavonoids, including anthocyanes, flavonoli and flavan-3-oli, form the most significant group of polyphenols (Lorrain et al., 2013). Phenolic acids are divided according to the structure into hydroxybenzoic and hydroxymethylic acids (Garrido and Borges, 2013). Stilbens, which act as phytoalexins in the vine, are becoming

increasingly interesting due to their positive impact on human health (Flamini et al., 2013). Anthocyanins are found in the skin of grapes as free and acylated derivatives of 3-*O*-glycosides. They play a key role in determining the colour of grapes (Guidoni and Hunter, 2012). The elicitor is defined as a substance that stimulates or increases the biosynthesis of certain compounds in order to adapt plants to stress conditions when applied in small quantities to the living system. They are classified as biotic and abiotic, depending on their origin and molecular structure (Radman et al., 2003). Among biological elicitors, yeast extracts contain several compounds (Ferrari, 2010). Yeast extracts can change the polyphenol content of the treated berries and are considered biological elicitors (Giacosa et al., 2019). In this case, the plant's defense mechanism relies on the berry's epidermis, which serves as a barrier against physical damage and pathogens attack. (Santamaría et al., 2011), improving the secondary metabolism of the ripening fruit (Zhao et al., 2005). Portu et al. (2016) reported an increase in the content of anthocyanes in grapes and consequently in wines of the Tempranillo variety in Spain following a foliar treatment with oenological yeasts. These findings are supported by the research of Villangó et al. (2015), where foliar treatments on Syrah in Hungary with yeast improved the maturation process of grapes in warmer and colder harvesting, resulting in a higher accumulation of anthocyanes in treated grapes and thus creating more balanced, tasty and complex wines. Aim of this study was to examine the impact of foliar application of inactive yeasts of the species *Saccharomyces cerevisiae* on the grape chemical composition of the indigenous Croatian grapevine variety 'Babić crni'.

Material and methods

Grape samples and experimental design

The experiment was carried out during 2023 growing season in commercial vineyard of variety 'Babić crni' in Sibenik wine growing area (Jadrtovac), Dalmatia, Croatia. The experiment was set according to the random block schedule method in three replicates with two variants: control and treatment. The treatment consisted of a foliar application of inactive yeast „LalVigne® MATURE“ as instructed by the manufacturer (at the beginning of the veraison and after 15 days) (Lallemant). Each variant is represented with 50 vines per replicate. For chemical analysis ten clusters were randomly chosen. The berries were removed with small scissors and were left with attached pedicels. Part of the berries were immediately frozen for the analysis of secondary metabolites. The rest of the berries were used for basic parameter analysis (total soluble solids, titratable acidity, pH) according to the methods of International Organisation for Vine and Wine (OIV, 2019). The same samples were used for organic acid analysis (Zoecklein et al., 1995).

Analysis of polyphenolic compounds

The grape skins were manually removed from the pulp and freeze-dried. Freeze-drying was performed at 0.1 mbar for 72 h. Dry skins were grinded and obtained powder (500 mg) was extracted by a 10 mL of 70% aqueous ethanol, containing 1% formic acid for one day in the dark at room temperature. The extract was centrifuged in LC-321 centrifuge (Tehtnica, Železnik, Slovenia) for 20 min at 5000 rpm at room temperature. Supernatant was collected, concentrated under vacuum to remove ethanol (40 °C) on rotary evaporator and brought to final volume of 10 mL with a mobile phase A. The extract was filtered with Phenex-PTFE 0.20 µm syringe filter (Phenomenex, Torrance, CA, USA) and analyzed by HPLC.

Separation and identification of polyphenol compounds was performed with the method described by Tomaz and Maslov (2016). The analyses were performed on an HPLC Agilent 1100 (Agilent Technologies, Palo Alto, CA USA) comprising a binary pump, an auto-sampler, a diode array detector and Agilent 1200 fluorescence detector. Separation was performed on Luna Phenyl-Hexyl (Phenomenex, Torrance, CA USA) column (250 mm × 4.6 mm i.d., 5 µm particle size) with Phenyl guard column (4.0 × 3.0). Column was heated at 50 °C. The injection

volume for all samples was 20 μ L. Gradient consists of two phases: (A) water/phosphoric acid (99.5/0.5, v/v), and (B) acetonitrile/water/phosphoric acid (50/49.5/0.5, v/v/v). For detection and quantification of compounds, the chromatograms were recorded at 280, 360, and 518 nm by diode array detector and at excitation wave-length 225 nm and emission wavelengths at 320 nm by fluorescence detector. UV-VIS spectra were recorded in range of 200–700 nm. Quantification of non-commercial available standards of anthocyanins was made accordingly to the calibration curves of mal-vidin-3-O-glucoside. Samples were analyzed in triplicate.

Statistical analysis

ANOVA was used to test the significance of the experimental treatments on measured parameters. In the case of significant results obtained by ANOVA, means were compared using Duncan's multiple range test among experimental treatments. All the analysis were carried out using XLSTAT software (Addinsoft, USA).

Results and discussion

Basic chemical parameters and cluster weight

The results of the analysis show significant differences in the cluster weight between the treatment and control. The weight of treated grapes of the “Babic crni” variety is significantly increased. The average cluster weight in the treatment was 24,88% higher compared to control. A higher weight of clusters can lead to an increased the productivity of vineyards, which is of great importance to winemakers. Suklje et al. (2016) did not record significant changes in the weight of clusters 'Sauvignon blanc'. Basic chemical analysis of must shows that the treatment had no significant effect on the TSS, titratable acidity and pH. Authors of Suklje et al. (2016) and Porto et al. (2016) did not record significant changes in sugar content, which coincides with the results of this study. Villangó et al. (2015) investigated the impact of the use of inactive dry yeasts on the maturation of Syrah grapes. In their research, too, the sugar content was not significantly altered by the yeast treatment.

Table 1. Average values of cluster weight and chemical parameters of experimental variants

Variant	Cluster weight (g)	TSS (Oe ⁰)	Titratable acidity (g L ⁻¹)	pH	Citric acid (g L ⁻¹)	Tartaric acid (g L ⁻¹)	Malic acid (g L ⁻¹)
Control	116,87 b	99,67 a	4,72 a	3,35 a	0,3 a	7,05 a	0,48 a
Treatment	155,57 a	99,67 a	4,6 a	3,31 a	0,27 a	6,6 a	0,78 a

* Means with different superscript letters in the same row differ significantly ($p \leq 0.05$). Treatment - foliar application of inactive yeast, Control – no application

Based on the results of the analysis of the organic acids of must, it has been established that there was no significant differences in their content in relation to treated and control samples. Suklje et al. (2016) recorded a significant increase in the tartaric acid content, which is not in line with the research. However, similarly to this study, there were no significant changes in the malic acid content. Malic acid content in treatment was 39 % higher compared to control which is contrary to Porto et al. (2016) .

Polyphenolic compounds

The results show that total anthocyanes in the treatment were significantly higher than in the control variant which is in line with research of Porto et al (2016). Total stilbens also have significantly more in the trial than in the control variant. Total phenolic acids content was slightly higher in the treatment, but without statistically significant difference. Furthermore, total flavonol glycosides in the control variant are significantly higher than in the treatment. Total flavan-3-ols were also higher in the control variant than in the treatment, but the difference is not significant which is in line with research of Villangó-a et al. (2015.).

Table 2. Average content of polyphenol groups of experimental variants expressed in $\mu\text{g g}^{-1}$

Variant	Total anthocyanans	Total flavonol	Total fenolic acids	Total stilbens	Total flavan-3-ols
Control	357,70 a	107,61 b	101,65 a	8,91 a	148, 96 a
Treatment	290, 82 b	127,16 a	94,15 a	7,04 b	162,02 a

* Means with different superscript letters in the same row differ significantly ($p \leq 0.05$).

Conclusions

These results suggest that treatment with inactive yeasts can have a significant impact on the grape chemical composition of the 'Babic crni' variety and potentially improve the quality of wines. Comparison of results of this study with previous works shows consistency in several key parameters, but also emphasizes the need for further research in order to better understand specific conditional variations, especially in changes in organic acids and polyphenol composition.

References

- De Orduna, R.M. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*. 43(7): 1844-1855. Available from: <https://doi.org/10.1016/j.foodres.2010.05.001>
- En-Qin, X., Gui-Fang, D., Ya-Jun, G., Hua-Bin, L. (2010). Biological Activities of Polyphenols from Grapes. *Int. J. Mol. Sci.* 11(2): 622-646. Available from: DOI: 10.3390/ijms11020622
- Ferrari, S. (2010.) Biological elicitors of plant secondary metabolites: Mode of action and use in the production of nutraceuticals. *Biofarmas for nutraceuticals: Functional food and safety control by biosensors*. 698: 152–166. Available from: DOI: 10.1007/978-1-4419-7347-4_12
- Flamini, R., Mattivi, F., De Rosso, M., Arapitsas, P., Bavaresco, L. (2013). Advanced Knowledge of Three Important Classes of Grape Phenolics: Anthocyanins, Stilbenes and Flavonols. *International Journal of Molecular Sciences*, 14(10): 19651-19669. Available from: DOI: 10.3390/ijms141019651
- Garrido, J. and Borges, F. (2013). Wine and Grape Polyphenols - a Chemical Perspective. *Food Research International*. 54(2): 1844-1858. Available from: <https://doi.org/10.1016/j.foodres.2013.08.002>
- Giacosa, S., Ossola, C., Botto, R., Segade, S.R., Paissoni, M.A., Pollon, M., Gerbi, V., Rolle, L. (2019.). Impact of specific inactive dry yeast application on grape skin mechanical properties, phenolic compounds extractability, and wine composition. *Food Research International*. 116: 1084 – 1093. Available from: DOI: 10.1016/j.foodres.2018.09.051
- Guidoni, S. and Hunter, J.J. (2012). Anthocyanin profile in berry skins and fermenting must/wine, as affected by grape ripe ness level of *Vitis vinifera* cv. Shiraz/R99. *Eur Food Res Technol*. 235: 397–408. Available from: DOI: 10.1007/s00217-012-1744-5
- Keller, M., (2010). The science of grapevines. In *Anatomy and Physiology*, 1st ed., Elsevier Academic Press. London, UK.
- Lazoglou, G., Anagnostopoulou, C., Koundouras, S., (2018). Climate change projections for Greek viticulture as simulated by a regional climate model. *Theoretical and Applied Climatology*, 133(1-2): 551–567. Available from: DOI: 10.1007/s00704-017-2198-2
- Lorrain, B., Ky, I., Pechamat, L. Teissedre, P. L. (2013). Evolution of Analysis of Polyhenols from Grapes, Wines, and Extracts. *Molecules*, 18(1): 1076-1100. Available from: <https://doi.org/10.3390/molecules18011076>
- New Foliar Spray. *South African Journal of Enology and Viticulture*. 36 (3): 303-315. Available from: DOI: 10.21548/36-3-964
- Niculescu, V-C., Nadia Paun, N., Ionete, R-E. (2018). The Evolution of Polyphenols from Grapes to Wines. In *Grapes and Wines – Advances in Production, Processing, Analysis and*

- Valorization, Jordão, A.M. and Cosme, F. (ed), 119-141. Rijeka, Croatia: InTech. Available from: DOI: 10.5772/intechopen.72800
- Oiv (2019). COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS, France.
- Ollat, N., Van Leeuwen C., Garcia De Cortazar Atauri I., Touzard J. M. (2017). The challenging issue of climate change for sustainable grape and wine production. *OENO One*. 51(2): 59–60. Available from: <https://doi.org/10.20870/oeno-one.2017.51.2.1872>
- Portu, J., López, R., Baroja, E., Santamaría, P., Garde-Cerdán, T. (2016). Improvement of grape and wine phenolic content by foliar application to grape- vine of three different elicitors: methyl jasmonate, chitosan, and yeast extract. *Food Chemistry*. 201: 213 – 221. Available from: DOI: 10.1016/j.foodchem.2016.01.086
- Quideau, S., De eux, D., Douat-Casassus, C., Pouysegu, L. (2011). Plant Polyphenols: Chemical Properties Biological Activities, and Synthesis. *Ang. Chem*. 50(3): 586–621. Available from: DOI: 10.1002/anie.201000044
- Radman, R., Saez T., Bucke C., Keshavarz T. (2003). Elicitation of plants and microbial cell systems. *Biotechnol Appl Biochem*. 37(1): 91-102. Available from: DOI: 10.1042/ba20020118
- Santamaría, A.R., Mulinacci, N., Valletta, A., Innocenti, M., Pasqua, G. (2011). Effects of elicitors on the production of resveratrol and viniferins in cell cultures of *Vitis vinifera* L. cv Italia. *Journal of Agricultural and Food Chemistry*. 59 (17): 9094 – 9101. Available from: DOI: 10.1021/jf201181n
- Šuklje K., Antalick G., Buica A., Coetzee Z.A., Brand J., Schmidtke L.M., Vivier M.A. (2016). Inactive dry yeast application on grapes modify Sauvignon Blanc wine aroma. *Food Chemistry*. 197: 1073 – 1084. Available from: DOI:10.1016/j.foodchem.2015.11.105
- Tomaz, I., and Maslov, L. (2016). Simultaneous Determination of Phenolic Compounds in Different Matrices using Phenyl-Hexyl Stationary Phase. *Food analytical methods*. 19(2): 401-410. Available from: DOI: 10.1007/s12161-015-0206-7
- Van Leeuwen, C., Friant, P., Chone, X., Tregoat, O., Koundouras, S., Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *American Journal of Enology and Viticulture*. 55(3): 207-217. Available from: DOI:10.5344/ajev.2004.55.3.207
- Villangó, Sz., Pásti, Gy., Kállay, M., Leskó, A., Balga, I., Donkó, A., Ladányi, M., Pálfi, Z., Zsófi, Zs. (2015). Enhancing Phenolic Maturity of Syrah with the Application of a new foliar spray. *South African Journal of Enology and Viticulture*. 36(3): 304-315. Available from: <https://www.scielo.org.za/pdf/sajev/v36n3/06.pdf>
- Zoecklein, B.K., Fugelsang, K.C., Gump, B.H., Nury, F.S. (1995). *Wine Analysis and Production*. New York, SAD: Chapman & Hall.

EXPERT PAPER

Agrotechnical Approaches in Potato Production: A SWOT-Based Review

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This study examines the effects of weather conditions and agrotechnical practices on potato yield, based on a retrospective three-year production analysis conducted on a family-owned farm. The research evaluated data on plant protection methods, fertilization, soil cultivation, and irrigation, and their respective impacts on disease incidence and final yield. Additionally, the influence of weather variability during the growing season on disease development and yield fluctuations was explored. The results indicate that the timely application of fungicides and insecticides, combined with biological control measures, effectively reduces disease occurrence and enhances the market quality of tubers. Across the observed years, yields ranged between 35 and 52,3 t ha⁻¹. Consistent irrigation proved beneficial for yield stability during drought conditions, mitigating plant stress and promoting uniform tuber development. Based on the findings of the SWOT-based review, it can be concluded that adjusting agrotechnical measures in accordance with weather patterns, including the implementation of an efficient irrigation strategy, has a significant impact on both the yield and quality of potatoes, while also reducing potential economic losses.

Key words: potato, yield, weather conditions, agrotechnical practices, plant protection

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important agricultural crops globally, with significant economic and nutritional value. Of the total global production, 52% is used for human consumption, 21% for animal feed, 10% for seed purposes, and 12% for processing, while the remainder accounts for post-harvest losses (FAO, 2009). Its yield depends on a variety of factors, including applied agrotechnical measures, prevailing weather conditions, and the occurrence of plant diseases (Haverkort and Struik, 2015). Tunio et al. (2020) state that the global average yield for potato production is about 17.4 t ha⁻¹, and that the United States leads the way with an average of 44.2 t ha⁻¹, closely followed by the United Kingdom.

Nutritionally, potatoes are a valuable food source. According to data from the USDA National Nutrition Database (2025), raw potatoes are rich in antioxidants and consist of approximately 79% water. They provide about 325 kcal of energy, 7.6 g of protein, 0.04 g of fat, and 72.8 g of carbohydrates. They also contain 42 mg of calcium, 231 mg of phosphorus, 2.7 mg of iron, 70 IU of vitamin A, 0.15 mg of riboflavin, 4.4 mg of niacin, and 64 mg of vitamin C (ascorbic acid).

Potato varieties are classified by vegetation period into early, medium-early, medium-late, and late types (Šuljaga, 2005). Increasing drought frequency threatens conventional agriculture, reducing yields by up to 60%, making irrigation essential (Ferreles and Soriano, 2007). According to Sito et al. (2015) the genetic potential of today's potato varieties exceeds a yield of 60 t ha⁻¹.

A SWOT analysis is a simple yet powerful tool for understanding the objective overview in any business or project and reducing risks (Nuga and Asimea, 2014; Goffart et al., 2022).

The aim of this study is to analyze potato production on a family farm, focusing on agrotechnical measures, e.g., the effects of fertilization, plant protection, and irrigation on final yield.

Particular attention was paid to weather conditions during the growing season, in order to assess their influence on plant development and disease incidence. Based on the analysis of the agrotechnical measurements and the obtained potato yield, a SWOT analysis was the second aim of the present study.

Material and methods

This study was conducted on the family-owned agricultural farm “Bešlić Marin” over the period from 2022 to 2024. The farm is located in Bokšić Lug, in the municipality of Đurđenovac. It manages a total of 340 hectares of land, with the largest share dedicated to potato production, followed by wheat and barley, soybeans, sugar beet, and fava beans.

Soil tillage begins after harvest in July with shallow cultivation and sowing of leguminous cover crops to improve fertility. Primary tillage is conducted in autumn by plowing at a depth of 25–30 cm to retain winter moisture. Additional tillage with a cultivator is performed in early spring to improve soil looseness, especially for heavy soils. Final preparation and planting occur simultaneously in March using a rotary harrow combined with a planter in a single pass.

Planting occurred between March and April, depending on the year. The farm cultivates Opal, Napoleon, Sorentina, and Kibitz for chip production, and Camelia exclusively for fresh consumption. The total potato area varied from 30 ha (2022) to 52 ha (2024).

Fertilization was based on soil analysis and adjusted to tuber development stages. Manure was applied every 2–3 years, and nitrogen was distributed in multiple forms to ensure availability throughout the season. Foliar feeding with micronutrients occurred in May, and calcium ammonium nitrate (CAN) was applied in two doses prior to rainfall or irrigation to improve uptake. Irrigation dose provided 20

During vegetation, 10–20 treatments were conducted against late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) using contact and systemic fungicides. Insecticides were rotated to prevent resistance, and weed control was achieved using herbicides at standard timings. Colorado potato beetles were controlled both mechanically and chemically. Plant protection strategies were adapted to weather conditions and crop phenology.

Potato harvesting using a combine harvester marked the final technological step, with efficiency ranging from 30–90 tons per day, depending on yield.

Results and discussion

The distribution of cultivated area by variety and year is presented in Table 1. The total potato production area increased progressively from 30 ha in 2022 to 52 ha in 2024. The Opal variety was dominant throughout all three years, with the highest area in 2023 (42.5 ha), indicating its agronomic reliability and market demand. In 2022, the area was more evenly distributed among three varieties: Opal (20 ha), Kibitz (5 ha), and Sorentina (5ha). In contrast, 2024 introduced Camelia, cultivated on 4.5 ha, solely for table consumption.

Table 1. Cultivated area by potato variety and year (ha)

Year	Opal	Napoleon	Sorentina	Kibitz	Camelia	Total area (ha)
2022	20.0	-	5	5.0	-	30.0
2023	42.5	0.5	-	-	-	43.0
2024	26.5	21.0	-	-	4,5	52.0

Napoleon showed a significant increase from only 0.5 ha in 2023 to 21 ha in 2024, likely due to positive agronomic or economic performance. These data reflect the farm's adaptive variety selection strategy based on yield performance, market needs, and agronomic suitability across growing seasons.

The goal of irrigation is to maintain optimal soil moisture, with irrigation beginning earlier due to dry springs. In recent years, the system has been activated in April, with approximately ten weekly irrigation cycles. Though the "tifon" system used is not energy efficient due to high pressure and water loss, it remains functional. Five wells, 90–100 m deep, supply water at 45–75 Ls⁻¹, with those closer to the Drava River being more productive. In years with a wet spring, irrigation began later and was applied less frequently. Uneven water supply can cause plant stress and secondary tuber growth, rendering investments in seed, fertilizers, and crop protection ineffective (Shock, 2002). For optimal yields, understanding soil water-holding capacity and moisture reserves is essential (Opena and Porter, 1999). The relationship between irrigation frequency and yield is clearly visible: 10 irrigation cycles in 2024 contributed to the highest overall yield, especially for the Opal and Napoleon varieties. In contrast, fewer cycles in 2022 and 2023 were associated with lower yields, indicating the vital role of regular water supply during tuber bulking.

Uneven water availability can cause secondary tuber growth and quality issues, as also emphasized by Shock et al. (2002). Adequate knowledge of soil water capacity and the timing of irrigation was crucial in preventing stress and maximizing nutrient uptake.

The monthly distribution of fungicide applications reveals distinct seasonal patterns. The most intensive protection occurred in June, with five treatments in 2023 and six in 2024, coinciding with peak disease pressure. Treatment frequency declined in August, reflecting either plant senescence or reduced infection risk. The total number of fungicide treatments was higher in 2024, aligning with increased rainfall during the critical growth stages. This confirms the need for timely and adaptive plant protection strategies under variable weather conditions.

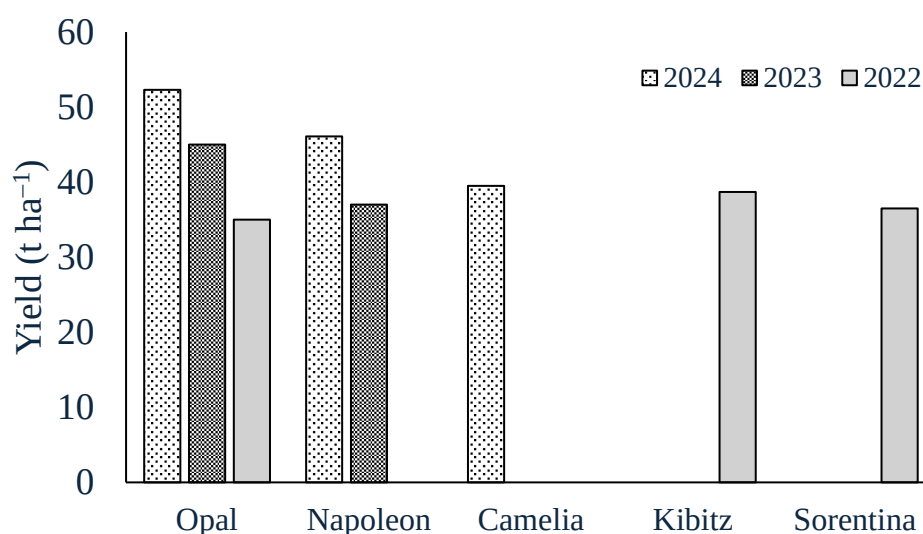


Figure 1. Potato yield by variety and year (t/ha)

The average yield during the 2022–2024 period was 40.7 t ha⁻¹. As shown in Figure 1, potato yield by variety and year (t ha⁻¹) is visually presented. The average potato yield across the three-year period ranged between 35 and 52.3 t ha⁻¹, depending on variety and seasonal conditions. The Opal variety consistently achieved the highest yields, culminating in 52.3 t ha⁻¹ in 2024, under favorable weather and optimal irrigation. Napoleon also showed notable yield improvement, from 37.0 t ha⁻¹ in 2023 to 46.1 t ha⁻¹ in 2024, suggesting a positive response to intensified agrotechnical measures. Camelia, introduced in 2024, recorded a moderate yield of 39.5 t ha⁻¹, appropriate for its exclusive use as a table variety. Kibitz and Sorentina, cultivated only in 2022, yielded 38.7 and 36.5 t ha⁻¹, respectively, indicating satisfactory performance despite less intensive management in the earlier season. The Opal variety consistently

outperformed others, reaching its highest yield in 2024 (52.3 tha^{-1}), likely due to optimal weather and input conditions. Napoleon showed notable improvement, while Camelia, grown only in 2024, had moderate results. The early cultivars Kibitz and Sorentina, recorded only in 2022, demonstrated lower but acceptable yields.

It is recommended to harvest under dry conditions, with minimal exposure of tubers to sunlight to prevent solanine accumulation (Kumar, 2008). In heavy soils, irrigation a few days before harvest is necessary to soften soil clods and minimize tuber damage. Optimal soil moisture ensures a clean harvest and better crop preservation.

Based on the three-year analysis of potato production, the SWOT analysis was done (Table 2). This SWOT analysis highlights the strong potential of potato production, especially in regions with favorable climates, good soil, and growing consumer demand. However, challenges like high input costs, labor demands, and vulnerability to climate change and pests need to be addressed. By investing in education, modern technologies, and leveraging support such as EU funds, producers can reduce weaknesses and take advantage of growing market opportunities while minimizing external threats.

Table 2. The SWOT analysis of potato production on the Family farm

Strengths	Weaknesses
Profitability of production with adequate management	Lack of expertise and education among some producers
Favorable climatic conditions for potato cultivation	High investments (tubers for sowing, fertilizers, protection)
Existing systems for purchase and processing	High labor demands during the season
Good soil quality for potato production	Dependence on irrigation systems due to increasingly frequent droughts
Possibility of growing specialized varieties for various purposes (consumption potatoes, chips, industrial processing)	Price fluctuations and market uncertainty
Demand for quality domestic potatoes	Increased competition from imported potatoes
The possibility of applying modern technologies in cultivation and storage	Limited investment in research and development of domestic varieties
Opportunities	Threats
Growing demand for processed potato products (French fries, chips)	Climate change (adverse weather conditions, extreme droughts)
Increased consumer awareness of the quality of domestic products	Risk of diseases and pests attack
Possibility of developing exports to regional markets	High energy and fuel costs for irrigation and mechanization
Support from EU funds for the modernization of production and irrigation	Competition from imported potatoes with lower prices and unverified quality

Conclusions

Potato is a demanding crop with high investment requirements in seed material, rising input costs, and substantial expenditures for irrigation. During the growing season, it requires intensive labor, particularly at harvest, where at least six workers are needed for tuber cleaning. Despite these challenges, production remains profitable, with yields exceeding 40 tha^{-1} . Success largely depends on technical expertise, experience, and continuous crop monitoring.

References

- FAO. (2009). *International Year of the Potato 2008: New light on a hidden treasure*. Food and Agriculture Organization of the United Nations. Retrieved from: <https://www.fao.org/potato-2008> (19 March 2025)
- Fereres, E., Soriano, M. A. (2007). Deficit irrigation for reducing agricultural water use. *Journal of Experimental Botany*, 58(2), 147–159. <https://doi.org/10.1093/jxb/erl165>
- Goffart, J. P., Haverkort, A., Storey, M., Haase, N., Martin, M., Lebrun, P., Ryckmans, D., Florins, D., Demeulemeester, K. (2022). Potato production in northwestern Europe (Germany, France, the Netherlands, United Kingdom, Belgium): characteristics, issues, challenges and opportunities. *Potato research*, 65(3), 503-547.
- Haverkort, A. J., Struik, P. C. (2015). Yield levels of potato crops: Recent achievements and future prospects. *Field Crops Research*, 182, 76–85. <https://doi.org/10.1016/j.fcr.2015.06.002>
- Kumar, D., Singh, B. P., Kumar, P. (2008). An overview of the factors affecting accumulation of glycoalkaloids in potatoes. *Potato Journal*, 35(1-2), 1–12.
- Nuga, B. O., Asimea, A. O. (2014). Value chain and SWOT analysis of the Manitoba potato sector. *Journal of Agriculture and Social Research (JASR)*, 14(1), 1-7.
- Opena, G. B., Porter, G. A. (1999). Soil management and supplemental irrigation effects on potato: II. Root growth. *Agronomy Journal*, 91(3), 426–431. <https://doi.org/10.2134/agronj1999.00021962009100030014x>
- Sito, S., Džaja, V., Kušec, V., Ciler, K., Palinić, B., Glogovšek, T. (2015). Suvremena tehnika u proizvodnji krumpira. *Glasnik Zaštite Bilja*, 38 (5), 70-83.
- Shock, C. C., Feibert, E. B. G., Saunders, L. D. (2002). Irrigation management for optimum potato quality and yield. *Oregon State University Agricultural Experiment Station*, Special Report 994.
- Šuljaga, N. (2005). Proizvodnja krumpira u Zavodu za krumpir Stara Sušica. *Sjemenarstvo*, 22(1–2), 61–72.
- Tunio, M. H., Gao, J., Shaikh, S. A., Lakhari, I. A., Qureshi, W. A., Solangi, K. A., Chandio, F. A. (2020). Potato production in aeroponics: An emerging food growing system in sustainable agriculture for food security. *Chilean journal of agricultural research*, 80(1), 118-132.
- USDA National Nutrition Database (2025), Retrieved from: <https://snaped.fns.usda.gov/resources/nutrition-education-materials/seasonal-produce-guide/potatoes> (19 March 2025).

ORIGINAL SCIENTIFIC PAPER

A bibliometric study on crop yield models

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Summary

The aim of this paper is to review global crop models and identify those most suitable, with the long-term goal of establishing a continuous national crop yield monitoring system under changing climate conditions in Slovenia. The crop model selection was based on their use in European agriculture and relevance to maize and wheat. A bibliometric analysis (2014–2024) of publication and citation counts highlighted the twelve most frequently cited models, where results suggest that six models, DSSAT, APSIM, AquaCrop, WOFOST, CROPWAT, and JULES, are the most promising. These simulation tools are indispensable for decision-making, optimizing practices, conducting economic analyses, and assessing the impact of climate change on crop yields.

Key words: crop models, yields, bibliometric analysis, climate change

Introduction

The climate change is already having a significant impact on a multitude of extreme weather and climate events across all regions of the world. Between 2018 and 2022, the global mean surface temperature was approximately 1.2 °C higher compared to the period 1850–1900, while in Europe, it was about 2.2 °C higher (EEA, 2024). The recent IPCC report (2022) indicates that climate-related risks are increasing and causing crop yield losses, with studies on crop simulation models and climate scenarios (excluding technological advancements) predict negative impacts on crop yields in the 21st century, with yield reductions per decade: –3.3% for soybeans, –2.3% for corn, –1.3% for wheat, and –0.7% for rice. Also, the climate projections for Slovenia indicate a high probability of continued temperature increases across all seasons and regions (Bertalanč et al., 2018). Monitoring climate change impacts is a challenge for Slovenia, made more difficult by the lack of systematic use of crop yield prediction models. A wide range of crop simulation models can be found in the literature (Gavasso-Rita et al., 2023; Feng et al., 2023). In Slovenia, simulation models that predict crop yields in relation to climate change have yet to be established. Although some individual studies have been conducted (e.g., Ceglar, 2011; Pogačar, 2015), the country still lacks a comprehensive system of simulation models capable of providing long-term predictions for the agricultural sector. The implementation of these models in Slovenia is crucial, as their use could address self-sufficiency challenges and enhance the resilience of Slovenian agriculture to changing climatic conditions.

The aim of this paper is to outline an approach for reviewing existing global models and selecting those that are potentially the most suitable for Slovenia, with a particular focus on maize and wheat—two of the most important arable crops in the country, and the ones for which the most comprehensive datasets are available.

Material and methods

As a first step in our methodological approach, we compiled a comprehensive checklist of globally available crop simulation models used for yield prediction. The first compiled table of

identified global crop simulation models included the following key attributes for each model: model name, spatial scale of application (e.g., field, farm, regional, global), targeted crops, documented examples of applications, and reference literature. In this process, around 100 different simulation models for various crops were identified, each addressing climate or climate change factors in different ways.

The selection criteria for narrowing down the number of models suitable for the Slovenian case were based on their previous application in European agricultural systems and their relevance to maize and wheat. For the crop models that fulfilled these two selection criteria, an additional bibliometric analysis was conducted for the past ten years (from 2014 to 2024). This further reduced the number of potential crop models under consideration. This analysis involved searching the ScienceDirect and Web of Science databases, applying the required conditions. Within these databases, the number of publications and citations associated with each crop model was systematically analyzed. The aim of this bibliometric screening was to evaluate the scientific relevance, visibility, and research engagement of each crop model over the last decade.

Results and discussion

By searching for globally available crop simulation models for yield prediction in the context of climate change, we identified more than 100 different models. After applying two selection criteria – use in European agricultural systems and suitability for maize and/or wheat, the list of crop models was narrowed down to slightly more than 30 models.

In order to further reduce the selection of potentially suitable crop models for Slovenia, we conducted a bibliometric analysis to assess the scientific visibility, relevance, and research usage of each model over the past decade (2014–2024). Based on this analysis, a final selection was established, the results of which are presented in this paper.

Based on the database analysis of publications and citations (Web of Science and ScienceDirect), we identified the twelve most frequently published or cited models (Table 1). The ranking 1 indicates the highest frequency of occurrence according to the selected criteria. The search string included key terms such as the *model name*, *model*, *crop*, *yield*, *climate*, and other thematically most relevant expressions.

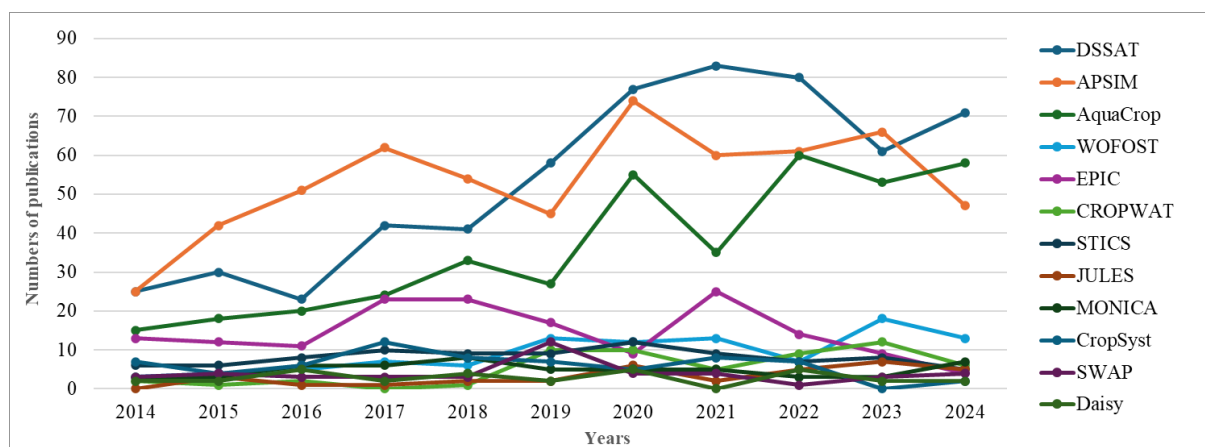
Table 1. Top 12 crop models ranked by number of publications or citations frequency

Crop model rank	Web of Science <i>number of publications</i>	Web of Science <i>number of citations</i>	ScienceDirect <i>number of citations</i>
1	DSSAT	APSIM	APSIM
2	APSIM	DSSAT	DSSAT
3	AquaCrop	AquaCrop	SWAP
4	EPIC	EPIC	EPIC
5	WOFOST	CropSyst	AquaCrop
6	STICS	WOFOST	STICS
7	CropSyst	STICS	WOFOST
8	CROPWAT	MONICA	CROPWAT
9	MONICA	LPJmL	MONICA
10	SWAP	Daisy	Daisy
11	JULES	SWAP	CropSyst
12	Daisy	SIRIUS	JULES

The results show no significant differences between the various bibliographic databases or approaches used. A total of 14 different models were identified among the top 12 ranked models (DSSAT, SWAP, EPIC, AquaCrop, STICS, WOFOST, CROPWAT, MONICA, Daisy, CropSyst, JULES, APSIM, LPJmL, and SIRIUS). Two models (LPJmL and SIRIUS) appeared

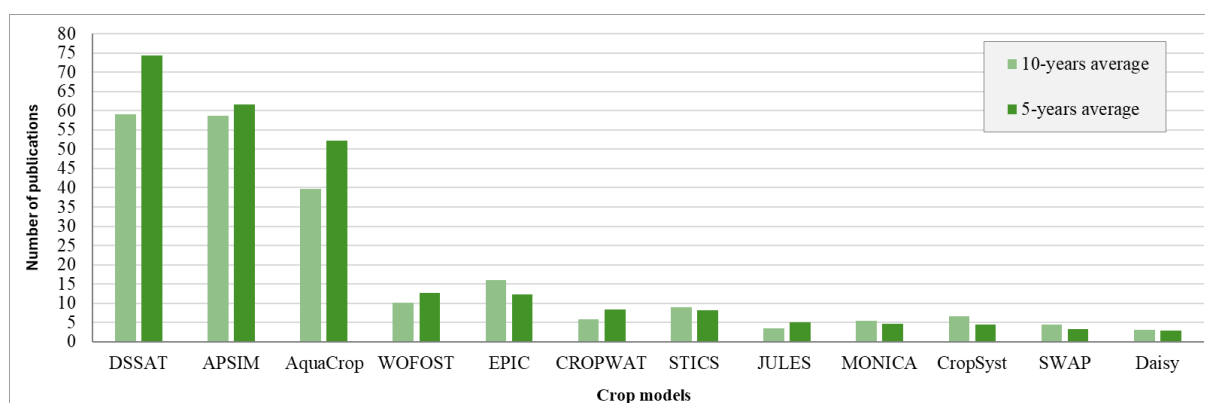
only once and were therefore excluded from further analysis, leaving 12 models for the subsequent analysis.

An analysis of publication frequency in Web of Science for the period 2014–2024 (Graph 1) reveals variation among models, with DSSAT, APSIM, and AquaCrop clearly standing out as the most frequently published. Similarly, citation counts from Web of Science and ScienceDirect rank APSIM and DSSAT as the top two models. Considering citations, AquaCrop and EPIC are highly ranked, while the others show lower and relatively uniform citation counts. These findings are supported by studies confirming APSIM and DSSAT as the most widely used crop models (e.g., Wimalasiri, 2023).



Graph 1. Number of crop models publications (Web of Science; 2014–2024)

Regarding trends in publication and citation counts, the results indicate a slight positive trend in both the number of publications and citations for almost all models (EPIC, AquaCrop, STICS, WOFOST, CROPWAT, MONICA, and JULES), with the most pronounced positive citation trend observed for AquaCrop. The most recent publication trends over the past five years have been also analyzed by comparing the five-year average (2019–2024) with the full period (2014–2024). As shown in Graph 2, DSSAT, APSIM, AquaCrop, WOFOST, CROPWAT, and JULES exhibited positive trends during the last five years.



Graph 2. Publication trends of crop models (5-/10-year averages; Web of Science)

Based on the results of this bibliographic analysis, six models emerge as the most promising: DSSAT, APSIM, AquaCrop, WOFOST, CROPWAT, and JULES. DSSAT, APSIM, and AquaCrop consistently rank highest in both publications and citations, with positive trends in

publication and citation growth in recent years. For WOFOST, CROPWAT, and JULES, the increasing number of publications and citations over the past five years suggests their growing relevance and potential for a more prominent role in the future.

The number and trend of peer-reviewed publications serve as indicators of a model's ongoing application and development within the scientific community. A growing body of literature suggests that models with increasing publications remain methodologically relevant, are actively maintained, and are frequently applied. These insights are crucial for identifying which models are currently recognized as reliable tools for assessing the impacts of climate change on crop yield. To determine the most suitable model for Slovenian agriculture, a more detailed evaluation of the six identified models will be necessary, which will be the focus of future studies. The final selection will likely depend on key criteria, including the complexity of model use, the scope and type of required input data, and the relevance and usability of the model outputs.

Conclusions

In conclusion, this paper presents a bibliometric review of global crop simulation models to identify those most suitable for Slovenia, focusing on maize and wheat. The goal is to establish a continuous national monitoring system for crop yields under changing climate conditions, ensuring the models' long-term use beyond short-term projects. A comprehensive checklist of available crop models was compiled. This process serves as a foundation for selecting the most appropriate models for future application in Slovenia's agricultural sector. Analysis of bibliographic databases, considering publication count and citation frequency, identified the twelve most frequently published or cited models. A more detailed review of the results indicates that six models within this group are the most promising: DSSAT, APSIM, AquaCrop, WOFOST, CROPWAT, and JULES. While DSSAT, APSIM, and AquaCrop lead in publications and citations, the increasing number of publications and citations in recent years for WOFOST, CROPWAT, and JULES highlights their increasing relevance and potential for a more significant role in the future.

The development of crop models has been an ongoing process for the past fifty years, and they are continuously improved to integrate new variables and outputs (Roja et al., 2023). These models are valuable tools for decision-making under various management scenarios, as they can be used to analyze problem situations, optimize management practices, assess crop yields, conduct economic risk analyses, and project climate impacts on different systems. However, their performance relies on high-quality datasets, making it essential to identify crop parameters, platforms, and multiple datasets (Ahmed, 2020).

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References

- Ahmed M., Raza M.A, Hussain T. (2020). Dynamic Modeling In: Ahmed, M. (eds) Systems Modeling. Springer, Singapore. DOI: https://doi.org/10.1007/978-981-15-4728-7_9
- Bertalančič R., Dolinar M., Draksler A., Honzak L., Kobold M., Kozjek K., Lokošek N., Medved A., Vertačnik G., Vlahović Ž., Žust A. (2018). Ocena podnebnih sprememb v Sloveniji do konca 21. stoletja: Sintezno poročilo – prvi del. Ministrstvo za okolje in prostor, Agencija Republike Slovenije za okolje.

- Ceglar A. (2011). The use of dynamic crop model for simulation of plant growth and development for prediction of crop yield in changed climate conditions: Doctoral Dissertation. Biotehniška fakulteta, Oddelek za agronomijo, Ljubljana.
- EEA (2024). European Climate Risk Assessment (ECRA). EEA Report 01/2024. European Environment Agency. DOI: <https://doi.org/10.2800/8671471>
- Feng X., Tian H., Cong J., Zhao C. (2023). A method review of the climate change impact on crop yield. *Front. For. Glob. Change* 6:1198186. DOI: 10.3389/ffgc.2023.1198186
- Gavasso-Rita Y.L., Papalexiou S.M., Li Yanping, Elshorbagy A., Li Z., Schuster-Wallace C. (2023). Crop models and their use in assessing crop production and food security: A review. *Food and Energy Security* published by John Wiley & Sons Ltd. DOI: 10.1002/fes3.503
- IPCC (2022). Pörtner, H.-O., Roberts, D. C., Tignor, M., Poloczanska, E. S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., & Rama, B., eds. *Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. DOI: <https://doi.org/10.1017/9781009325844>
- Pogačar T. (2015). Modelling of weather and climate impacts on growth and yield of grass sward in Slovenia: Doctoral Dissertation. Biotehniška fakulteta, Oddelek za agronomijo, Ljubljana.
- Roja M., Gumma M.K., Reddy M.D. (2023). Crop modelling in agricultural crops. *Current Science*. 124: 910. DOI: <https://doi.org/10.18520/cs/v124/i8/910-920>
- Wimalasiri E.M., Ariyachandra S., Jayawardhana A., Dharmasekara T., Jahanshiri E., Muttill N., Rathnayake U. (2023). Process-Based Crop Models in Soil Research: A Bibliometric Analysis. *Soil Systems*. 7: 43. DOI: <https://doi.org/10.3390/soilsystems7020043>.

ORIGINAL SCIENTIFIC PAPER

Effects of magnetic field seed treatment on early growth of white clover (*Trifolium repens* L.)

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Summary

The aim of this study was to determine the effect of pretreatment of white clover seeds with permanent magnetic fields (MF) of different strengths and exposure times on the early growth of white clover. Clover seeds were exposed to MF of 250 and 350 mT for 5, 15 and 30 minutes and then were sown in pots filled with commercial substrate, along with untreated seeds used for the control variant. After sowing, the plants were exposed to sunlight and watered when needed. After 14 days of vegetation, the plants were removed and the roots were washed with water. The number of developed plants was counted, the length of the roots and of the above-ground part was measured. The total length of the plants was determined by adding them, and the root-to-stem ratio was also determined. A significant effect ($p < 0.01$) of MF on all tested plant properties was observed. The time of exposure of clover seeds significantly ($p < 0.01$) affected the number of developed plants, root length and root/stem ratio. Given that pretreatment of seeds with MF affects germination and plant growth, MF represents one of the possibilities (*options*) for (*improvement of*) organic farming and thus environmental conservation.

Key words: white clover growth, plant height, root length, root to shoot ratio

Introduction

Due to its high nutritional value, white clover is a regular component of pasture mixtures. After sowing, it grows slowly in the first three months and is therefore suppressed in pasture mixtures (Brock and Hay, 2001). Due to its shallow root system and low seed germination rates, as noted by Norton et al. (2021) and Baxter et al. (2019), white clover is sensitive to drought, making it crucial to sow seeds with higher germination rates for achieving high crop yields. Pretreatment of field crop seeds with MF is a non-toxic method of increasing their germination as well as growth and development, especially under stressful agro-ecological conditions (Kataria et al., 2017). The application of seed pretreatment with MF increased seed germination and seedling properties of wheat (Hussain et al., 2020), corn (Ferroni et al., 2023), soybean and pea (Kataria et al., 2017, Ahmad et al., 2023), and sunflower (Bukhari, et al., 2021). Research by Radhakrishnan (2019) and Sarraf i et al. (2020) showed that pretreatment of seeds with MF, in addition to seed germination, increases plant tolerance to stressful growing conditions, better uptake of nutrients from the soil, and resistance to diseases and pests. The effect of MF depends on the genotype, frequency of alternating fields, magnetic flux density, seed exposure time, absolute exposure dose, and polarity (Teixeira da Silva and Dobránszki, 2016). Of particular importance are the strength of MF and the exposure time, with the time of exposure to MF being more significant than its strength (Phirke et al., 1996). The aim of this study was to determine the influence of pretreatment of white clover seeds with MF of different strengths

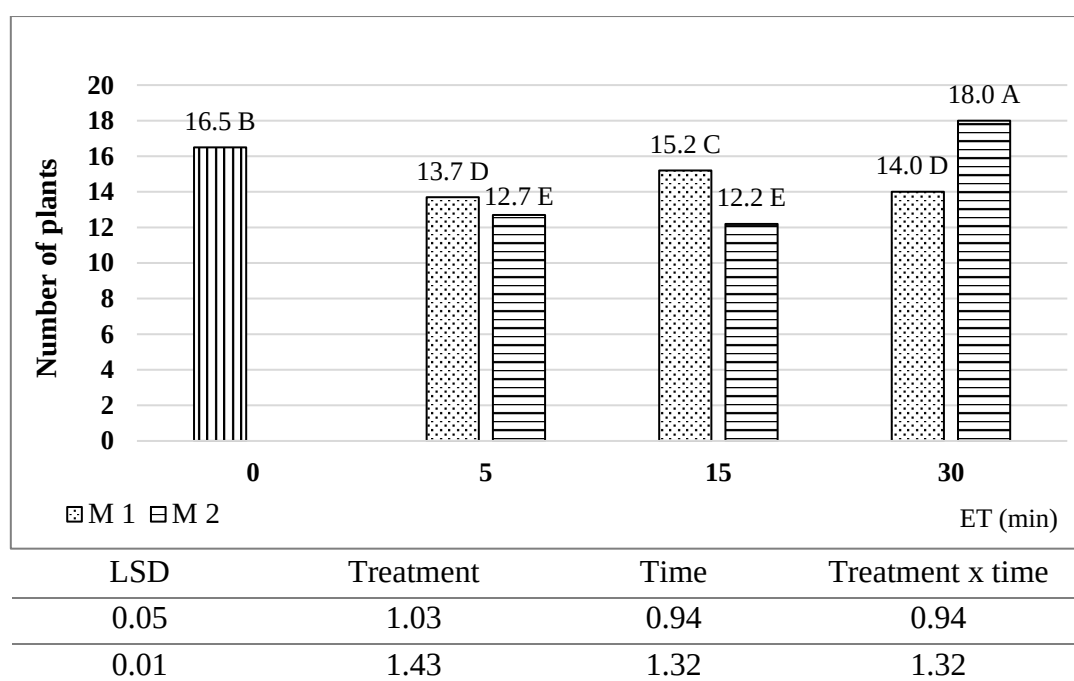
and different exposure times on the morphological properties of white clover in order to possibly determine the optimal value of the strength and time of exposure of seeds to MF.

Material and methods

The research was conducted at the location Osijek (Croatia, latitude 45°32', longitude 18°44', altitude 90 m). Pretreatment of white clover seeds (cv "Apolo") was performed with MF 250 mT (M1) and 350 mT (M2). Each MF treatment was performed at an exposure time (ET) of 5, 15, 30 minutes. Control (M0) and treatments were set up in 4 replications. Untreated (control) as well as MF-treated seeds were sown in pots with a seeding site volume of 20 cm³ filled with commercial substrate "Klasman Potground P". Each replication had 8 seeding sites, and 3 seeds were sown in each site (24 per replication). The experiment was conducted under conditions of exposure to natural light, protected from precipitation. Watering was performed as needed. After 14 days, plants were counted and removed, and roots were washed. Root length (RL) and plant height (PL) were measured. Total plant length (TLP) was determined by adding them together, and the ratio of root length to aboveground part (R/A) was calculated. ANOVA analysis of variance was performed using SAS 9.4 software (SAS Institute Inc.). The LSD test was used to calculate differences in mean values at $p < 0.05$ and $p < 0.01$.

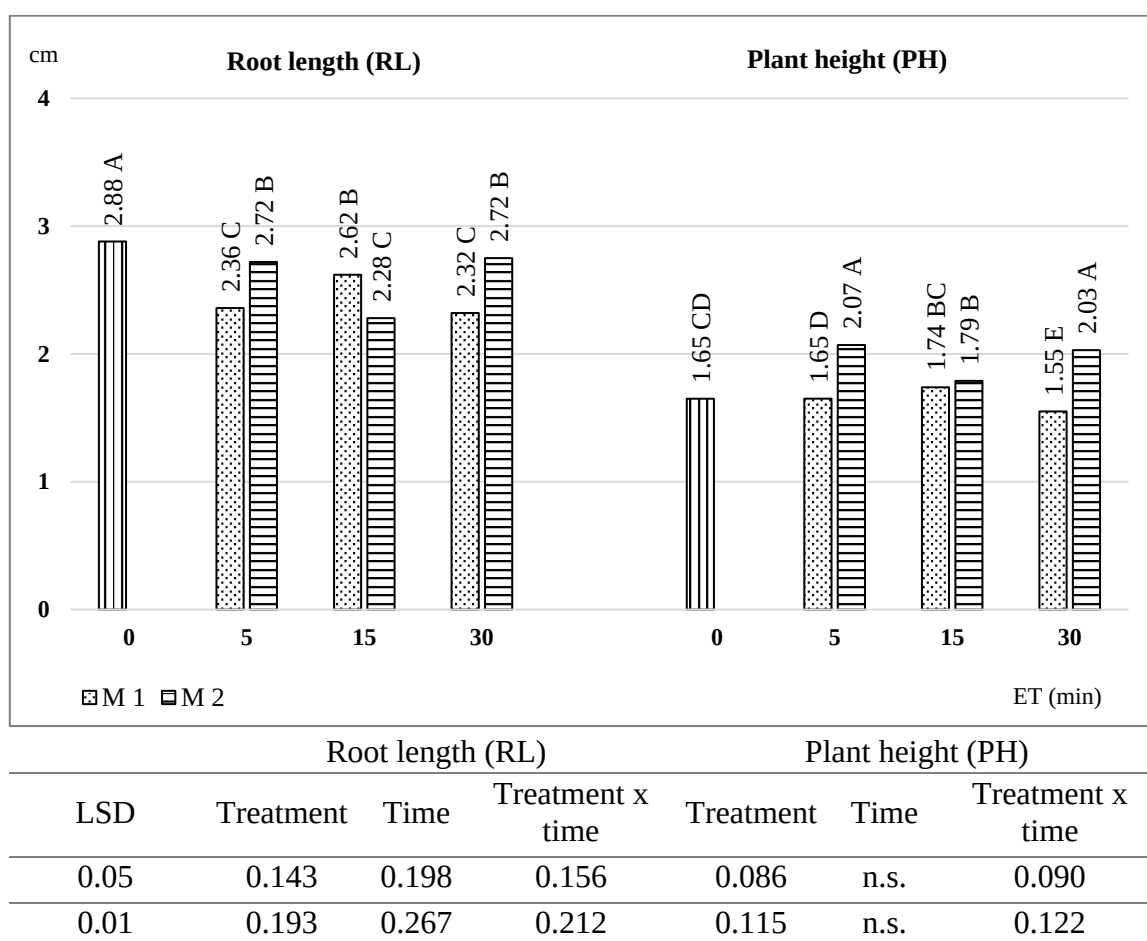
Results and discussion

A significant effect ($p < 0.01$) on the number of developed plants was achieved by the effect of MF, ET and their interaction. The largest number of plants developed in control, and the average value between the control and MF did not differ (14.3). The control (16.5) and ET seeds of 30 (16) had the highest average value. The differences between 5 and 15 minutes of exposure were not significant. White clover seeds have a low germination. Chu et al. (2022) achieved values of 54–69% in their study. When sowing untreated seeds, 16 plants (66%) developed from a total of 24 sown seeds. The largest number of plants (75%) developed when sowing seeds were exposed to MF M2 for 30 minutes (9% more than in the control).



Graph 1. Influence of MF strength and exposure time of white clover seeds on the number of developed plants

Root length was significantly ($p < 0.01$) influenced by MF treatment, seed ET and their interaction (Graph 2). In the average of treatments and control, the highest value for RL was obtained in control (2.88 cm), then in treatment M2 and the lowest in M1 (2.43 cm). In the average of magnetic strength, the lowest value was obtained at ET of 15' (2.45 cm) and the highest at control (2.88 cm). There was no difference between ET of 5' and 30' (2.54 and 2.52 cm). In magnet M1, the longest root developed at an ET of 15'. In contrast, the clover root was the shortest at the aforementioned ET and magnet M2. The research results of other researchers regarding the influence of MF on RL differ depending on the crop, MF strength and ET. Similarly, Harb et al. (2021) treated lentil seeds with 10 levels of MF strength and 6 different ET and found different effects of MF strength and ET on root length. An increase in root length with MF treatment was found by Hussain et al. (2020) in wheat and Bukhari et al. (2021) in sunflower.

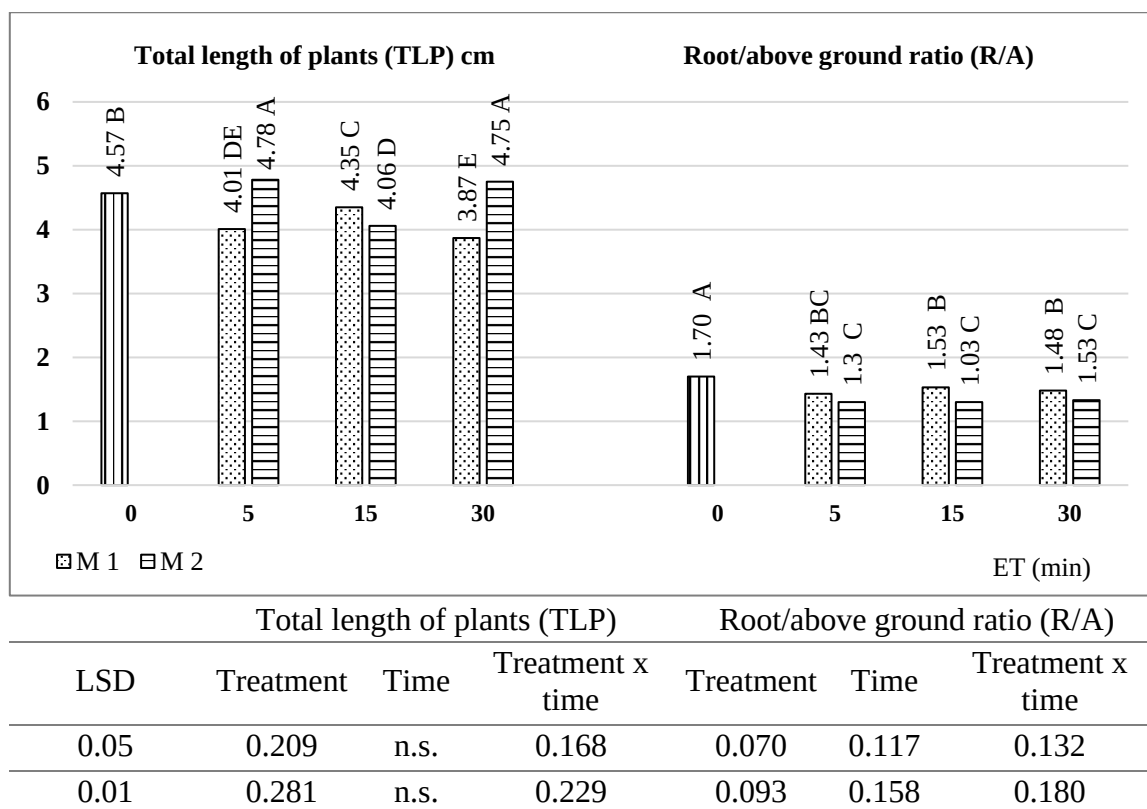


Graph 2. Influence of MF strength and ET of white clover seeds on root length (RL) and plant height (PH)

The PH value was significantly ($p < 0.01$) influenced by the strength of the MF and the interaction of MF and ET (Graph 2). On average, plants had the greatest length during MF exposure when treated with magnet M2 (1.96 cm). There was no significant difference between the control and M1. The highest value for PH was obtained in pretreatment M2 and exposure times of 5' and 30'. Plants were significantly lower in the MF M1 pretreatment for 30'. Research by Harb et al. (2021) showed a different effect of pretreatment of lentil seeds in treatments with 10 strengths of MF and 6 exposure times. In fodder pea, pretreatment of seeds with MF (125

and 250 mT) and germination Carbonell et al. (2011) resulted in stems that were 35% and 32% longer compared to the control.

TLP significantly ($p < 0.01$) depended on the strength of MF and the interaction of MF with exposure time. On average, there was no difference in exposure time between the control (4.57 cm) and the M2 treatment (4.53 cm). The highest values for TLP (Graph 3) were obtained by pretreatment of M2 seeds at ET of 5' and 30'. An increase in TLP by 37 and 35% by pretreating fodder pea seeds with MF (125 mT and 250 mT) by 37% and 35% compared to the control was obtained in the study by Carbonell et al. (2011).



Graph 3. Influence of MF intensity and exposure time of white clover seeds on total plant length (TLP) and the ratio of root to shoot length (R/A)

According to Forde et al. (1989), white clover has a low root:shoot ratio (1:4). The R/A ratio was significantly ($p < 0.01$) influenced by MF and ET and their interaction. The highest mean value was found for the control (1.7) and the lowest for M2 (1.44). There were no differences between exposure times for MF, and the highest value was also obtained for the control. As for the values obtained by the interaction of the strength of MF and ET (Graph 3), the lowest and equal value was obtained for M2 for all tested exposure times.

Conclusions

The obtained research results showed the influence of MF strength on all investigated properties of white clover. ET had no influence on PH and TLP. Based on the data obtained, especially for TLP and the number of developed plants, the best seed pretreatment was shown to be 350 mT and ET of 30'. However, further studies of the treatments applied are needed, both under controlled conditions and in the field.

References

- Ahmad G., Ali M., Usman M., Rasheed S. (2023). Effect of magnetic field on germination and early growth of soybean (*Glycine max*) seed. *International Journal of Advance Industrial Engineering*. 13 (2): 1-6.
- Baxter L. L., Grey T. L., Tucker J. J., Hancock D. W. (2019). Optimizing temperature requirements for clover seed germination. *Agrosystems, Geosciences & Environment*. 2 (1): 1-7.
- Brock J. L., Hay M. J. M. (2001). White clover performance in sown pastures: A biological/ecological perspective. In *proceedings of the new Zealand Grassland association* (pp. 73-83).
- Bukhari S. A., Tanveer M., Mustafa G., Zia-Ud-Den N. (2021). Magnetic field stimulation effect on germination and antioxidant activities of presown hybrid seeds of sunflower and its seedlings. *Journal of Food Quality*. 2021: 1-9.
- Carbonell M. V., Florez M., Martínez E., Maqueda R., Amaya J. M. (2011). Study of stationary magnetic fields on initial growth of pea (*Pisum sativum* L.) seeds. *Seed Science and Technology*. 39 (3): 673-679.
- Chu L., Gao Y., Chen L., McCullough P.E., Jespersen D., Sapkota S., Bagavathiannan M., Yu J. (2022). Impact of environmental factors on seed germination and seedling emergence of white clover (*Trifolium repens* L.). *Agronomy*. 12 (1): 190.
- Ferroni L. M., Dolz M. I., Guerra M. F., Makinistian L. (2023). Static magnetic field stimulates growth of maize seeds. *arXiv preprint arXiv:2303.00512*.
- Forde M. B., Hay M. J. M., Brock J. L. (1989). Development and growth characteristics of temperate perennial legumes. *Persistence of forage legumes*. 91-109.
- Hussain M. S., Dastgeer G., Afzal A. M., Hussain S., Kanwar R. R. (2020). Eco-friendly magnetic field treatment to enhance wheat yield and seed germination growth. *Environmental nanotechnology, monitoring & management*. 14: 100299.
- Harb A. M., Alnawateer B. A. M., Abu-Aljarayesh I. (2021). Influence of Static Magnetic Field Seed Treatments on the Morphological and the Biochemical Changes in Lentil Seedlings (*Lens Culinaris* Medik). *Jordan Journal of Biological Sciences*. 14 (1): 179-186.
- Kataria S., Baghel L., Guruprasad K. N. (2017). Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean. *Biocatalysis and agricultural biotechnology*. 10: 83-90.
- Norton M. R., Li G. D., Xu B., Price A., Tyndall P., Hayes R. C. (2021). Differences in dehydration tolerance affect survival of white clover (*Trifolium repens*) and lucerne (*Medicago sativa*) during a drying cycle. *Crop and Pasture Science*, 72 (8-9): 723-730.
- Phirke P. S., Patil M. N., Umbarkar S. P., Dudhe Y. H. (1996). The application of magnetic treatment to seeds: methods and responses. *Seed Science and Technology*. 24 (2): 365-373.
- Radhakrishnan R. (2019). Magnetic field regulates plant functions, growth and enhances tolerance against environmental stresses. *Physiology and Molecular Biology of Plants*. 25 (5): 1107-1119.
- Sarraf M., Kataria S., Taimourya H., Santos L. O., Menegatti R. D., Jain M., Ihtisham M. and Liu S. (2020). Magnetic field (MF) applications in plants: An overview. *Plants*. 9 (9): 1139.
- Teixeira da Silva J. A., Dobránszki J. (2016). Magnetic fields: how is plant growth and development impacted?. *Protoplasma*. 253 (2): 231-248.

ORIGINAL SCIENTIFIC PAPER

The Role of Biostimulants in Alleviating Drought Stress in Maize

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A pot experiment was conducted to investigate the effects of biostimulants on maize plants grown under varying water supply conditions. Biostimulant treatments, especially BS02 at 0.25 L ha⁻¹, significantly enhanced maize photosynthetic performance, water use efficiency (WUE), biomass, and root development, particularly under droughty conditions. Initial effects were shown six days after treatment, with net CO₂ assimilation rate increases up to 54.36%. Under drought, the 0.25 L ha⁻¹ dose led to a 350.19% increase in photosynthetic intensity over the positive control while higher doses under drought reduced effectiveness. The WUE was highest with the 1.0 L ha⁻¹ BS02 treatment. Biomass and root length were greatest in the 0.25 L/ha treatments, with drought-treated plants achieving result similar to normally irrigated controls. Overall, 0.25 L ha⁻¹ BS02 proved most consistently effective.

Key words: biostimulants, photosynthesis, transpiration, water stress, water use efficiency

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide with more than 208.2 million ha area (FAOStat, 2023), serving as a staple food, animal feed, and industrial raw material. It plays a critical role in global food security due to its high productivity and versatility. Maize is cultivated across a wide range of climatic zones and is a key component in the agricultural economies of many countries. Plant biostimulant products contain substances and/or microorganisms that, when applied to plants or the rhizosphere, stimulate natural processes that enhance or positively influence nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality. These include compounds, substances, and other products that, even in small amounts, can enhance plant growth and development by improving the efficiency of physiological processes within the plant (Soltani et al., 2015; Sangiorgio et al., 2020; Yuldasheva and Karabayeva, 2023).

Environmental factors and crop production technologies can influence the effectiveness of plant biostimulants. The optimal conditions vary depending on the type of biostimulant used (Caradonia et al., 2022). The timing of application is an important factor that affects the performance of biostimulants, as does their type. Using biostimulants can improve resource use efficiency in crop production, enhance fertilizer utilization, and increase the effectiveness of non-chemical weed control methods and herbicide applications. In addition, they can lead to higher yields and improved quality (Gazoulis et al., 2023). According to Privantu et al. (2023), recent research shows that the use of biostimulants boosts plant physiological performance both under optimal environmental conditions and under abiotic stress caused by water deficiency. The application of biostimulants in agriculture can be a useful tool in mitigating the harmful effects of climate change. Based on numerous in vitro, climate chamber, greenhouse, and field trials conducted with different environmental conditions and potato varieties, Caradonia et al. (2022) found that all tested plant biostimulants had a beneficial effect on yield and quality parameters.

The objective of the experiment was to assess the potential of biostimulant applications to alleviate the adverse effects of drought stress in maize. In a pot experiment, we investigated the extent to which the application of the biostimulant could mitigate the negative effects of water deficit-induced stress in maize.

Material and methods

A climate chamber pot experiment with genotype Pioneer P0023 (FAO420) was set in 2023 in Nyíregyháza. The experimental setup was 12 treatments, 6 replicates, in sum 72 pots (3 liters each). The growth medium was quartz sand. Environmental conditions: Climate chamber with fluorescent lighting, PAR 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature maintained at 20 °C. Sowing date: September 20, 2023. Irrigation was done as nutrient solution irrigation, standard watering ("N"), and reduced (50%) watering ("D") to simulate water stress. Treatments applied on October 11, 2023 (BBCH14 growth stage). Termination of the experiment: November 6, 2023. Treatments were shown in the Table 1. Negative control was normal irrigation, no treatment, positive control was reduced irrigation, no treatment.

Table 1. The experiment treatments

Negative control	Normal irrigation (N)
Positive control	Water stress (D)
Biostimulant 01 (BS01) 0.25 L/ha	Normal irrigation (N)
Biostimulant 01 (BS01) 0.25 L/ha	Water stress (D)
Biostimulant 02 (BS02) 0.25 L/ha	Normal irrigation (N)
Biostimulant 02 (BS02) 0.5 L/ha	Normal irrigation (N)
Biostimulant 02 (BS02) 0.75 L/ha	Normal irrigation (N)
Biostimulant 02 (BS02) 1.0 L/ha	Normal irrigation (N)
Biostimulant 02 (BS02) 0.25 L/ha	Water stress (D)
Biostimulant 02 (BS02) 0.5 L/ha	Water stress (D)
Biostimulant 02 (BS02) 0.75 L/ha	Water stress (D)
Biostimulant 02 (BS02) 1.0 L/ha	Water stress (D)

Photosynthesis parameters and transpiration were measured in intact leaves using the LI-6800 (LI-COR, USA) portable photosynthesis system. It has two high-precision infrared gas analyzers to measure CO₂ and H₂O mole fraction in air. The instrument calculates net assimilation, transpiration, stomatal conductance, intercellular CO₂ concentration, and other physiological parameters. The light was controlled in the sample chamber; we used 1500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ PAR, with 90% red (625 nm) and 10% blue (475 nm) light. The CO₂ concentration was controlled in the chamber: 400 $\mu\text{mol mol}^{-1}$ using an injector and carbon dioxide patrons. We measured light-adapted leaves six times per leaf on two plants per plot. Water use efficiency was calculated from the measured data on the leaves, applying the formula proposed by Tanner and Sinclair (1983):

$$WUE = \frac{Ass*44}{Emm*18}$$

WUE: water use efficiency (kg m^{-3})

Emm: transpired H₂O ($\text{mmol m}^{-2} \text{s}^{-1}$)

Ass: assimilated CO₂ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)

At the end of the experiment, we measured the root length and dry weight of shoots and roots. IBM SPSS Statistics 26.0 (IBM Corp., Chicago, IL, USA) statistical software package was used to process and evaluate the data. The GLM (univariate) model was used to compare the means of the different measured values between treatments. The prerequisites to the analysis of variance were checked on the dependent variables. Homogeneity of variance was tested using

Levene's test. LSD post hoc tests were conducted for the pair-wise comparisons of the means. The significance level (alpha) was set to $p = 0.05$.

Results and discussion

The first measurements were taken on the day of treatment, immediately before the application of the biostimulants (DAT0). No significant differences were observed between the treatments regarding photosynthetic parameters. Plants under optimal irrigation exhibited photosynthetic values indicative of normal physiological functioning. In contrast, plants irrigated with a reduced water supply showed photosynthetic parameters (net assimilation rate, stomatal conductance, intercellular CO_2 concentration, electron transport rate, and transpiration) indicative of water deficiency. At the second measurement (DAT6), six days after treatment, significant differences in photosynthetic activity were recorded (Figures 1, 3, 5). In all treatments, the net assimilation rate was higher compared to the both controls, with increases ranging from 9.16% to 54.36%. The 0.25 L ha^{-1} BS02 treatment resulted in a 54.28% greater CO_2 fixation compared to the negative control under normal water conditions (N treatments) and a 73.08% increase compared to the positive control under drought stress (D treatments). Under drought conditions, the intensity of photosynthesis in maize increased with higher doses of BS02 up to 0.75 L ha^{-1} . Significant differences were also observed in other measured parameters (electron transport rate, and transpiration) between the BS01 and BS02 treatments compared to the controls. On DAT14, photosynthetic intensity in all treatments remained higher than in the controls. Under normal water supply, the highest values were observed with the 0.75 L ha^{-1} BS02 treatment, while under drought stress, the 1.0 L ha^{-1} BS02 treatment showed the highest activity. Similar trends were found for electron transport rate. By the 20th day after treatment (DAT20), the situation had changed. Treated plants exhibited higher photosynthetic intensity than the controls (Figure 2), with increases of 18.85–75.78% under normal irrigation and 20.17–68.06% under drought stress, depending on the treatment. The highest value was recorded for the 0.75 L ha^{-1} BS02 treatment under normal conditions and for the 0.25 L ha^{-1} BS02 treatment under drought stress. Doses higher than 0.25 L ha^{-1} of BS02 decreased photosynthetic intensity and other assimilation parameters under drought conditions. This trend was visible in the results from the DAT26. The positive control plants had the lowest photosynthetic intensity among all treatments. Assimilation activity was more intense under normal irrigation than under drought conditions; however, the 0.25 L ha^{-1} BS02 treatment almost completely compensated for drought-induced losses. Under drought stress, the photosynthetic intensity of the 0.25 L ha^{-1} BS02-treated plants was 350.19% higher than that of the positive control. Higher doses significantly reduced net assimilation rate, stomatal conductance, electron transport rate, and transpiration under drought (Figures 2, 4, 6). Under normal water supply, photosynthetic intensity in BS02-treated plants exceeded that of the controls by 57.63–81.29%, depending on the treatment. Under drought stress, the positive effect of BS02 treatments compared to controls ranged between 110.15% and 350.19%. When evaluating water use efficiency (WUE), we found that at the second measurement (DAT6), the highest WUE under drought stress was achieved in the 1.0 L ha^{-1} BS02 treatment, significantly exceeding other treatments (Figure 7). The second-highest WUE was measured in the positive control group under drought conditions. At this time, the average WUE of normally irrigated maize plants across treatments was $27.63 \pm 1.19 \text{ kg CO}_2 \text{ m}^{-3} \text{ H}_2\text{O}$, while under drought stress it averaged $28.55 \pm 1.26 \text{ kg CO}_2 \text{ m}^{-3} \text{ H}_2\text{O}$. On DAT20, the average WUE of normally irrigated plants was significantly lower than that of drought-stressed plants (Figure 8). No significant differences were found between the BS02 treatments (0.25 , 0.50 , and 1.0 L ha^{-1}) under drought stress. The negative control plants exhibited the lowest water use efficiency. Analysis of dry plant weight showed that plants under normal irrigation produced greater biomass (Figure 9). Treatments resulted in significant differences compared to the controls. Under normal water

supply, the BS01 0.25 L ha⁻¹ and BS02 0.25 L ha⁻¹ treatments produced 24.58% and 22.17% greater biomass, respectively, compared to the controls. Under drought, differences ranged between +3.63% and 37.05% relative to the controls. The greatest biomass under drought was observed in the 0.25 L ha⁻¹ BS02 treatment, which was comparable to that of the control plants under normal irrigation (6.9 and 6.7 g, respectively). Among the BS02 treatments, plants treated with 0.25 L ha⁻¹ had the longest root length under both water conditions. The longest root length among all treatments was recorded in the BS01 0.25 L ha⁻¹ treatment under normal irrigation. Under drought stress, increasing the BS02 dose from 0.25 to 1.0 L ha⁻¹ progressively reduced root length.

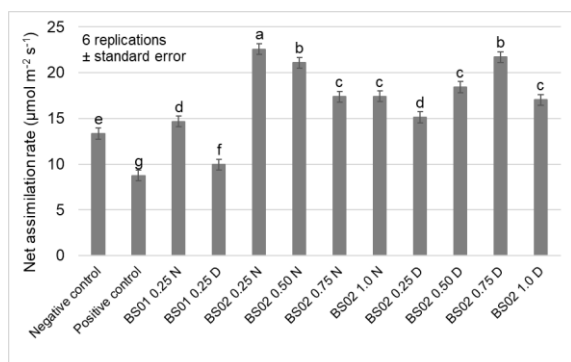


Figure 1 Net assimilation rate in maize, DAT6 (Nyíregyháza, 2023)

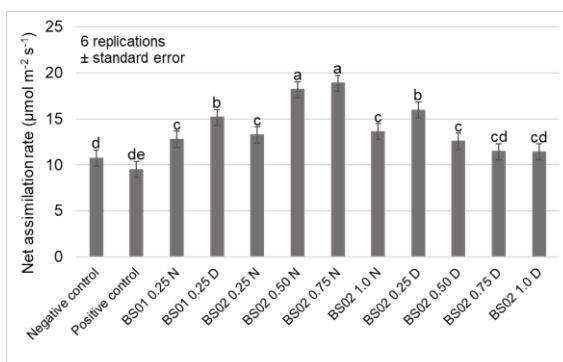


Figure 2 Net assimilation rate in maize, DAT20 (Nyíregyháza, 2023)

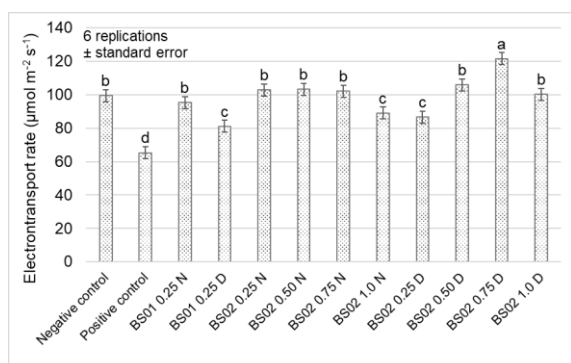


Figure 3 Electron transport rate in maize, DAT6 (Nyíregyháza, 2023)

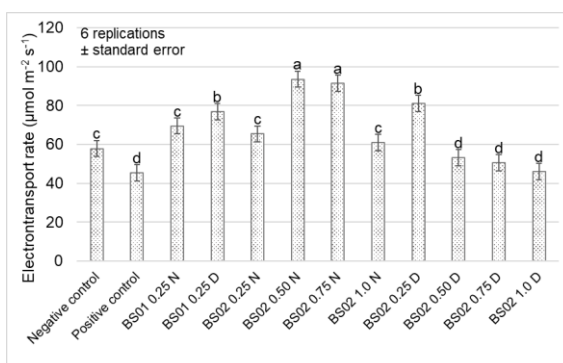


Figure 4 Electron transport rate in maize, DAT20 (Nyíregyháza, 2023)

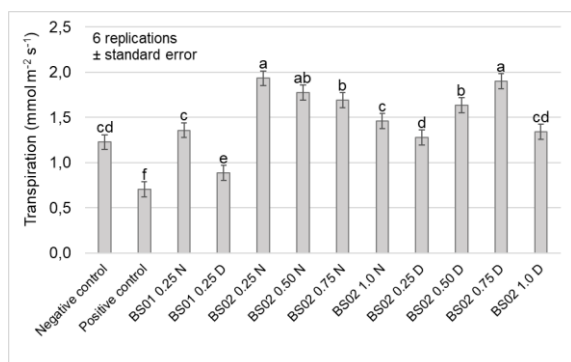


Figure 5 Transpiration in maize, DAT6 (Nyíregyháza, 2023)

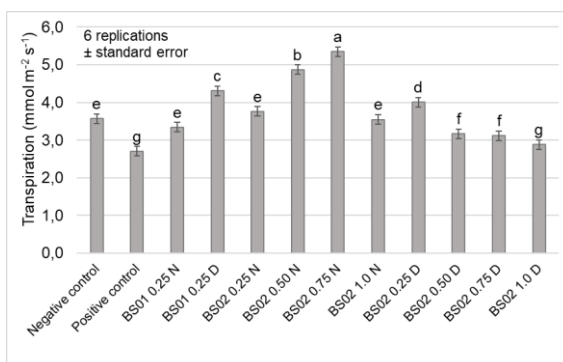


Figure 6 Transpiration in maize, DAT20 (Nyíregyháza, 2023)

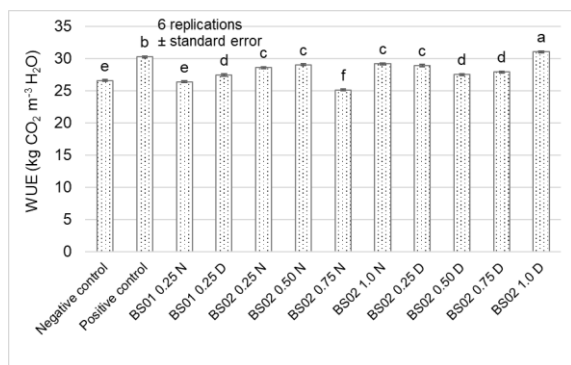


Figure 7 Water use efficiency (WUE) in maize, DAT6 (Nyíregyháza, 2023)

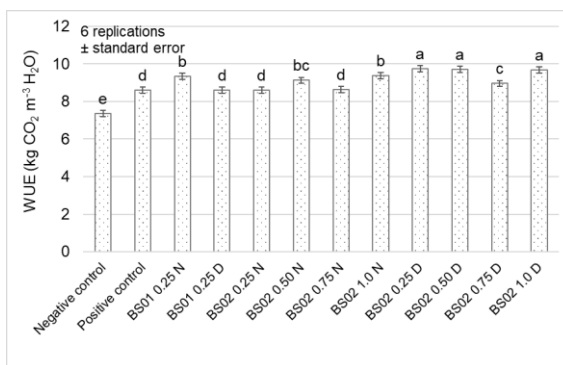


Figure 8 Water use efficiency (WUE) in maize, DAT20 (Nyíregyháza, 2023)

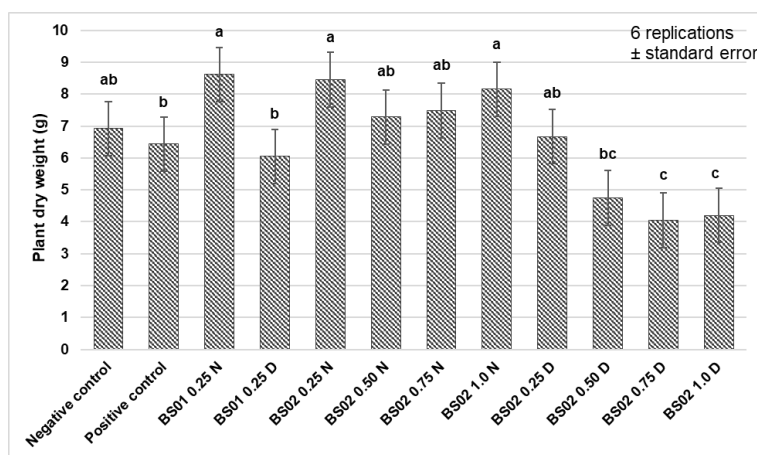


Figure 9: The effect of treatments on the plant dry weight (root +shoot) in maize (Nyíregyháza, 2023)

Conclusions

The biostimulants tested had demonstrable effects on the metabolic processes and photosynthetic system of maize. In particular, BS02 biostimulant had a positive effect on maize photosynthesis and water use efficiency. Significant differences in NDVI values were also observed between the treatments. Under well-watered conditions, increasing doses of the biostimulant led to higher NDVI values, whereas under water stress, higher doses resulted in a decrease in NDVI values. Under water stress conditions, the differences in plant dry biomass resulting from the treatments ranged from +3.63% to −37.05% compared to the stressed control. The highest dry biomass was observed in the DEV0085 treatment at a dose of 0.25 L ha⁻¹, which did not differ significantly from the control plants grown under well-watered conditions.

References

- Soltani, N., Shropshire, C., Sikkema, P.H. (2015). Effect of biostimulants added to postemergence herbicides in corn, oats, and winter wheat. *Agricultural Sciences*, 6, 527–534. DOI: 10.4236/as.2015.65052
- Sangiorgio, D., Cellini, A., Donati, I., Pastore, C., Onofrietti, C., Spinelli, F. (2020). Facing Climate Change: Application of Microbial Biostimulants to Mitigate Stress in Horticultural Crops. *Agronomy*, 10, 794. DOI: 10.3390/agronomy10060794
- Yuldasheva, Z.F., Karabayeva, D.J. (2023). The effect of different doses of different biostimulants on the yield of oily sunflower. *IOP Conference Series: Earth and Environmental Science*, 1142, 012097, DOI: 10.1088/1755 1315/1142/1/012097

- Caradonia, F., Ronga, D., Tava, A., Francia, E. (2022). Plant Biostimulants in Sustainable Potato Production: An Overview. *Potato Research*, 65, 83–104, DOI: 0.1007/s11540-021-09510-3
- Gazoulis, I., Kanatas, P., Antonopoulos, N., Kokkini, M., Tsekoura, A., Demirtzoglou, T., Travlos, I. (2023). The Integrated Effects of Biostimulant Application, Mechanical Weed Control, and Herbicide Application on Weed Growth and Maize (*Zea mays* L.) Yield. *Agronomy*, , 13, 2614. DOI: 10.3390/agronomy13102614
- Privantu, A.E., Olaru, A.L., Murtaza, D.F., Dobre, S.C. (2023). Biostimulants and their role in the development of maize crops. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 53(1), 237–247, DOI: 10.52846/aamc.v53i1.1467
- Tanner C.B., Sinclair T.R. (1983). Efficient water use in crop production: research or re-search. In: *Limitations to efficient water use in crop production* (Taylor H.M et al., eds). ASA, Madison, WI, USA. pp 1-27.
- FAOStat 2023: <https://www.fao.org/faostat/en/#data/QCL>.

REVIEW ARTICLE

Irrigation scheduling for strawberry crops under different management and climate conditions

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Summary

Strawberry (*Fragaria × ananassa* Duch.) production is highly dependent on efficient water management due to the crop's sensitivity to soil moisture fluctuations and climatic variations. Proper irrigation scheduling is essential for optimizing water use efficiency, maintaining soil moisture balance, and ensuring sustainable agricultural practices. This work provides an analysis of crop evapotranspiration (ET_c) and crop coefficient (K_c) in strawberry cultivation under varying climatic and growing systems. It examines use of different irrigation techniques and growing system, such as open field, with and without mulch, and the use of protected and semi protected structures such as greenhouses and plastic tunnels.

Key words: irrigation, climate, evapotranspiration, crop coefficient, strawberry

Introduction

Strawberry (*Fragaria × ananassa* Duch.) is one of the most widely cultivated fruit crops globally, valued for its high economic return and nutritional benefits (FAOSTAT, 2021). However, strawberry production is highly sensitive to water availability due to a shallow root system and high transpiration rates, making irrigation management a crucial factor for optimizing yield, fruit quality, and water use efficiency (Yuan et al., 2004; Garcia-Tejero et al., 2018; Ariza et al., 2021; Amini et al., 2022).

Given the increasing global challenges posed by water scarcity, climate variability, and the need for sustainable agricultural practices, developing efficient irrigation scheduling strategies is essential to ensure the sustainability of strawberry cultivation. Irrigation practices for strawberries vary significantly depending on environmental conditions, soil type, and the chosen irrigation system. Various management techniques, such as mulching, drip irrigation, and protected cultivation (greenhouses and tunnels), have been employed to optimize water use and improve crop performance (Yuan et al., 2004; Garcia-Tejero et al., 2018; Ariza et al., 2021; Amini et al., 2022).

Understanding the crop water requirement across different climatic regions and management systems is necessary to improve irrigation efficiency, reduce water losses, and ensure high productivity under different cultivation scenarios. The strawberry irrigation requirements must be simple and easy to understand from the irrigation planning strategy in order to help farmers to easily implement and satisfy water demand. For that reason, accurate knowledge of crop evapotranspiration is essential (Allen et al., 1998), as strawberries are grown under the different climate and management conditions. The reference (ET_0) and crop evapotranspiration (ET_c) always depend on the climate conditions (Allen et al., 1998; Srdić et al., 2023).

To understand the difference in the climate and management conditions is crucial for sustainable strawberry production to overcome over irrigation and balance crop needs due to strawberries specific sensitivity to improper irrigation. With ET_c estimate and irrigation scheduling improvements, strawberry can be better managed, ensuring adequate water use which is a critical factor in sustainable water management (Amini et al., 2022). Using ET_0 and crop coefficient (K_c) to determine the irrigation schedule is a common method that allows precise management of water resources, since it takes into account the specific needs of crops for water, as well as the climatic conditions that occur at different stages of growth.

The K_c is an important as represents differences between ET_c and ET_0 , explaining how plants of different species and under different climates use water during their growth. According to Allen et al. (1998), standard K_c values for strawberry are presented. However, recent research shows that these values must be revised, because agronomic practices have changed, as well as climatic conditions. The estimation of K_c for strawberries reveals significant variability across different methods, climatic conditions, and measurement techniques. While Allen et al. (1998) values provide a general guideline, locally observed K_c values tend to exhibit deviations, particularly during the initial growth stages. This discrepancy is primarily attributed to differences in canopy development, measurement methodologies, management conditions and climatic influences.

With the intensifying effects of climate change, including rising temperatures and altered precipitation patterns, there is a growing need to refine irrigation strategies to enhance resilience in strawberry production. This review aims to provide an insight of irrigation scheduling strategies for strawberry crops across diverse climate conditions and management practices. By synthesizing research findings from various studies, this paper will assess the impact of different management conditions on ET_c and K_c . The objective is to support decision-making for farmers, researchers, and policymakers in developing more sustainable irrigation strategies tailored to specific environmental and agronomic conditions.

Material and methods

To compare strawberry water requirements values across various growing and management conditions, a literature review was conducted. Relevant studies were identified through multiple academic databases, including Scopus, Web of Science (WoS), Google Scholar, Elsevier, Springer, Wiley, and ResearchGate. The search was performed using the following keywords: strawberry, evapotranspiration, crop coefficient, irrigation scheduling, water use, water requirements, drought, climate change, and decision support system. The following selection criteria were applied: studies conducted in different climate zones and under diverse crop management conditions, long-term research (ideally spanning multiple growing seasons) and ET_0 measurement methods. The selected studies focused on strawberries grown under different management conditions, including open field with and without mulch, tunnel, and greenhouse environments.

Results and discussion

The water requirements for strawberry vary significantly under different growing systems, such as open field, open field with mulch, tunnel, and greenhouse conditions. The field experiment study (Amini et al., 2022) determined the water requirements and K_c for strawberries in a semi-arid climate using lysimeter experiments. The average daily strawberry evapotranspiration (ET_c) was **3.8 mm day⁻¹**, with total seasonal water consumption of **873.4 mm (2018)** and **932.1 mm (2019)**. Peak ET_c occurred in early August, while the lowest was in late November. Compared to grass, where **ET_0 was 4.7 mm day⁻¹, with a total of 1,143.5 mm**, strawberries had lower water consumption, emphasizing the need for precise irrigation scheduling. These

findings support **efficient water use strategies** for sustainable strawberry production in water scarce regions.

Mulching on open field conditions is widely used in strawberry cultivation to reduce evaporation, improve soil moisture retention, and regulate soil temperature shown that the choice of mulch can significantly impact growth and overall yield. In particular, mulching with materials like black polyethylene generally results in improved fruit size and quality, more uniform fruit development, and higher overall yields. Moreover, the use of plastic mulch can significantly decrease total evapotranspiration while enhancing yield. For example, Ariza et al. (2021) reported that field-grown strawberries with mulch required 224-510 mm of water.

The use of plastic tunnels further influences irrigation needs as tunnels provide a semi-protected environment, reducing exposure to wind and direct solar radiation. Study from Spain (García-Tejero et al., 2018) reported variations in water consumption under different years 2014/15 and 2015/16. The difference in water consumption for cultivar ‘Sabrina’ was 100 mm, stated as, 430 mm and 350 mm, from where 54.7 mm and 70.4 mm were lost by drainage, making water requirements for cultivar ‘Sabrina’ as 375.3 and 279.6 mm for growing seasons, respectively. These differences coincidence with the accumulated ET_0 , therefore, the difference were attributed to the difference in climate between the seasons. Greenhouse strawberry production presents unique microclimatic conditions. Compared to open field cultivation, greenhouse environments moderate temperature fluctuations, increase humidity, and reduce wind speed, ultimately lowering evapotranspiration. The study of Yuan et al. (2004) determined that the optimal irrigation amount for greenhouse grown strawberries as 380 mm, corresponding to a pan factor of 1.1 (Ep 1.00). This level provided the best balance between yield and water use efficiency, ensuring adequate soil moisture (5–17 kPa) while maximizing fruit production. Lower irrigation (Ep 0.75) significantly reduced yield, while higher irrigation (Ep 1.25) offered only marginal gains with lower efficiency. For efficient water management and optimal strawberry growth, a drip irrigation system set to 1.1 times pan evaporation is recommended. The data compiled in Table 1 provides a detailed summary of the water requirements observed in different experiments, which can be used to compare the impacts of these management systems on water use efficiency and overall crop performance.

Table 1. Summary of strawberry water requirements under different conditions

Condition	Water requirement (mm)	Reference
Field experiment, no mulch	873.4	Amini et al., 2022
Field experiment, mulch	224-510	Ariza et al. 2021
Plastic tunnel	280-376	García-Tejero et al., 2018
Greenhouse	380	Yuan et al., 2004

The results highlights the need for region-specific irrigation strategies to optimize water use and improve strawberry production efficiency. Irrigation scheduling for strawberries must be adapted to specific environmental and management conditions to optimize water use efficiency. The integration of mulching, precise irrigation methods, and climate-based scheduling can significantly enhance water conservation while maintaining high yields.

The reviewed studies on K_c (Table 2) demonstrated that $K_{c\text{ ini}}$ values ranged from 0.4 to 0.6, with lower values commonly observed in greenhouse conditions, and reflecting minimal canopy coverage immediately after planting. As the crop develops, $K_{c\text{ mid}}$ values increased to a range of 0.6-1.1, corresponding to peak canopy coverage and maximum transpiration. At the end of the growing season, K_c values declined to 0.4-0.8, reflecting reduced physiological activity and transpiration. However, in the study in tunnels a slightly different values are found, due to the different methodologies applied in K_c estimation. The use of two weather stations

(inside vs. outside the tunnel) for ET_o calculations in this study resulted in the notable differences.

Table 2. Strawberry crop coefficient (K_c) under different growing systems compared to FAO 56 (Allen et al., 1998) K_c values during the initial stage ($K_{c\text{ ini}}$), the mid-season stage ($K_{c\text{ mid}}$) and at the end of the late season stage ($K_{c\text{ end}}$).

Management	$K_{c\text{ ini}}$	$K_{c\text{ mid}}$	$K_{c\text{ end}}$	Reference
FAO single K_c	0.40	0.85	0.75	Allen et al., 1998
Open field no mulch	0.45	0.86	0.80	Amini et al., 2022
Open field mulch	0.40	0.69	0.40	Hanson and Bendixen, 2004
Tunnel	0.40-0.60	0.60-1.10	0.70-1.20	Garcia-Tejero et al., 2018
Greenhouse	0.15	0.90	-	Gavilan et al., 2024

Future research should focus on advanced irrigation modeling and real-time monitoring technologies to further improve scheduling practices. The integration of advanced scheduling tools, such as the Riego Berry App (Gavilan et al., 2024), further enhances water-use efficiency, minimizing unnecessary water losses while ensuring sustainable strawberry production in controlled environments.

Conclusions

Strawberry production is highly dependent on efficient water management due to the crop's sensitivity to soil moisture fluctuations and climatic variations. Proper irrigation scheduling is essential for optimizing water use efficiency, maintaining soil moisture balance, and ensuring sustainable agricultural practices. The findings from the evaluated studies indicate that water requirements for strawberries vary significantly across different climatic conditions and management practices, ranging from approximately 300-400 mm in protected conditions such as tunnels and greenhouse, 200-500 mm under open field conditions with mulch and 900 mm under field conditions without mulch. By evaluating strawberry water requirement, this paper aims to assist farmers, researchers, and policymakers in adopting more precise and sustainable irrigation scheduling methods.

Acknowledgement

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References

- Allen R.G., Pereira L.S., Raes D., Smith M. (1998). Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements; FAO Irrigation and drainage paper 56; Food and Agriculture Organization of the United Nations: Rome, Italy, 1998; Volume 56, p. 300.
- Amini A., Karami F., Sedri M. H., Khaledi V. (2022). Determination of water requirement and crop coefficient for strawberry using lysimeter experiment in a semi-arid climate. *H2Open Journal* (2022) 5 (4): 642-655. <https://doi.org/10.2166/h2oj.2022.030>
- Ariza M.T., Miranda L., Gómez-Mora J.A., Medina J.J., Lozano D., Gavilán P., Soria C., Martínez-Ferri E. (2021). Yield and Fruit Quality of Strawberry Cultivars under Different Irrigation Regimes. *Agronomy* 11, 261. <https://doi.org/10.3390/agronomy11020261>
- Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database. [Rome]: FAO, 2021.
- García-Tejero I.F., López-Borralló D., Miranda L., Medina J.J., Arriaga J., Muriel-Fernández J.L., Martínez-Ferri E. (2018). Estimating Strawberry Crop Coefficients under Plastic

- Tunnels in Southern Spain by Using Drainage Lysimeters. *Sci. Hortic.* 231; 233-240. <https://doi.org/10.1016/j.scienta.2017.12.020>
- Gavilan P., Higuera J. L., Lozano D., Ruiz N. (2024). The Riego Berry mobile application: A powerful tool to improve on-farm irrigation performance in berry crops. *Agricultural Water Management*, 292: 233-240. <https://doi.org/10.1016/j.agwat.2024.108682>
- Hanson B., Bendixen W. (2004). Drip irrigation evaluated in Santa Maria Valley strawberries. *California Agriculture*, 58(1). <https://escholarship.org/uc/item/3sk3691f>
- Srđić S, Srđević Z, Stričević R, Čereković N, Benka P, Rudan N, Rajić M, Todorović M. (2023). Assessment of Empirical Methods for Estimating Reference Evapotranspiration in Different Climatic Zones of Bosnia and Herzegovina. *Water* 15, 3065. <https://doi.org/10.3390/w15173065>
- Yuan B.Z., Sun J., Nishiyama S. (2004). Effect of drip irrigation on strawberry growth and yield inside a plastic greenhouse. *Biosyst. Eng.* 87; 237-245. <https://doi.org/10.1016/j.biosystemseng.2003.10.014>

Allelic variation at *Ppo-A1* and *Ppo-D1* loci in Croatian wheat varieties

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Summary

Polyphenol oxidase (PPO) is an enzyme responsible for the undesirable darkening of wheat-based products. Breeding wheat varieties with low PPO activity is the best strategy for reducing product darkening so the identification of varieties with desirable *Ppo* genes is of a great importance. The goal of this research was to examine the distribution of alleles at *Ppo-A1* and *Ppo-D1* loci in Croatian wheat germplasm. Total of 50 wheat varieties were analyzed. Three sets of primers were used for PCR analysis: PPO 18, PPO 16 and PPO 29. At *Ppo-A1* locus, the highest frequency was determined for *Ppo-A1b* allele (58%), and at the *Ppo-D1* locus, for *Ppo-D1b* allele (52%). The most frequent allelic combination was *Ppo-A1b* + *Ppo-D1b* (30%). Considerable allelic variability was observed at both tested loci, which offers possibilities for selecting wheat varieties with desirable alleles.

Key words: polyphenol oxidase, *Ppo* genes, wheat

Introduction

Polyphenol oxidase (PPO) is known as a key enzyme responsible for the undesirable darkening of wheat-based products, resulting in their reduced quality (Demeke and Morris, 2002). Polyphenol oxidase is involved in the oxidation of phenolic acids to quinones, which then react with amines and thiol groups leading to the darkening of final products (Demeke et al., 2001). PPO activity can vary depending on the genotype and environmental conditions since it is a complex quantitative trait influenced by multiple genes and environmental factors (Harisha et al., 2024). Comprehensive knowledge of the genetics behind PPO activity in wheat enables the development and application of improved strategies in breeding programs aimed at improving the quality of wheat-based final products (Zhai et al., 2020). Demeke and Morris (2002) were the first to report on the molecular characterization of wheat *Ppo* gene and its successful sequencing and cloning. Most of the *Ppo* genes are located on the wheat homoeologous group 2 chromosomes, among which *Ppo-A1* and *Ppo-D1* are the most researched. These genes are activated during the development of the wheat kernel and can impact the PPO activity and the color stability of wheat flour and dough (Liu et al., 2020). Breeding wheat varieties with low PPO activity is an effective strategy to minimize the darkening of wheat-based products. Since PPO activity is both physiological and biochemical trait, selection cannot be based solely on the morphological traits. Application of molecular markers linked to PPO activity can significantly increase selection efficiency and accelerate the breeding process (Sun et al., 2005). The main goal of this research was to examine the distribution of alleles at *Ppo-A1* and *Ppo-D1* loci in Croatian wheat germplasm using functional molecular markers.

Material and methods

In total, 50 wheat varieties were analyzed, which are part of the gene collection of the Faculty of Agrobiotechnical Sciences Osijek (Table 1). Genomic DNA was extracted from fresh leaf

tissue according to CTAB method (Doyle and Doyle, 1987). The concentration and purity of the extracted DNA was verified by spectrophotometric and electrophoretic analysis. Three sets of primers were used for PCR analysis, PPO 18 for the detection of *Ppo-A1a* and *Ppo-A1b* alleles (Sun et al., 2005), and PPO 16 and PPO 29 for the detection of *Ppo-D1a* and *Ppo-D1b* alleles (He et al., 2007). The PCR reaction consisted of 2 μL of 50 ng μL^{-1} genomic DNA template, 2.3 μL of 5x Promega Green GoTaq Flexi Buffer, 0.9 μL of 15 mM MgCl_2 , 0.12 μL of 10 mM dNTPs mixture, 0.11 μL of 10 μM forward and reverse primer, 0.06 μL of 5U μL^{-1} Promega GoTaq DNA polymerase and 9.4 μL of d.d. H_2O for the total reaction volume of 15 μL . Thermocycling conditions for PPO 18 were: initial denaturation at 95 $^{\circ}\text{C}$ for 5 min; 35 cycles of 95 $^{\circ}\text{C}$ (45 s), 63 $^{\circ}\text{C}$ (45 s), and 72 $^{\circ}\text{C}$ (60 s) and a final extension step at 72 $^{\circ}\text{C}$ for 8 min (Zhang et al., 2016). Thermocycling conditions for PPO 16 and PPO 29 were: initial denaturation at 95 $^{\circ}\text{C}$ for 5 min; 41 cycles of 94 $^{\circ}\text{C}$ for 30 s, 66 $^{\circ}\text{C}$ – 54 $^{\circ}\text{C}$ for 30 s (–0.3 $^{\circ}\text{C}$ per cycle), and 72 $^{\circ}\text{C}$ for 95 s and a final extension step at 72 $^{\circ}\text{C}$ for 10 min (Zhang et al., 2008). All amplifications were performed on Applied Biosystems Veriti™ Thermal Cycler. Amplification products were analyzed using a horizontal agarose gel electrophoresis (Bio-Rad Sub-Cell® GT) and visualized using a Syngene G:BOX F3 gel imaging system.

Results and discussion

Based on the results of the PCR analysis, the presence of the *Ppo* alleles was determined for all the varieties tested (Table 1).

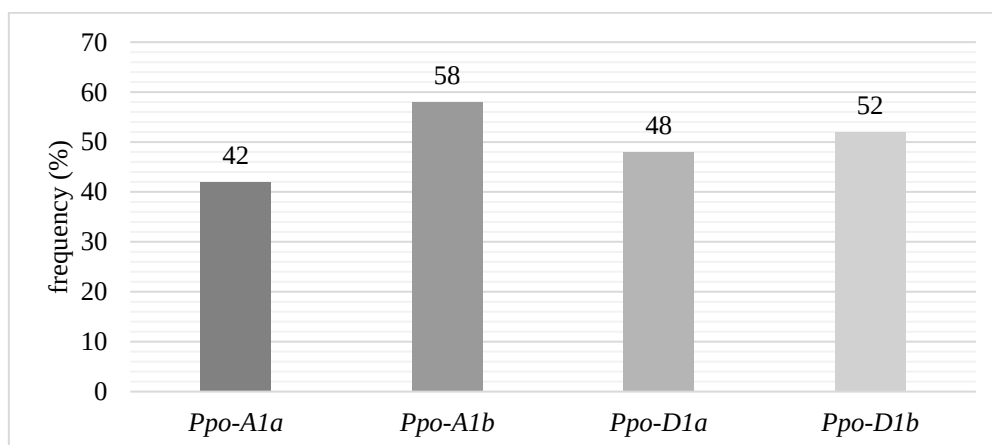
Table 1. Wheat varieties with detected alleles at *Ppo-A1* and *Ppo-D1* loci

No.	Variety	Year	<i>Ppo-A1</i> locus	<i>Ppo-D1</i> locus
1	U1	1936	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
2	MURA	1967	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
3	ZLATNA DOLINA	1971	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
4	TENA	1973	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
5	SANA	1983	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
6	ANA	1988	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
7	ADRIANA	1988	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
8	MARIJA	1988	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
9	DEMETRA	1991	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
10	SNAŠA	1993	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
11	BC PATRIA	1994	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
12	DIVANA	1995	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
13	KUNA	1995	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
14	MIHELCA	1996	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
15	KATA	1997	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
16	KRUNA	1997	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
17	MONIKA	1997	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
18	LARA	1997	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
19	FIESTA	1998	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
20	GABI	1999	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
21	HANA	2000	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
22	PRIMA	2001	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
23	PANONIJA	2002	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
24	BC ELVIRA	2002	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
25	BELA	2003	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
26	MARTA	2003	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
27	ZRNKA	2003	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
28	INES	2004	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>

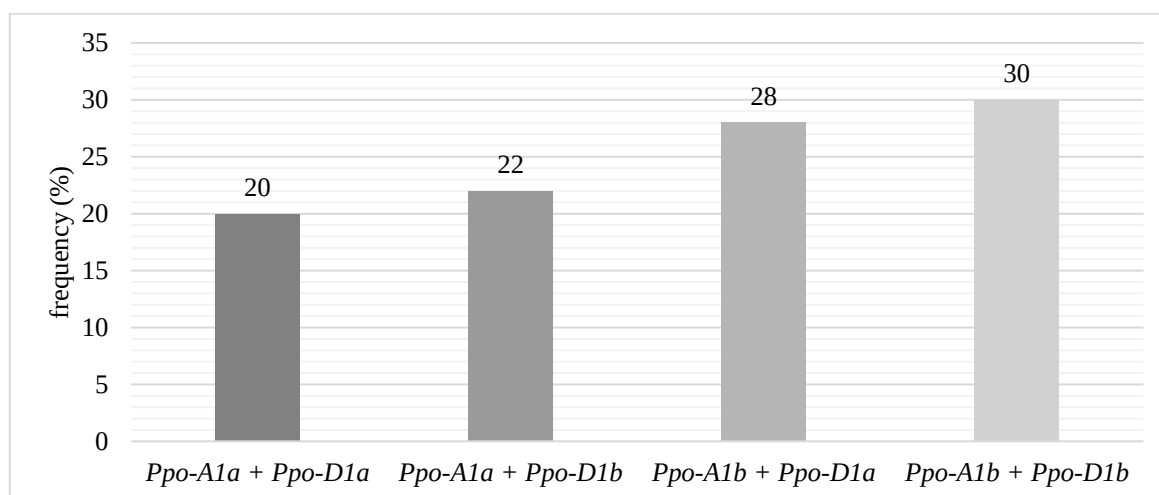
29	MATEA	2005	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
30	KALISTA	2005	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
31	HELIA	2005	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
32	ELVIRA	2005	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
33	PIPI	2006	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
34	SEKA	2006	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
35	DONNA	2007	<i>Ppo-A1a</i>	<i>Ppo-D1a</i>
36	FELIX	2008	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
37	MIHAELA	2008	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
38	NEVENA	2009	<i>Ppo-A1b</i>	<i>Ppo-D1a</i>
39	MIA	2009	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
40	OLIMPIJA	2009	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
41	VULKAN	2009	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
42	FICKO	2009	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
43	DEA	2009	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
44	NOVA ŽITARKA	2010	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
45	BIANCA	2010	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
46	EMA	2010	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
47	BC ANICA	2010	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
48	REBEKA	2011	<i>Ppo-A1a</i>	<i>Ppo-D1b</i>
49	BC MANDICA	2014	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>
50	OLUJA	2014	<i>Ppo-A1b</i>	<i>Ppo-D1b</i>

In general, the presence of *Ppo-A1a* allele at the *Ppo-A1* locus is usually associated with increased PPO activity, and the presence of the *Ppo-A1b* allele is associated with decreased PPO activity. Conversely, at the *Ppo-D1* locus, the *Ppo-D1a* allele is usually associated with decreased PPO activity, and the *Ppo-D1b* allele is associated with increased PPO activity (He et al., 2007, Wang et al., 2008). Among Croatian wheat varieties, the highest frequency at the *Ppo-A1* locus was found for the *Ppo-A1b* allele (58%), associated with low PPO activity. At the *Ppo-D1* locus, the highest frequency was determined for the *Ppo-D1b* allele (52%), associated with high PPO activity. Based on the frequency distribution of each of the detected *Ppo* alleles, it is evident that within the tested wheat germplasm there is considerable variability present at both tested loci (Graph 1). Regarding the frequencies of certain allelic combinations, the highest frequency was determined for *Ppo-A1b* + *Ppo-D1b* allelic combination (30%), and the lowest frequency was determined for *Ppo-A1a* + *Ppo-D1a* allelic combination (20%). Graph 2 shows the frequency distribution of all individual allelic combinations, demonstrating that all combinations are represented in the examined wheat germplasm with more or less similar frequencies. The identification of alleles present in a certain germplasm is of great importance as it provides valuable insights into the genetic potential and variability within the population and enables the improvement of plant breeding efficiency. Singh et al. (2009) determined a prevalence of 77% for *Ppo-A1a* allele and 71% for *Ppo-D1a* allele in a sample of 57 Indian wheat varieties. Zhang et al. (2008) analyzed 70 Chinese wheat varieties and determined the presence of *Ppo-A1b* allele in 54.3% of tested genotypes and the presence of *Ppo-D1a* allele in 64.3% of them, revealing the existence of variability at both tested loci. Same authors claim that although both alleles are associated with reduced PPO activity, the *Ppo-D1a* allele shows less efficacy in reducing PPO activity than the *Ppo-A1b* allele. The same findings were confirmed by Nilthong et al (2012). Chen et al. (2013) analyzed 118 wheat varieties from the Huanghuai region and detected all four allelic combinations at *Ppo-A1* and *Ppo-D1* loci, which showed significant differences in polyphenol oxidase activity ($P < 0.05$), where varieties with *Ppo-A1a* + *Ppo-D1b* combination exhibited the lowest PPO activity, and those with *Ppo-A1b* + *Ppo-D1a* the highest PPO activity. He et al. (2007) analyzed 217 wheat genotypes to validate

the connection between the *Ppo-A1* and *Ppo-D1* genes and grain PPO activity. It was concluded that molecular markers used to assess PPO activity are reliable and can be used in wheat breeding programs. Zhai et al. (2020) state that several QTL studies confirmed that the main genes responsible for PPO activity are located on homoeologous group 2 chromosomes and in addition to them some minor QTLs associated with PPO activity were also found on other wheat chromosomes.



Graph 1. Frequency distribution of detected *Ppo* alleles



Graph 2. Frequency distribution of detected allelic combinations

Varieties with low PPO activity are preferred both by food producers and final consumers, making it essential for breeding programs to develop wheat varieties with reduced PPO activity (Sun et al., 2005). Molecular markers for the identification of *Ppo* genes can be useful for the selection of varieties with low PPO activity, hereby increasing the efficiency of selection for low PPO activity in wheat breeding programs (Singh et al., 2009).

Conclusions

Based on the conducted research it can be concluded that there is considerable allelic variability present at *Ppo-A1* and *Ppo-D1* loci within the tested Croatian wheat germplasm, where the presence of all alleles and allelic combinations was determined. The observed variability offers possibilities for selecting wheat varieties with the desirable alleles, specifically those associated with low PPO activity.

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References

- Chen J., Chen F., Zhan K. H., Cui D. Q. (2013). Molecular identification of the polyphenol oxidase genes in bread wheat cultivars from Huanghuai wheat region. *Journal of Plant Genetic Resources*. 14 (5): 900-907.
- Demeke T., Morris, C. (2002). Molecular characterization of wheat polyphenol oxidase (PPO). *Theoretical and Applied Genetics*. 104: 813-818.
- Demeke T., Morris C. F., Campbell K. G., King G. E., Anderson J. A., Chang H. G. (2001). Wheat polyphenol oxidase: distribution and genetic mapping in three inbred line populations. *Crop science*. 41 (6): 1750-1757.
- Doyle J. J., Doyle J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*. 19: 11-15.
- Harisha R., Balakrishnan A. P., Manjunath K. K., Bhavya B., Singhal S., Shivakumar H. B., Singh A. M. (2024). Polyphenol oxidase (PPO) in wheat (*Triticum aestivum*): a systematic review on bioactivity, inheritance, functionality and their effects on end products. *Cereal Research Communications*. 2024: 1-13.
- He X. Y., He Z. H., Zhang L. P., Sun D. J., Morris C. F., Fuerst E. P., Xia X. C. (2007). Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. *Theoretical and Applied Genetics*. 115: 47-58.
- Liu C., Zhou Z., Wu Z., Qin M., Shi X., Wang, Y., Zhao R. (2020). In-depth genetic analysis reveals conditioning of polyphenol oxidase activity in wheat grains by cis regulation of TaPPO2A-1 expression level. *Genomics*. 112 (6): 4690-4700.
- Nilthong S., Graybosch R. A., Baenziger P. S. (2012). Inheritance of grain polyphenol oxidase (PPO) activity in multiple wheat (*Triticum aestivum* L.) genetic backgrounds. *Theoretical and applied genetics*. 125 (8): 1705-1715.
- Singh R., Goutam U., Gupta R. K., Pandey G. C., Shoran J., Tiwari R. (2009). Allelic variations of functional markers for polyphenol oxidase (PPO) genes in Indian bread wheat (*Triticum aestivum* L.) cultivars. *Journal of genetics*. 88 (3): 325-329.
- Sun D. J., He Z. H., Xia X. C., Zhang L. P., Morris C. F., Appels R., Wang H. (2005). A novel STS marker for polyphenol oxidase activity in bread wheat. *Molecular Breeding*. 16: 209-218.
- Wang X. B., Ma C. X., Si H. Q., Qiao Y. Q., Chang C., He X. F., Xia Y. X. (2009). Gene markers for grain polyphenol oxidase activity in common wheat. *Molecular breeding*. 23: 163-170.
- Zhai S., He Z., Wen W., Liu J., Jin H., Yan J., Xia X. (2020). Genetic architecture of polyphenol oxidase activity in wheat flour by genome-wide association study. *Crop Science*. 60 (3): 1281-1293.
- Zhang X. K., Liu L., He Z. H., Sun D. J., He X. Y., Xu Z. H., Xia X. C. (2008). Development of two multiplex PCR assays targeting improvement of bread-making and noodle qualities in common wheat. *Plant Breeding*. 127 (2): 109-115.
- Zhang Y., Wang X., Wang X., Jiang L., Liu F., He X., Zhang X. (2016). Development of multiplex-PCR systems for genes related to flour colour in Chinese autumn-sown wheat cultivars. *Quality Assurance and Safety of Crops & Foods*. 8 (2): 231-241.

ORIGINAL SCIENTIFIC PAPER

Green extraction techniques for high-quality nettle (*Urtica dioica* L.) tinctures

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Summary

The objective of this study was to optimize sustainable extraction processes for the production of nettle tinctures rich in phenolic compounds. Fresh nettle leaves were extracted by classical and ultrasound-assisted extraction (UAE) for 30 minutes. As a solvent 50% and 80% ethanol was used. The highest content of total phenolic, flavonoid and non-flavonoid compounds as well as the highest antioxidant capacity were recorded in sample extracted with 80% EtOH and UAE. The obtained results show that the type of extraction and the solvent have a significant effect on nettle tincture quality with a higher alcoholic concentration and ultrasound improving the yield of phenolic compounds.

Key words: stinging nettle tinctures, green extraction, green solvents, ultrasound-assisted extraction, phenolic compounds

Introduction

The use of tinctures and extracts in the food industry is a dynamic field with significant potential for growth and innovation. Herbal tinctures and extracts have long been utilized in the food industry because they can enhance flavor, aroma, color and functional properties and are therefore often added to beverages, baked goods, and confectionery. They not only provide improved sensory characteristics, but also exhibit strong antioxidant activity that can extend the shelf life of foods and provide health benefits to consumers (Bolouri et al., 2022). Due to the rich composition of phenolic compounds and other antioxidants, nettle tinctures are used in the food industry as functional ingredients in various food products (beverages, soups, sauces, and herbal teas). In addition, when diluted in water and orally consumed, nettle tincture can be used to treat some health conditions such as urinary tract infections, benign prostatic hyperplasia, anemia, hair loss, etc. (Johnson et al., 2013; Pachisia, 2023). The increasing demand for natural and sustainable food additives has led to an increased interest in environmentally friendly methods of producing alcoholic tinctures. These techniques prioritize the efficient use of resources while minimizing environmental impact. Key approaches often include the use of green solvents and ultrasound-assisted extraction (UAE). Green solvents, such as ethanol, offer a sustainable alternative to traditional organic solvents due to their non-toxicity, making them ideal for food-grade applications. UAE is an innovative technique that can easily be combined with green solvents to create a fully sustainable extraction process. It offers several advantages, including shorter extraction time, lower solvent consumption, and improved yield of target compounds (Martins et al., 2023). As this method enhances the release of bioactive compounds into the solvent, it enables the production of high-quality tinctures. Since such high-quality tinctures can be incorporated into functional foods and nutraceuticals (Rutkowska and Pasqualone, 2025), it is important to determine the optimal extraction methods and conditions to obtain a high proportion of phenolic compounds. Therefore, this study aimed to produce phenolic-rich nettle tinctures by evaluating the extraction method. Two types of extraction

process (classical and ultrasound-assisted) were employed and different concentrations of ethanol (50% and 80%) were used.

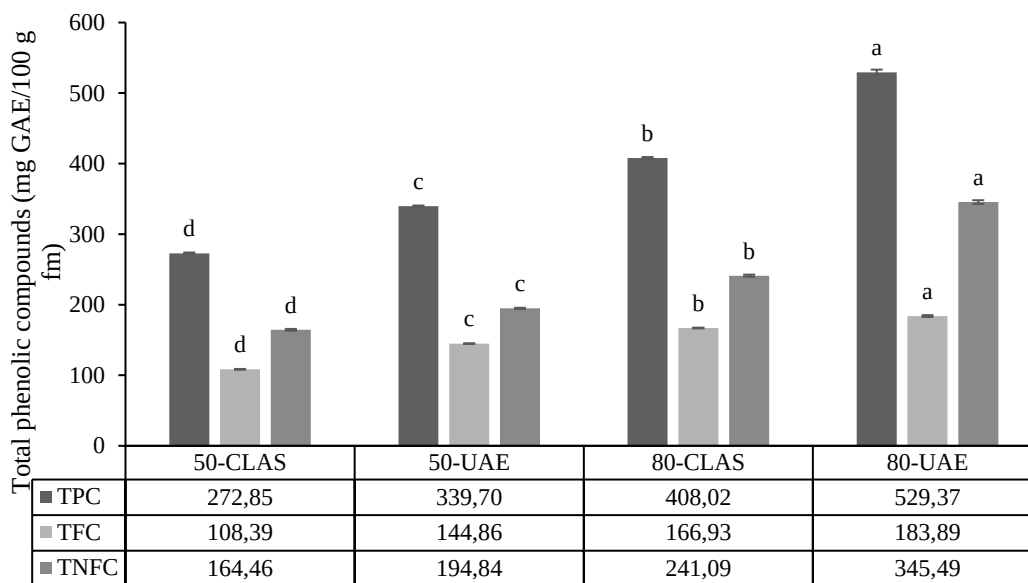
Material and methods

For this experiment a cultivated stinging nettle grown in a hydroponic ebb and flow system was used. The nettle was grown in a greenhouse at the University of Zagreb Faculty of Agriculture with a nutrient solution containing a combination of salts suitable for growing leafy vegetables (pH 5.8 – 6.2, dissolved oxygen 4 – 9 mg/L, and EC 2.5 – 3.2 dS/m). Harvest was conducted in August 2023, and only fresh leaves were used for the experiment. Tinctures were prepared by mixing and homogenizing $10 \text{ g} \pm 0.01$ of fresh nettle leaves with 100 mL of extraction solvent. Two different concentrations of ethanol were varied: 50% (v/v) and 80% (v/v). Also, part of the samples was used for classical extraction on a magnetic stirrer, while the rest was used for treatment in an ultrasonic bath (Bandelin RK 103H, Germany). The extraction process took place at room temperature for 30 minutes. Afterwards, the obtained tinctures were filtered through Whatman filter paper and used for further analysis. The content of total phenolic, total flavonoid and non-flavonoid compounds, as well as the antioxidant capacity (FRAP assay) were determined spectrophotometrically (Shimadzu, 1900i, Kyoto, Japan) in three repetitions, as previously described in Dujmović et al. (2023). The results were statistically analyzed by SAS software version 9.4. (SAS Institute Inc., USA) with ANOVA procedure and the LSD post-hoc test.

Results and discussion

Phenolic compounds are vital bioactive components that contribute to health benefits and biological activities of plant extracts and tinctures. According to the statistical analysis, the interaction of tested factors (solvent concentration and extraction method) strongly affected total phenolic content as can be seen from the statistically significant differences between the samples shown in Graph 1. The highest yield of total phenolics (TPC), total flavonoids (TFC) and total non-flavonoids (TNFC) was detected in sample 80-UZV that was extracted using ultrasound and higher concentrated alcoholic solvent. On average, a 28% higher content of TPC, a 19% higher content of TFC, and a 33% higher content of TNFC was recorded in UAE treated samples compared to the classically treated ones, regardless of the solvent concentration. These results highlight the advantages of using ultrasound in maximizing the yield of phenolic compounds during nettle tincture production. In accordance with this present research many previous studies (Kumar et al., 2021) have emphasized the positive effects of ultrasound in the extraction of chemical compounds and the production of alcoholic tinctures from various plants. This is because ultrasonic waves create microbubbles in the solvent through a process called cavitation. When these bubbles collapse, they generate strong forces that break down plant cell walls, releasing phenolic and other intracellular chemical compounds into the solvent (Ashokkumar, 2015). The difference in the results of phenolic compounds between different concentrated ethanolic solvents was even more pronounced. Namely, if observing solvent concentration, as much as 53%, 38% and 63% higher levels of TPC, TFC, and TNFC respectively were detected in the samples extracted with 80% EtOH. This clearly indicates that the concentration of the solvent significantly influences the extraction efficiency of phenolic compounds from plants. According to Setford et al. (2017), the type and polarity of the solvent are critical factors that affect the extraction of phenolic compounds. Since substances with similar polarity tend to dissolve in one another, solvents with intermediate polarity, such as aqueous ethanol mixtures, are particularly effective for extracting moderately polar phenolic compounds (Galanakis et al., 2013; Kaczorová et al., 2021). In this context, a mixture containing 80% ethanol, being less polar than 50% EtOH, favors the extraction of less polar phenolic compounds such as flavonoid aglycones, certain flavonols, and other lipophilic or moderately hydrophobic phenolics. However, the literature data on the influence of ethanol

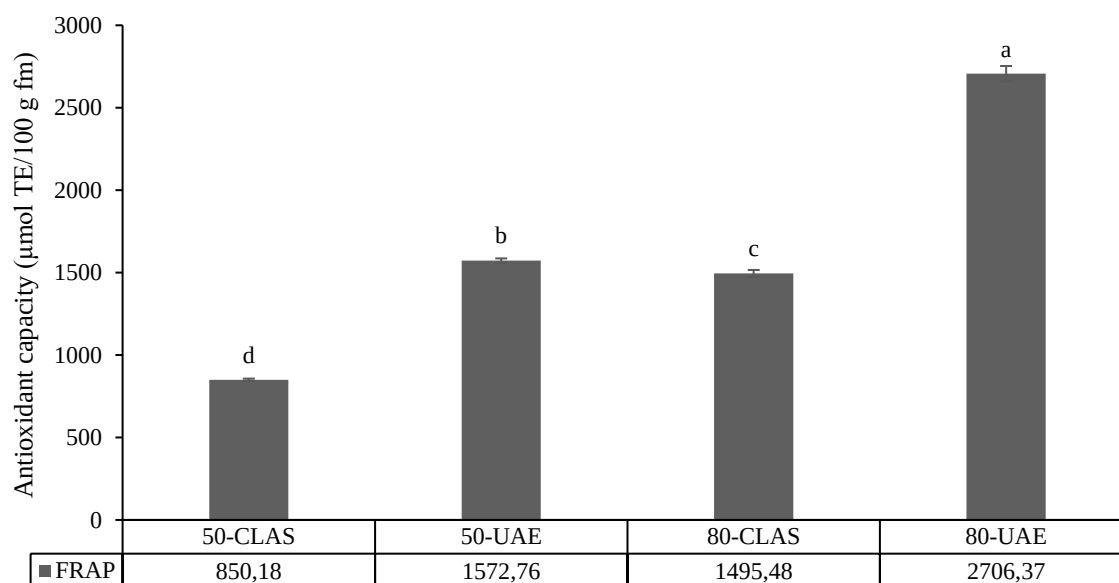
concentration on the extraction of phenolic compounds are inconsistent, with some studies (Kobus et al., 2017; Bucić-Kojić et al., 2009) reporting that a lower concentration is more favorable, while others (Mandache et al., 2024) demonstrate that a higher concentration is more effective in phenolic extraction. Considering that different plant materials have varying phenolic profiles, which are strongly influenced by numerous factors, it can be concluded that the nettle leaves used in this experiment are particularly rich in the aforementioned less polar phenolic compounds. These findings underscore the importance of optimizing the solvent concentration and employing techniques such as ultrasound-assisted extraction to maximize the yield of target phenolic compounds.



50-CLAS – tinctures extracted with 50% EtOH and classic method, 50-UAE – tinctures extracted with 50% EtOH and ultrasound-assisted method, 80-CLAS – tinctures extracted with 80% EtOH and classic method, 80-UAE – tinctures extracted with 80% EtOH and ultrasound-assisted method.

Graph 1. Total phenolics (TPC), total non-flavonoids (TNFC), and total flavonoid content (TFC) of nettle tinctures extracted with different solvent concentrations and extraction methods, $p \leq 0.05$.

The antioxidant capacity of alcoholic plant extracts and tinctures refers to the ability of the compounds within to neutralize harmful free radicals, preventing oxidative stress and cellular damage. This property is essential for promoting human health and extending the shelf life of foods (Sadowska-Bartosz and Bartosz, 2022). The antioxidant capacity of nettle tinctures extracted with different methods and solvent concentrations ranged from 850.18 to 2706.37 $\mu\text{mol TE}/100 \text{ g fm}$ (fresh mass) (Graph 2). It is evident that the highest and lowest values of antioxidant capacity corresponded to the highest and lowest content of phenolic compounds in samples (80-UZV and 50-CLAS). This leads to the conclusion that the phenolic compounds are the strongest antioxidants in tested nettle tinctures. This statement is consistent with several studies (Dujmović et al., 2023; Đurović et al., 2024) that emphasize that phenolic compounds are among the most important antioxidants in nettle leaves. The effects of extraction method and solvent concentration were significant and the same previous explanations regarding the positive effects of these two tested factors on phenolic compounds also apply to antioxidant capacity. In fact, the conditions with higher solvent concentration and ultrasound-assisted extraction (80-UAE) apparently favored the easier extraction of the less polar antioxidants due to leaf cell wall disruption.



50-CLAS – tinctures extracted with 50% EtOH and classic method, 50-UAE – tinctures extracted with 50% EtOH and ultrasound-assisted method, 80-CLAS – tinctures extracted with 80% EtOH and classic method, 80-UAE – tinctures extracted with 80% EtOH and ultrasound-assisted method.

Graph 2. Antioxidant capacity of nettle tinctures extracted with different solvent concentrations and extraction methods, $p \leq 0.05$.

Conclusions

The results show significant influence of the type of extraction method and the solvent concentration on phenolic compounds content and the antioxidant capacity of the nettle tinctures. The highest quality tincture was the one obtained with UAE and 80% EtOH with the values of 529.37 mg GAE/100 g fm for total phenolic compounds and 2706.37 $\mu\text{mol TE}/100 \text{ g fm}$ for antioxidant capacity. This indicates that ultrasound and higher concentration of ethanol enhanced the extraction process and increased the yield of phenolic compounds and other antioxidants. The obtained results can contribute to the further production of tinctures with sustainable methods.

Acknowledgement

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References

- Ashokkumar M. (2015). Applications of ultrasound in food and bioprocessing. *Ultrasonics Sonochemistry*. 25: 17–23.
- Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J, Astatkie T. (2022). Applications of essential oils and plant extracts in different industries. *Molecules*. 27 (24): 8999.
- Bucić-Kojić A., Planinić M., Tomas S., Jakobek L., Šeruga M. (2009). Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. *International Journal of Food Science and Technology*. 44 (12): 2394–2401.

- Dujmović M., Opačić N., Radman S., Fabek Uher S., Voća S., Šic Žlabur J. (2023). Accumulation of stinging nettle bioactive compounds as a response to controlled drought stress. *Agriculture*. 13 (7) :1358.
- Đurović S., Kojić I., Radić D., Smyatskaya Y.A., Bazarnova J.G., Filip S., Tosti T. (2024). Chemical constituents of stinging nettle (*Urtica dioica* L.): A comprehensive review on phenolic and polyphenolic compounds and their bioactivity. *International Journal of Molecular Sciences*. 25 (6) :3430.
- Galanakis C. M., Goulas V., Tsakona S., Manganaris G. A., Gekas V. (2013). A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties*. 16 (2): 382–396.
- Johnson T. A., Sohn J., Inman W. D., Bjeldanes L. F., Rayburn K. (2013). Lipophilic stinging nettle extracts possess potent anti-inflammatory activity, are not cytotoxic and may be superior to traditional tinctures for treating inflammatory disorders. *Phytomedicine*. 20 (2): 143-147.
- Kaczorová D., Karalija E., Dahija S., Bešta-Gajević R., Parić A., Čavar Zeljković S. (2021). Influence of extraction solvent on the phenolic profile and bioactivity of two *Achillea* species. *Molecules*. 26 (6): 1601.
- Kobus Z., Wilczynski K., Nadulski R., Rydzak L., Guz T. (2017). Effect of solvent polarity on the efficiency of ultrasound-assisted extraction of polyphenols from apple pomace. In *IX International Scientific Symposium*, 158-163. Lublin, Poland: University of Life Science.
- Kumar K., Srivastav S., Sharanagat V.S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrasonics Sonochemistry*. 70: 105325.
- Mandache M.B., Stoenescu A.-M., Cosmulescu S. (2024). Influence of solvent concentration on the amount of individual phenolic compounds in apple and sour cherry pomace. *Horticulturae*. 10 (9): 900.
- Martins R., Barbosa A., Advinha B., Sales H., Pontes R., Nunes J. (2023). Green extraction techniques of bioactive compounds: A state-of-the-art review. *Processes*. 11 (8): 2255.
- Pachisia J. (2023). Therapeutic effects of nettle leaf: an indigenous vegetable. *International Journal for Multidisciplinary Research*. 5 (6): 1-7.
- Rutkowska J., and Pasqualone A. (2025). Plant extracts as functional food ingredients. *Foods*. 14 (3): 374.
- Sadowska-Bartosiz I., and Bartosz G. (2022). Evaluation of the antioxidant capacity of food products: Methods, applications and limitations. *Processes*. 10 (10): 2031.
- SAS (SAS Institute, Inc.). SAS/STAT® (Version 9.4): User's guide, [Computer software], (2018). USA: Cary, NC.
- Setford P.C., Jeffery D.W., Grbin P.R., Muhlack R.A. (2017). Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process modelling. *Trends in Food Science and Technology*. 69 (Part A): 106-117.

ORIGINAL SCIENTIFIC PAPER

Effect of silicon and sulphur fertilization on LAI values and yield of different winter oat genotypes

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Summary

The aim of our study was to describe the effects of silicon and sulphur foliar fertilization on the leaf area index (LAI) and yield of winter oat in the growing season of 2024 in north-eastern Hungary. The examined varieties were GK Arany, Mv Hópehehely, Mv Imperiál, Mv Kincsem, Mv Istráng and Mv Hóka, we applied silicon and sulphur based foliar fertilizers at three leaf, flag leaf and milk stage. Our results showed that both sulphur (S) and silicon (Si) treatments had positive effects on LAI values of the winter oat, and the combined treatment (Si+S) significantly increased seed yield. Among the winter oat cultivars, we measured mostly the highest LAI values for the cultivar Mv Istráng at all measurement time, and the lowest value for GK Arany, in contrast to the fact that we measured outstanding yield, as well as for Mv Kincsem.

Key words: leaves, seed yield, winter oat, growth dynamics, foliar fertilization

Introduction

Silicon (Si) and sulfur (S) as nutrients can potentially improve grain yield and the leaf area index (LAI). Several studies have shown that silicon treatment may enhance plant growth, increase tolerance to abiotic stress, and improve physiological characteristics, which ultimately leads to higher yields in winter oats (Xu et al., 2001; Toledo et al., 2012). Enhancing plant resistance is particularly important for winter oats, which are frequently exposed to cold temperatures and waterlogging. Studies have demonstrated that Si fertilization can increase the biomass and grain yield of winter cereals, including oats (Hossain et al., 2002; Toledo et al., 2012). Silicon improves leaf architecture, resulting in increased LAI, which promotes better light interception and photosynthesis (Toledo et al., 2012). Sulfur fertilization also plays a significant role in increasing yield due to its vital function in the synthesis of amino acids and proteins essential for growth and development. Rubia et al. (2016) indicates that adequate S supply can improve plant performance, including grain and biomass production, especially under nutrient-limited conditions. The interaction between nitrogen (N) and sulfur is particularly noteworthy, as S may enhance nitrogen uptake efficiency, which in turn promotes the growth and yield of winter oats (Šeremešić et al., 2019).

Different winter oat genotypes respond differently to Si and S fertilization, suggesting that selecting varieties based on nutrient use efficiency could optimize productivity. For example, certain genotypes have shown better responses in terms of LAI and yield when treated with Si, indicating a genetic basis for nutrient sensitivity (Baxevanos et al., 2020). Furthermore, the timing and method of nutrient application are also critical. Studies suggest that foliar application of Si and S can effectively improve plant nutrient status during key growth stages, thereby influencing yield characteristics (Chwil, 2014; Ayalew et al., 2018). Integrating these nutrients into agronomic practices may enhance sustainable oat production, particularly in regions where oats are an integral part of cropping systems.

The aim of this study was to examine the effect of silicon and sulphur fertilizer foliar application effects on leaf area index (LAI) and yield in different winter oat varieties, also examining the individual sensitivities of each variety.

Material and methods

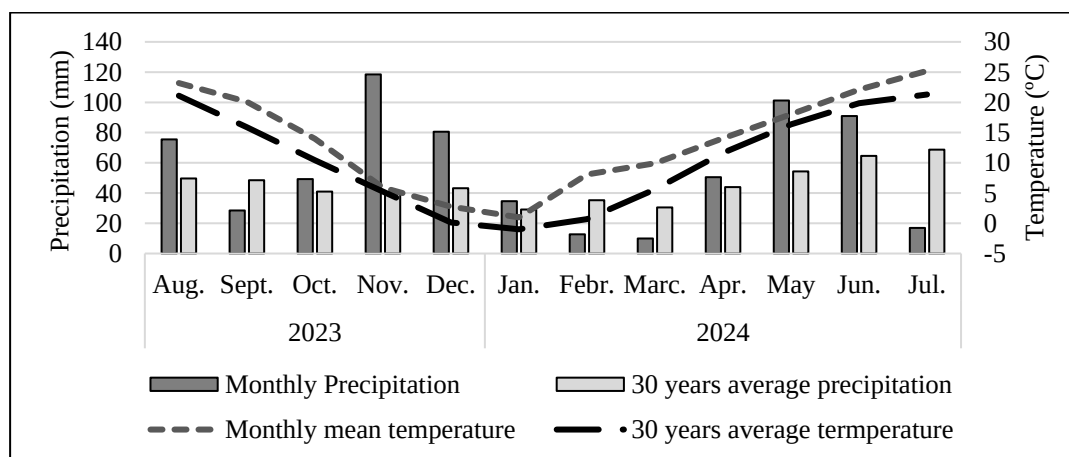
The experiment was set up in the 2024 growing season in the Experimental Garden of the University of Debrecen, under field conditions in 3 replicates. The plot area was 3 m x 4 m. The soil of the experimental area is chernozem type, enriched in phosphorus (1020-1538 mg kg⁻¹ P₂O₅ [AL] in the top 60 cm layer of soil) and potassium (366-638 mg kg⁻¹ K₂O [AL] in the top 60 cm layer of soil) with good humus content (2.37-2.89% in the top 100 cm layer of soil) (AL: ammonium lactate-soluble). These values means very good supply for the plants (Stefanovits et al., 1999). The soil analysis was made at the Agricultural Laboratory Centre of the University of Debrecen. The laboratory is accredited by the National Accreditation Board of Hungary. Due to the rich supply of phosphorus and potassium and the remaining amount of nitrogen from the previous crop (bean) application of additional fertilizer was not necessary. The primary tillage was ploughing. The sowing date was October 3, 2023, with a 600 seeds per square meter seeding rate, and the depth of the sowing was 5 cm.

In the experiment, Hungarian-bred varieties were tested (GK Arany, Mv Hópehehely, Mv Imperiál, Mv Kincsem, Mv Istráng, Mv Hóka).

The use of 4 treatments was investigated (labelled as follows):

- Control: no active ingredient was applied.
- Si: Foliar fertilizer containing silicon (200g SiO₂ L⁻¹, applied dosage: 0,5 L ha⁻¹)
- S: Foliar fertilizer containing sulphur (30g N L⁻¹, 1000g SO₃ L⁻¹, 30g MgO L⁻¹, 27g B L⁻¹, 0,003g Mo L⁻¹, applied dosage: 5 L ha⁻¹)
- Si+S (Combined treatments): Silicon and sulphur foliar fertiliser applied simultaneously (Si: 0,5 L ha⁻¹ + S: 5 L ha⁻¹)

During the growing season, we also applied an insecticide against Cereal Leaf Beetle (*Oulema melanopus*). The active ingredient is lambda-cyhalothrin, sprayed on 10/04/2024 (at BBCH 32) and 15/05/2024 (at BBCH 47). The weather conditions of the growing season are shown in Graph 1. The autumn weather was ideal for the development of the winter oats. The winter was mild enough that we did not have to expect any significant frost damage; the average monthly temperature in February was more than 4.6 °C warmer than the 30-year average. The spring period also proved optimal, although the average monthly temperature was above the 30-year average in every month. From the second decade of June, however, a drought period began, testing the abiotic stress tolerance of the plants.



Graph 1: Weather conditions of the growing season of the winter oat

In the experiment, the leaf area index (LAI) was measured 7 times using the LICOR LI-2000 Plant Canopy Analyzer, a portable instrument that measures leaf area and has the advantage of measuring without damaging the vegetation. Four measurements per plot were taken and averaged to obtain the plot average. To determine the yield averages, the plants were harvested separately per plot using a special plot combiner (Wintersteiger 125), the moisture content of the samples per plot was determined using a drying cabinet, and the yield per hectare was determined by correcting the samples for 14% moisture content.

The programme IBM SPSS version 22 was used to analyse the data. The analysis was performed using one-factor analysis of variance and an LSD post-hoc test for pairwise comparisons.

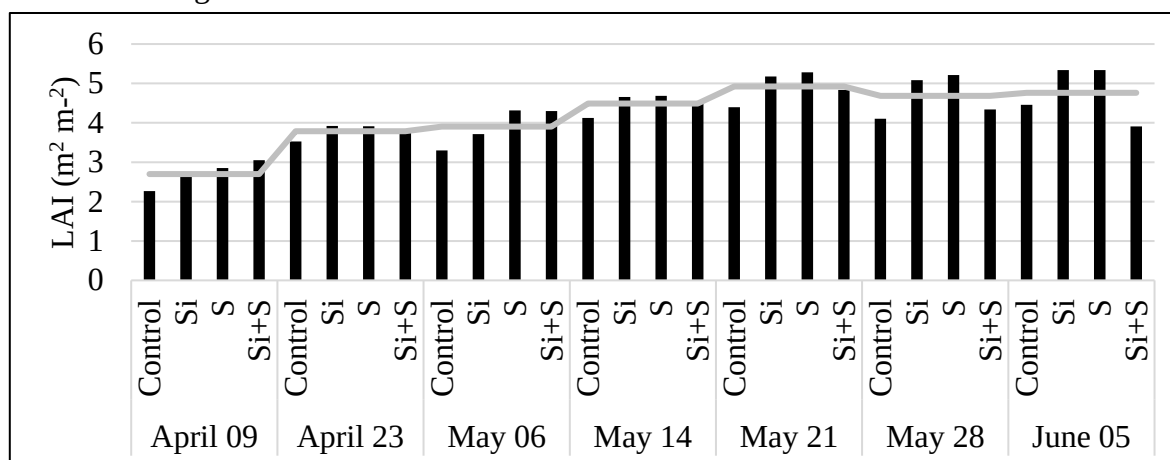
Results and discussion

Leaf area was measured 7 times during the growing period, once a week from 2 nodes (BBCH 32) to milk maturity (BBCH 75) stages. Typically, no statistically supported differences were found between varieties at each measurement time. However, with the exception of the last measurement date, Mv Istráng had the highest leaf area index on all occasions, and GK Arany typically had the lowest values (although it had the highest yield) (Table 1). LAI increased until flowering, after which a slight decrease in values was observed.

Table 1: LAI of the varieties averaged over treatments at each measurement date ($\text{m}^2 \text{m}^{-2}$)

	April 09	April 23	May 06	May 14	May 21	May 28	June 05	Avg.
GK Arany	2,54	3,34	3,70	3,93	4,43	4,29	4,56	3,83
Mv Hópehely	2,63	3,53	3,93	4,49	5,00	4,93	5,67	4,31
Mv Imperiál	2,65	3,82	4,11	4,70	5,26	4,87	4,69	4,30
Mv Kincsem	2,70	3,75	3,68	4,53	4,73	4,31	4,61	4,04
Mv Istráng	2,96	4,39	4,35	5,01	5,44	5,04	4,90	4,58
Mv Hóka	2,72	3,90	3,67	4,26	4,68	4,68	4,14	4,01

For the treatments, the lowest LAI values were measured in the control plots in all cases except the last measurement date. In their research, Salvagiotti and Miralles (2008) found that sulphur had a positive effect primarily on leaf area index values. As can be seen in Graph 2, for most of the dates we measured, the plots receiving the sulphur treatment (S) showed the highest values, and from the second measurement date onwards, both S and Si plots showed values above the average value for that date.

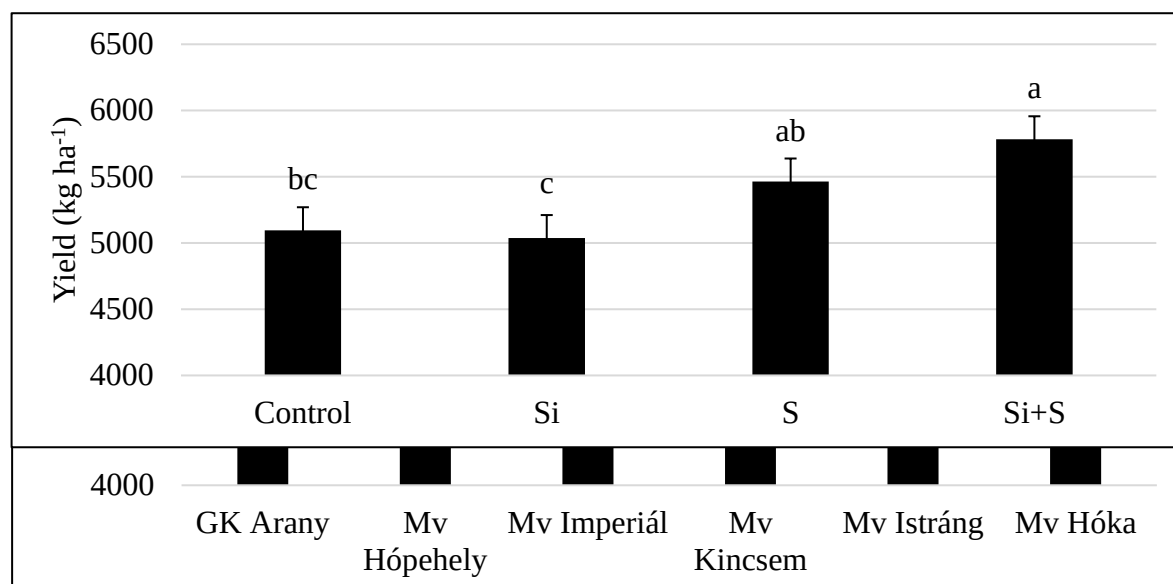


Graph 2: The effect of treatments on LAI values averaged across varieties. Note: The gray line represents the average of the values measured at one measuring time

We also examined the effect of varieties and treatments on yield. The highest values for the varieties were measured for the Mv Kincsem variety, with a yield of just over 6 tonnes (6080 kg ha^{-1}). This value was statistically different from the other varieties, except for one, the GK Arany variety, which had a yield of 5884 kg ha^{-1} . The lowest value was recorded for the variety Mv Hóka (4719 kg ha^{-1}), which did not differ significantly from the results of the varieties Mv Istráng (5077 kg ha^{-1}) and Mv Imperiál (5078 kg ha^{-1}) (Graph 3). The average yield value for the treatments was 5345 kg ha^{-1} .

Graph 3: Effect of varieties on yields averaged over treatments. Note: different lower case letters (a-c) indicate significant differences ($p < 0.05$) between varieties at each measurement time. $\pm$ standard error of means. 3 replicates.

Among the treatments, the highest yields were measured for the combined treatment plots (Si+S) (5.78 t ha^{-1}), which were significantly higher than the control and silicon-treated (Si) plots. The sulphur treatment also resulted in higher yields (5.46 t ha^{-1}) compared to the control plots (5.10 t ha^{-1}), but this could not be significantly confirmed, nor could it be confirmed that the silicon (Si) treatment resulted in lower yields compared to the control plots. Despite the fact that White et al. (2017) found that silicon slag application at all doses increased yields compared to control plots. It can be said that values above 5 t ha^{-1} were measured for all treatments (Graph 4).



Graph 4: Effect of the treatments on yields averaged over genotypes. Note: different lower case letters (a-c) indicate significant differences ($p < 0.05$) between varieties at each measurement time. $\pm$ standard error of means. 3 replicates.

Conclusions

Based on our results, we found significant differences in both varieties and treatments. In terms of LAI among the genotypes, the Mv Istráng variety produced the highest LAI in almost all measurement dates, compared to the GK Arany variety, which showed the lowest LAI values except for 2 measurement dates. Nevertheless, it performed very well in terms of yield, as it almost reached an average of 6 t ha^{-1} . In terms of yield, the Mv Kincsem variety had the highest yield among all the varieties. In terms of treatments, both S and Si treatments increased the LAI values of the plants, although this was more significant in the case of the S treatment. The highest yield was measured in the Si+S treatment plants, which was significantly different from the control plants.

Acknowledgement

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References

- Ayalew H., Anderson J., Kumssa T., Maulana F., Ma X. (2018). Screening oat germplasm for better adaptation to cold stress in the southern great plains of the united states. *Journal of Agronomy and Crop Science*, 205(2), 213-219.
- Baxevanos D., Tsialtas J., Voulgari O., Pankou C., Vlachostergios D., Lithourgidis A. (2020). Oat genotypic requirement for intercropping with vetch under mediterranean conditions. *The Journal of Agricultural Science*, 158(8-9), 695-706.
- Chwil S. (2014). A study on the effects of foliar feeding under different soil fertilization conditions on the yield structure and quality of common oat (*avena sativa* L.). *Acta Agrobotanica*, 67(1), 109-120.
- Hossain M., Mori R., Soga K., Wakabayashi K., Kamisaka S., Fujii S., Hoson T. (2002). Growth promotion and an increase in cell wall extensibility by silicon in rice and some other poaceae seedlings. *Journal of Plant Research*, 115(1), 23-27.
- Rubia D., José A., Emilio G., Osmar B., Angela T., Rafael Z., Dionatan K. (2016). Simulation of oat grain (*avena sativa*) using its panicle components and nitrogen fertilizer. *African Journal of Agricultural Research*, 11(40), 3975-3983.
- Salvagiotti F., Miralles D. J. (2008). Radiation interception, biomass production and grain yield as affected by the interaction of nitrogen and sulfur fertilization in wheat. *European Journal of Agronomy*. 28. 3: 282-290.
- Toledo M., Castro G., Crusciol C., Soratto R., Cavariani C., Ishizuka M., Picoli L. (2012). Silicon leaf application and physiological quality of white oat and wheat seeds. *Semina Ciências Agrárias*, 33(5), 1693-1702.
- White B., Tubana B. S., Babu T., Mascagni H., Agostinho F., Datnoff L. E., Harrison S. (2017). Effect of Silicate Slag Application on Wheat Grown Under Two Nitrogen Rates. *Plants*, 6(4), 47. <https://doi.org/10.3390/plants6040047>
- Xu G., Zhan X., Li C., Shi-dan B., Liu X., Chu T. (2001). Assessing methods of available silicon in calcareous soils. *Communications in Soil Science and Plant Analysis*, 32(5-6), 787-801.
- Šeremešić S., Manojlović M., Vojnov B., Babec B., Koren A., Mirjačić T., Sikora V. (2019). Comparative study of growing winter small grain cereals in organic and conventional production systems. *Journal on Processing and Energy in Agriculture*, 23(4), 176-179. <https://doi.org/10.5937/jpea1904176q>
- Stefanovits P., Filep Gy., Füleky Gy. (1999). Talajtan. Budapest: Mezőgazda Kiadó. In Hungarian, ISBN: 963-286-178-7.

ORIGINAL SCIENTIFIC PAPER

Microclimatic variability and its impact on water requirements in raspberry production

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Summary

Efficient water management is crucial for stable raspberry production, especially in regions with increasing temperature extremes and irregular precipitation. Serbia, a major raspberry producer, has faced yield fluctuations due to climate-driven water challenges. This study analyzes meteorological data from five stations (364 – 753 m a. s. l.) in central and western Serbia to assess irrigation needs. The results show significant temperature and precipitation variability across elevations, leading to uneven water availability. The findings emphasize the necessity of site-specific irrigation strategies to mitigate seasonal drought and sustain optimal yields.

Key words: Raspberry production, irrigation management, water stress, climate variability.

Introduction

Raspberry (*Rubus idaeus* L.) production is highly dependent on climatic conditions, particularly temperature and water availability. Climate change induced variability in temperature and precipitation significantly affects yield stability (Mazur et al., 2014). As global temperatures rise and precipitation patterns become increasingly irregular, traditional agricultural practices must adapt to ensure sustainable fruit production.

Serbia is among the world's top raspberry-producing countries, positioned in third place (FAOSTAT, 2025), with a long-standing tradition of cultivating this economically significant crop. However, unpredictable weather conditions have caused yield instability in recent years, creating economic challenges for growers. High summer temperatures, prolonged drought, and unevenly distributed rainfall contribute to water stress, directly affecting raspberry fruit quality and overall production output (Mittler et al., 2012).

Due to Serbia's diverse topography, microclimatic conditions vary significantly, even within small geographic areas. Elevation, exposure, and local weather patterns influence temperature fluctuations and precipitation levels, affecting the water balance in raspberry orchards. Lower-altitude regions experience higher evapotranspiration rates due to increased heat stress, making efficient irrigation strategies essential for maintaining optimal growing conditions. In contrast, higher-elevation plantations may receive more precipitation but still face risks from temperature extremes.

Accordingly, the aim of this study is to analyze the impact of microclimatic variability on water requirements in raspberry production by evaluating meteorological data from different elevations in central and western Serbia.

Material and methods

This study was conducted using meteorological data collected from five stations in central and western Serbia, strategically selected to represent different altitudinal zones, ranging from 364 m (Gornja Gorevnica near Čačak) to 753 m (Ovčinja near Bajina Bašta). The research covered

the 2024 growing season (May–October), during which temperature and precipitation were continuously monitored to assess climatic variability.

The meteorological parameters analyzed included daily maximum and minimum temperatures, which were used to identify heat stress events and assess temperature variations across different altitudes. The number of hot days, with temperatures exceeding 30 °C and 35 °C, was also examined to evaluate the frequency and intensity of extreme temperature events at each locations. Precipitation levels (mm) were analyzed to assess rainfall distribution and its adequacy for raspberry cultivation.

Temperature and precipitation data were collected using automatic meteorological stations equipped with sensors for measuring air temperature, humidity, and precipitation levels. Data analysis included: comparative analysis to identify differences in microclimatic conditions among the selected locations; correlation analysis to examine relationships between elevation, temperature fluctuations, and precipitation variability; and regression analysis to evaluate the impact of elevation on total precipitation using a linear regression model.

The correlation matrix was computed using the Pearson correlation coefficient to assess the strength and direction of relationships between elevation, the number of hot days (>30 °C and >35 °C), and total precipitation. A simple linear regression model was used to evaluate the relationship between total precipitation and altitude. The regression equation, *Total Precipitation* = $0.568 \times \text{altitude} + 69.66$, was derived, and the model fit was assessed using the coefficient of determination (R^2).

Results and discussion

Previous studies have demonstrated that climatic variations significantly affect the growth and productivity of raspberry crops. Research conducted in the Republic of Moldova (Parascovia, 2014) established that phenological phases such as bud break, flowering, and fruit ripening are directly influenced by temperature and precipitation patterns. Similar findings were observed in this study, where significant microclimatic differences were recorded across varying altitudes.

The results reveal notable temperature disparities among the locations (Table 1). The number of days exceeding +30 °C ranged from 23 to 39 at higher elevations, while lower-elevation plantations experienced between 42 and 69 such days. Extreme heat events, defined as days with temperatures surpassed +35 °C, occurred up to four times at high-altitude sites and up to 19 times in lowland orchards. Prolonged exposure to high temperatures in this lowland areas can severely disrupt plant physiological processes, leading to increased transpiration, reduced fruit set, and diminished overall yield quality (Sharma and Manjeet, 2020).

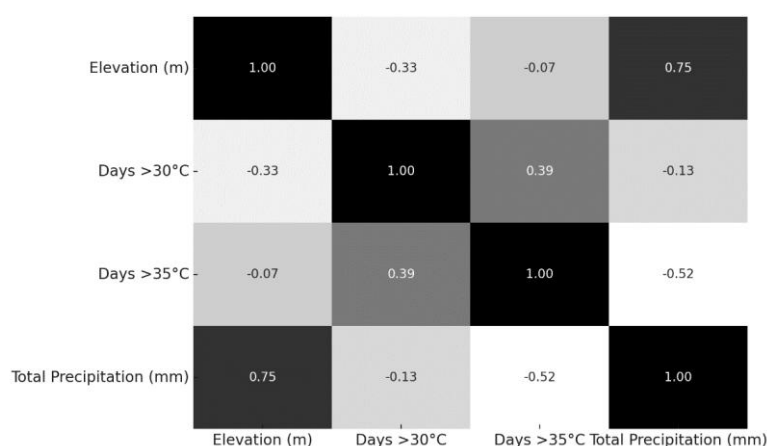
Table 1. Temperature and precipitation disparities between the locations

Location (Elevation)	Days >30°C	Days >35°C	Total Precipitation (mm)
Gornja Gorevnica (364 m)	42	19	268.6
Miličinica (372 m)	69	12	341.4
Lučani (643 m)	49	3	304.6
Brezovice (738 m)	23	0	448.8
Ovčinja (753 m)	39	4	631.0

Precipitation levels also displayed considerable variability (Table 1). Total rainfall ranged from 304.6 mm at the lowest sites to 631 mm at the highest elevations, with lower-elevation areas experiencing drier conditions and more frequent soil moisture deficits. Uneven rainfall distribution was particularly evident during the peak summer months, when prolonged dry

spells were recorded in lowland plantations. These findings are consistent with previous research indicating that insufficient and irregular precipitation exacerbates water stress, negatively impacting raspberry growth and fruit development (Mazur et al., 2014). In contrast, higher-altitude locations received more consistent rainfall, contributing to better soil moisture retention and reduced plant stress.

The correlation analysis provided valuable insights into the relationships among key climatic variables (Graph 1). Numerous studies have shown that temperature plays a crucial role in vegetative growth, flowering, and fruit quality in raspberries (Petrović et al., 2020). Carew et al. (2001) reported that the optimal temperature for raspberry growth is approximately 20 °C, with 24 °C being ideal for strong vegetative development. In contrast, temperatures above this range slow growth, while temperatures around 11 °C induce dormancy. The results indicate a strong positive correlation between elevation and total precipitation ($r = 0.75$), while a negative correlation was observed between elevation and the number of days with temperatures exceeding 30 °C ($r = -0.33$), indicating that rainfall generally increases with elevation. This suggests that higher-altitude locations benefit from greater natural water availability, while lower-altitude areas experience drier conditions. Additionally, the analysis confirmed that lower-elevation sites are more prone to higher temperatures, which exacerbate water stress and impact plant physiology. Previous research has also shown that photoperiod plays a crucial role in raspberry flowering, with the shortest time to flowering occurring at photoperiods between 11 and 14 hours (Lockshin and Elfving, 1981; Kershaw, 1991). The combined effects of temperature and light intensity further influence raspberry development, with optimal conditions accelerating both flowering and fruiting. These results emphasize the necessity of site-specific irrigation solutions, particularly in regions where natural precipitation levels are insufficient to support optimal raspberry growth.



Graph 1. Correlation matrix of climatic factors

The regression model (Graph 2) further quantified the relationship between altitude and precipitation, yielding the equation:

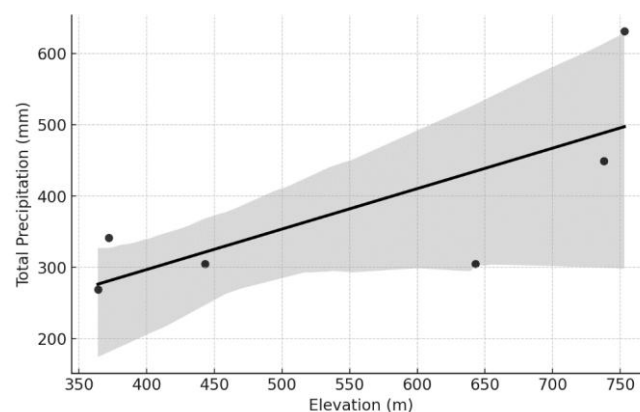
$$\text{Total Precipitation} = 0.568 \times \text{altitude} + 69.66$$

with an R^2 value of 0.57, suggesting that altitude alone explains a significant portion of precipitation variability (0.568 is the slope of the regression line (slope), which means that for every meter of elevation, the amount of precipitation increases by 0.568 mm; 69.66 is the Y-intercept, which means that, according to the model, the expected amount of precipitation at an elevation of 0 m above sea level would be 69.66 mm). The p-value (0.0848) indicates borderline statistical significance, aligning with previous climatological studies that highlight the role of topography in precipitation distribution. Sishodia et al. (2020) highlighted that terrain variations significantly affect local precipitation patterns, impacting soil moisture retention and water

availability for crops. Their study on remote sensing applications supports the need for site-specific irrigation strategies in regions with complex topography. These findings reinforce the conclusion that elevation plays a key role in shaping local climatic conditions and influencing water availability in raspberry orchards.

Compared to earlier studies (Jones and Tardieu, 1998), our findings reveal a key limitation in relying solely on natural precipitation. Although rainfall generally increases with altitude, its variability and uneven seasonal distribution make it an unreliable water source—particularly in lowland plantations. Additionally, results indicate that temperature stress at lower altitudes may further accelerate soil moisture loss, reinforcing the necessity of precision irrigation strategies. This aligns with findings from Bwambale (2022), which emphasize that combining soil-based, plant, and weather-based monitoring methods in a modelling environment with model predictive control can significantly improve water use efficiency.

The results indicate that relying solely on natural precipitation is insufficient for achieving optimal raspberry production, particularly at lower-elevation areas where water deficits are more pronounced. Mazur et al. (2014) found that raspberry fruit yield and quality significantly declined with increasing precipitation variability, underscoring the necessity for controlled irrigation strategies. Their findings also show that fruit sugar content and acidity are highly influenced by microclimatic conditions (Mazur et al., 2014). Site-specific irrigation strategies are essential to counteract seasonal drought effects and maintain stable yields. The integration of precision agriculture techniques, such as automated irrigation scheduling based on microclimatic monitoring, can optimize water management and improve long-term production stability (Sishodia, 2020).



Graph 2. Relationship between elevation and precipitation

Jones and Tardieu (1989) highlighted that temperature variations influence both cane development and fruit maturity in raspberries, reinforcing the importance of microclimatic management strategies. In addition, Williams (1959) found that young raspberry shoots are particularly sensitive to environmental conditions, requiring adequate water supply to sustain growth. Sishodia et al. (2020) emphasized the role of remote sensing technologies in precision agriculture, particularly for monitoring evapotranspiration, soil moisture levels, and crop water stress. The integration of satellite-based data can enhance irrigation management by providing real-time insights into plant water needs, reducing water wastage, and optimizing yield outcomes. These technologies are especially relevant to raspberry production, where microclimatic variability significantly influences water availability and overall plant performance. The results support the integration of precision agriculture techniques, such as automated irrigation scheduling based on microclimatic monitoring, to optimize water management and improve long-term production stability.

Conclusions

The study highlights the critical role of site-specific irrigation systems in mitigating the effects of seasonal drought on raspberry production. Given the observed climatic variations, implementing tailored irrigation strategies can enhance water-use efficiency and improve yield stability in Serbia's raspberry orchards. Future research should focus on integrating real-time soil moisture monitoring and optimizing irrigation scheduling based on microclimatic conditions to ensure sustainable raspberry production.

Acknowledgement

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References

- Bwambale E., Abagale F.K., Anornu G.K. (2022). Smart irrigation monitoring and control strategies for improving water use efficiency in precision agriculture: A review *Agricultural Water Management*, 260: 107324.
- Carew J.G., Mahmood K., Darby J., Hadley P., Battey N.H. (2001). The effects of low temperatures on the vegetative growth and flowering of the primocane fruiting raspberry 'Autumn Bliss'. *The Journal of Horticultural Science and Biotechnology*, 76:264–270.
- FAOSTAT (2025). <https://www.fao.org>.
- Jones H.G., Tardieu F. (1998). Modeling water relations of horticultural crops: a review. *Scientia Horticulturae*, 74(1): 21-46.
- Kershaw C. (1991). The effect of temperature on the rate of shoot development in the raspberry (*Rubus idaeus* L.) cultivar Autumn Bliss. PhD diss. Wye College, Univ. London.
- Lockshin L.S., Elfving D.C. (1981). Flowering response of 'Heritage' red raspberry to temperature and nitrogen. *Hortscience* 16:527–528.
- Mazur S.P., Sønsteby A., Nes A., Wold A.B., Foito A., Freitag S., Verrall S., Stewart D., Heide O.M. (2014). Effects of Post-Flowering Environmental Variation along an Altitudinal Gradient on Chemical Composition of 'Glen Ample' Red Raspberry (*Rubus idaeus* L.). *European Journal of Horticultural Sciences*, 79 (5): 267–277.
- Mittler R., Finka A., Goloubinoff P. (2012). How do plants feel the heat? *Trends in Biochemical Sciences*, 37(3): 118-125.
- Parascovia S. (2014). Resistance of some raspberry varieties on fluctuations climate conditions in Republic of Moldova. *Fruit Growing Research*, Vol. XXX, Practical Scientific Institute of Horticulture and Food Technology, Republic of Moldova.
- Petrović S., Milošević T., Jevremović D., Glišić I., Milošević N. (2020). Raspberry in *Berries*, growing technology, protection and processing. Published by Faculty of Agronomy in Čačak and Fruit research institute in Čačak. ISBN 978-86-87611-76-4, 97-226.
- Sharma S., Manjeet M. (2020). Heat stress impacts on small fruit crops: A review. *Agricultural Reviews*, 41(1): 73-78.
- Sishodia P.R., Ray L.R., Singh K.S. (2020). Applications of Remote Sensing in Precision Agriculture: A Review. 12: 3136. *Remote Sensing*, DOI: 10.3390/rs12193136.
- Williams I.H. (1959). Effects of environment on *Rubus idaeus* L. III Growth and dormancy of young shoots. *Journal of Horticultural Sciences*, 34:210–218.

EXPERT PAPER

Chlorophyll and Carotenoid Content in Radish Microgreens: Insights Across Different Cultivars

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Summary

Microgreens, harvested at the cotyledon stage, are nutrient-dense young plants recognized as functional superfoods, with popular varieties including radish, pea, red cabbage, red beet, garden cress, leek, amaranth, and sunflower. This study evaluated chlorophyll and carotenoid content in microgreens of three radish (*Raphanus sativus* L.) cultivars with different stem and cotyledon colors. Plants were grown under controlled conditions and harvested on day eight. Chloroplastic pigments were quantified using spectrophotometry. Results showed significant differences among cultivars, with Vienna radish exhibiting the highest pigment levels. Findings highlight the impact of cultivar selection on nutritional quality and underscore the value of pigment profiling in optimizing microgreen production.

Keywords: *Raphanus sativus* L., *Chl a*, *Chl b*, *Car*, young plants, spectrometry

Introduction

Microgreens represent young plants in the phase of fully expanded cotyledons at the time of harvest. These young plants are rich in nutrients and bioactive compounds, which gives them a special place as a functional superfood (Mir et al., 2017). The most common microgreens on the market are pea (*Pisum sativum* L.), radish (*Raphanus sativus* L.), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra* (L.) Thell.), red beet (*Beta vulgaris* L.), garden cress (*Lepidium sativum* L.), leek (*Allium ampeloprasum* var. *porrum* L.), amaranth (*Amaranthus* spp.), and sunflower (*Helianthus annuus* L.) (Opačić et al., 2018, Cerjak et al., 2019; Paraschivu et al., 2021; Singh et al., 2024).

Radishes microgreens are one of the most popular microgreens in the everyday diet. They are rich in vitamins A, B, C, E, and K, but also provide the human body with calcium, iron, magnesium, phosphorus, potassium, and zinc (Thuong et al., 2020; Kaimuangpak et al., 2022), tocopherols and carotenoids (Xiao et al., 2014). Depending on the cultivar, radish microgreens have beautiful red, white, rose, and purple stems and green or purple leaves. They will add a spicy kick to food, along with added vitamins and minerals. Both of these are beneficial for health and immune system function. Microgreens improve digestion and often they are used to help overcome some diseases. Generally, radish microgreens are considered the superfood chefs' choice over restaurants.

Plants contain four primary pigments. These are the compounds responsible for the color in plants and play a key role in regulating photosynthesis, as well as supporting growth and development (Sudhakara et al., 2016). The chlorophyll content of vegetables is important for both the health benefits and the visual appearance of the microgreens (Paradiso et al., 2018). Chlorophylls are important antioxidants found in foods and its derivatives, such as chlorophyllin, have demonstrated cancer-preventive properties through various mechanisms such as antioxidant activity, detoxification, and antimutagenic effects (Hsu et al., 2013). In plants, carotenoids have photoprotective and antioxidant properties (Swapnil et al., 2021). Also, they are becoming important in the human diet, as they are known for their protective properties

against various diseases due to their provitamin A activity and antioxidant effects in fruits and vegetables. They influence cell proliferation and differentiation, prevent free radical generation, aid in immune function, and promote intercellular communication (Jeevanandam et al., 2017.). Considering all the above advantages of plant pigments, this study aimed to investigate the microgreens chloroplastic pigment status of three different radish cultivars.

Material and methods

In this study, three different cultivars of radish (*Raphanus sativus* L.), with different stem and cotyledons colors were used: Red radish (purple stem and cotyledons) and Rose radish (red stem and green/red cotyledons) varieties from GEO Bavicchi Spa, Italy, and Vienna radish (white stem and green cotyledons) from Gartenland GmbH Aschersleben, Germany. Plastic containers (13 x 3.5 x 8 cm) were used for microgreen growing. The seeds were sown in the commercial substrate (Klasmann) and placed in the darkroom for 48 h for the Rose radish and Vienna variety and 72 h for the Red radish cultivar. Afterward, the containers were placed under LED white light on 16/8 photoperiod at 24 ± 1 °C. Plants were watered daily. On the 8th day, the plants were harvested for chloroplast pigment determination. The plants were cut 2 cm above the substrate in three replications. Stems and cotyledonous leaves were macerated to powder tissue using liquid nitrogen and weighed (0.05 g of tissue). Acetone and MgCO_3 were added, and the mixture was vortexed. The samples were then transferred to a centrifuge for 10 minutes at 4 °C and 4000 rpm. Absorbance was measured at three wavelengths: 440, 644, and 662 nm, using pure acetone as a blank.

The obtained absorption values (A_{662} , A_{644} , and A_{440}) were inserted into the Holm-Wetstein equations to calculate the pigment concentration in mg dm^{-3} . The final concentrations of the leaf pigments in radish microgreens are expressed as mg g^{-1} of the fresh weight (FW):

$$\text{Chlorophyll a (Chl a)} = 9.784 \times A_{662} - 0.990 \times A_{644}$$

$$\text{Chlorophyll b (Chl b)} = 21.426 \times A_{644} - 4.65 \times A_{662}$$

$$\text{Chl a+b} = 5.134 \times A_{662} + 20.436 \times A_{644}$$

$$\text{Carotenoids (Car)} = 4.695 \times A_{440} - 0.268 \times (\text{Chl a+b})$$

Results and discussion

The ANOVA analysis showed the significant influence of cultivars on pigment status and ratio (Table 1.).

Table 1. ANOVA of chloroplast pigments status regarding radish variety

Parameter	F Value	P value
<i>Chl a</i>	17.64	**
<i>Chl b</i>	3.79	*
<i>Chl a+b</i>	3.52	*
<i>Chl a/b</i>	5.91	*
<i>Car</i>	14.36	*
<i>Chl a+b / Car</i>	6.35	*

ns – non significant; * $p \leq 0.05$; ** $p \leq 0.01$

The average *Chl a* content of the radish microgreens was 0.38 mg g^{-1} FW (Figure 1a). The Vienna cultivar had the highest *Chl a* content (0.48 mg g^{-1} FW), which was significantly different ($p \leq 0.05$) from the other two cultivars. The average *Chl b* content was 0.18 mg g^{-1} FW (Figure 1b), with values ranging from 0.16 mg g^{-1} FW in Rose radish to 0.19 mg g^{-1} FW

in Vienna (Figure 1b). Significant differences in the *Chl a/b* ratio were observed ($p \leq 0.05$) among cultivars. The *Chl a+b* content in radish microgreens was $0.56 \text{ mg g}^{-1} \text{ FW}$ (Figure 1c), ranging from $0.50 \text{ mg g}^{-1} \text{ FW}$ in Rose radish to $0.67 \text{ mg g}^{-1} \text{ FW}$ in Vienna. The average *Chl a/b* ratio was 2.14 (Figure 1d). The carotenoid (*Car*) content significantly differed ($p \leq 0.05$) among the radish microgreens (Table 1), with an average *Car* content of $0.12 \text{ mg g}^{-1} \text{ FW}$ (Figure 1e). The lowest *Car* content was found in Red radish ($0.10 \text{ mg g}^{-1} \text{ FW}$), while Vienna radish had the highest *Car* content ($0.15 \text{ mg g}^{-1} \text{ FW}$). The average *Chl/Car* ratio was 4.76 (Figure 1f).

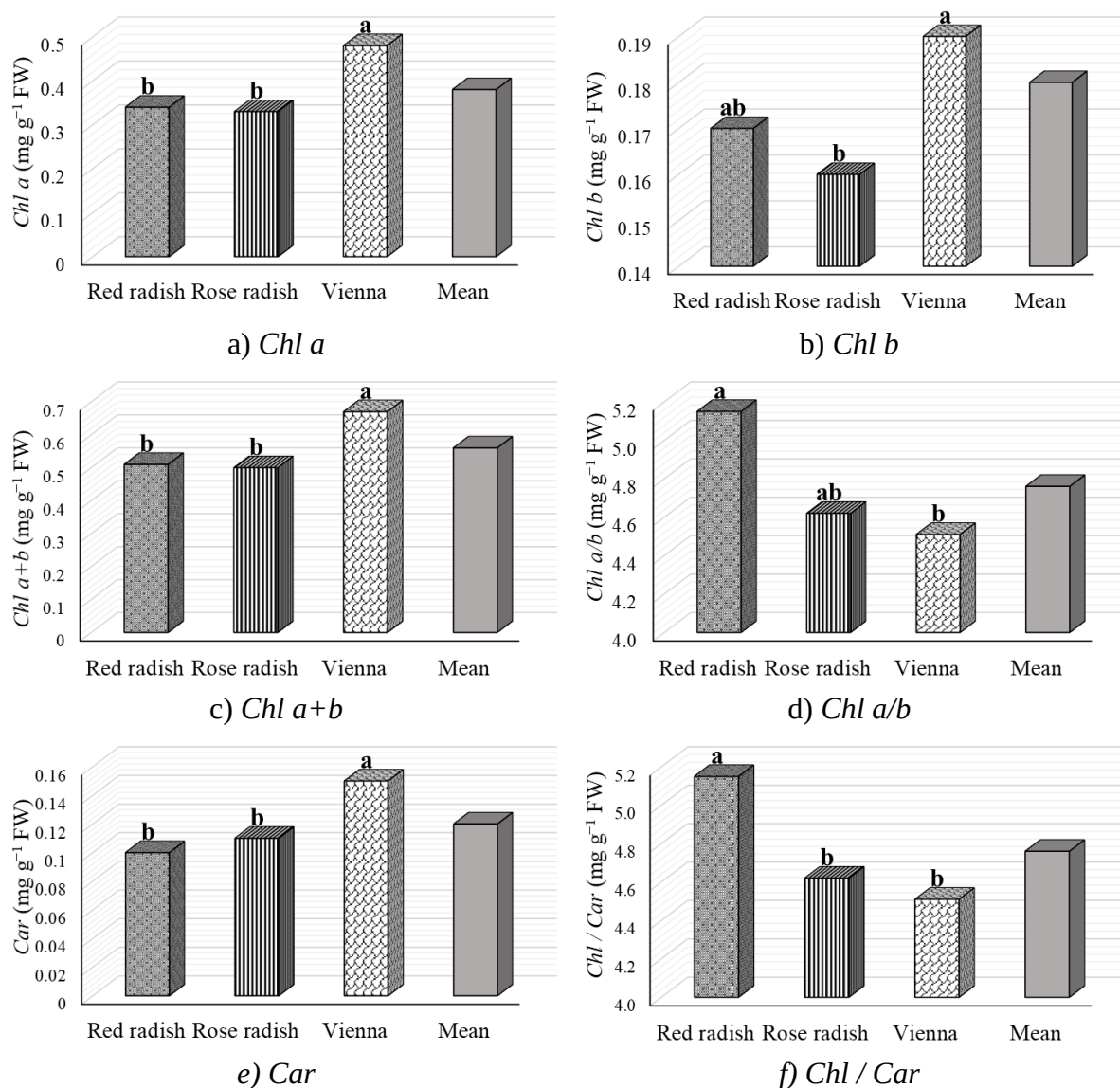


Figure 1. Chlorophyll and carotenoid content of different radish microgreens (*Chl a* – chlorophyll a, *Chl b* – chlorophyll b, *Car* – carotenoids). Means denoted by a different letter indicate significant differences between treatments ($p \leq 0.05$)

Mlinarić et al. (2023) reported similar differences in total chlorophyll (*Chl a+b*) and carotenoids (*Car*) content between the microgreen cultivars. Authors found that under the LED light (photoperiod 16/8 h), the average *Chl a+b* content in radish microgreens was highest in the green radish cultivar (Daikon radish) $0.320 \text{ mg g}^{-1} \text{ FW}$ and the lowest in Rose radish ($0.268 \text{ mg g}^{-1} \text{ FW}$). Also, *Car* content was highest in green cultivar ($0.059 \text{ mg g}^{-1} \text{ FW}$), compared to

the lowest content of 0.046 mg g^{-1} FW in Rose radish cultivar. This is in accordance with our research where the highest content of *Chl a+b* and *Car* was determined in the green cultivar. In the research of Toscani et al. (2023) content of *Chl a*, *Chl b*, *Chl a+b* and *Car* in radish microgreen was 0.199 ± 0.022 , 0.084 ± 0.019 , 0.283 ± 0.04 , $0.045 \pm 0.002 \text{ } \mu\text{g mg}^{-1}$ FW, respectfully. These values are smaller than those found in our study, especially for *Chl a+b* and *Car* content, which are more than twice as high in our research. In contrast, Rizvi et al. (2024) reported similar values of total chlorophyll content in green radish microgreens (0.6236 mg g^{-1}), but also much lower values of *Car* 0.054 mg g^{-1} compared to our research. Kowitcharoen et al. (2021) measured the total chlorophyll content, which is the sum of chlorophyll a and chlorophyll b, in various fresh radish varieties, with values ranging from 0.3661 mg g^{-1} FW to 0.5921 mg g^{-1} FW.

It is important to note that the cultivation conditions, particularly exposure to sunlight or artificial light sources, as well as the maturity of the microgreens, influence their chlorophyll content. The chlorophyll a/b ratio reflects these conditions, with a higher ratio indicating exposure to sunlight or intense light, as a more yellow appearance is caused by an increased chlorophyll b content (Stajčić et al., 2024). These findings suggest that cultivar choice, growing conditions and photoperiod play a significant role in pigment accumulation, contributing to a deeper understanding of the nutritional potential of radish microgreens, emphasizing their value as a source of bioactive compounds beneficial to human health.

Conclusions

In this study, three different radish cultivars were grown as microgreens to determine the chlorophyll and carotenoid content in the young plants. In general, the highest *Chl* and *Car* content was found in Vienna radish cultivar, while the pigment content in the Red and Rose cultivars was quite similar. This study provides an insight into leaf pigment content in relation to radish microgreens with different stem and cotyledon colors. It also gives a fundamental for further research in other bioactive compounds in the radish microgreens.

References

- Cerjak, M., Rustan, M., Juračak, J. (2019). Struktura i izgledi za razvoj hrvatskog tržišta mikro zelenja. *Agroeconomia Croatica*, 9(1), 142-152.
- Holm, G. (1954): Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica*, 4, 457–461.
- Hsu, C. Y., Chao, P. Y., Hu, S. P., Yang, C. M. (2013). The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. *Food and Nutrition Sciences*, 4(8), 1-8.
- Jeevanandam, J., Aing, Y. S., Chan, Y. S., Pan, S., & Danquah, M. K. (2017). Nanoformulation and application of phytochemicals as antimicrobial agents. In *Antimicrobial nanoarchitectonics* (pp. 61-82). Elsevier.
- Kaimuangpak, K., Tamprasit, K., Thumanu, K., Weerapreeyakul, N. (2022). Extracellular vesicles derived from microgreens of *Raphanus sativus* L. var. *caudatus* Alef contain bioactive macromolecules and inhibit HCT116 cells proliferation. *Scientific Reports*, 12(1), 15686.
- Kowitcharoen, L., Phornvillay, S., Lekham, P., Pongprasert, N., & Srilaong, V. (2021). Bioactive composition and nutritional profile of microgreens cultivated in Thailand. *Applied Sciences*, 11(17), 7981.
- Martins, T., Barros, A. N., Rosa, E., Antunes, L. (2023). Enhancing health benefits through chlorophylls and chlorophyll-rich agro-food: A comprehensive review. *Molecules*, 28(14), 5344.

- Mir, S. A., Shah, M. A., Mir, M. M. (2017). Microgreens: Production, shelf life, and bioactive components. *Critical Reviews in Food Science and Nutrition*, 57(12), 2730-2736.
- Mlinarić, S., Piškor, A., Melnjak, A., Mikuška, A., Šrajer Gajdošik, M., Begović, L. (2023). Antioxidant capacity and shelf life of radish microgreens affected by growth light and cultivars. *Horticulturae*, 9(1), 76.
- Opačić, N., Šagud, A., Skomrak, A., Đurak, J., Kos, F., Butković, M., Uher, S. (2018). Microgreens kao funkcionalna hrana. *Glasnik Zastite Bilja*, 41(3), 18-26.
- Paradiso, V. M., Castellino, M., Renna, M., Leoni, B., Caponio, F., Santamaria, P. (2018). Simple tools for monitoring chlorophyll in broccoli raab and radish microgreens on their growing medium during cold storage. *Progress in Nutrition*, 20, 415-422.
- Paraschivu, M., Cotuna, O., Sărățeanu, V., Durău, C. C., Păunescu, R. A. (2021). Microgreens-current status, global market trends and forward statements.
- Rizvi, A., Kumar, S., Sharma, M., & Saxena, S. (2024). Functional and Antioxidant Potential of Beetroot, Mustard and Radish Microgreens Using Spectroscopic Techniques. *Agriculture*, 70(2), 53-71.
- Singh, A., Singh, J., Kaur, S., Gunjal, M., Kaur, J., Nanda, V., Ullah, R., Ercisli, S., Rasane, P. (2024). Emergence of microgreens as a valuable food, current understanding of their market and consumer perception: A review. *Food Chemistry: X*, 23, 101527.
- Stajčić, S., Četković, G., Tumbas Šaponjac, V., Travičić, V., Ilić, P., Brunet, S., & Tomić, A. (2024). Bioactive compounds and the antioxidant activity of selected vegetable microgreens: A correlation study. *Processes*, 12(8), 1743.
- Sudhakar, P., Latha, P., & Reddy, P. V. (2016). *Phenotyping crop plants for physiological and biochemical traits*. Academic Press.
- Swapnil, P., Meena, M., Singh, S. K., Dhuldhaj, U. P., Marwal, A. (2021). Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Current Plant Biology*, 26, 100203.
- Thuong, V. T., Minh, H. G. (2020). Effects of growing substrates and seed density on yield and quality of radish (*Raphanus sativus*) microgreens. *Research on Crops*, 21(3), 579-586.
- Toscano, S., Romano, D., Patanè, C. (2023). Effect of application of biostimulants on the biomass, nitrate, pigments, and antioxidants content in radish and turnip microgreens. *Agronomy*, 13(1), 145.
- Wettstein, D. (1957), Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. *Experimental Cell Research*, 12, 427-487.
- Xiao, Z., Lester, G. E., Luo, Y., Xie, Z. K., Yu, L. L., Wang, Q. (2014). Effect of light exposure on sensorial quality, concentrations of bioactive compounds and antioxidant capacity of radish microgreens during low temperature storage. *Food chemistry*, 151, 472-479.

ORIGINAL SCIENTIFIC PAPER

Optimization of simultaneous detection of wheat and maize DNA using duplex PCRJelena Kovač¹, Renata Hanzer², Sunčica Kujundžić¹, Sonja Vila¹, Sonja Petrović¹¹*Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia (sonja.petrovic@fazos.hr)*²*Croatian Agency for Agriculture and Food, Usorska 19, Osijek, Croatia***Summary**

This study focuses on optimizing duplex PCR simultaneous detection of wheat and maize DNA, two essential crops in global agriculture. The polymerase chain reaction (PCR) is a powerful amplification technique that can generate a specific segment of DNA from a small amount of starting material. In this study, DNA was extracted from a seed mixture of maize and wheat, using the CTAB method. The duplex PCR was optimized with specific primer pairs targeting genes in wheat and maize. The amplification products were analyzed using agarose gel and single-channel capillary electrophoresis, and the results confirmed the successful amplification of the expected DNA fragments.

Key words: duplex PCR, wheat, maize, optimization, detection

Introduction

Maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) are two of the most important food crops worldwide, with each being cultivated on approximately 200 million ha (Igrejas et al., 2020). According to Yanagi (2024), wheat is not only extensively used as a raw material, fodder, and ingredient in modern food production, but it also plays multiple roles in the ecosystem, such as maintaining ecological balance and improving soil quality etc. Also, despite maize's later domestication due to geographical isolation, Erenstein et al. (2022) suggest that maize plays an important role in global agri-food systems and food and nutrition security. Given the significance of these crops in global agriculture and food systems, it is evident that their production is widespread. Due to their frequent cultivation near each other, as well as during harvesting and seed distribution, seed contamination often occurs.

The polymerase chain reaction (PCR) is a powerful amplification technique that can generate a specific segment of DNA from a small amount of starting material (Lorenz, 2012), making it an effective and accurate technique for detecting plant species in seed mixtures. Choosing the crop-specific markers needs special attention, representing biological markers that are used in commercial food/feed applications, and focusing on their stability, specificity, and copy number. The *ACC 1* gene in wheat that encodes acetyl-CoA carboxylase 1, a key multidomain enzyme involved in both fatty acid biosynthesis and secondary metabolism (Faris et al., 2001). The *ADH* gene, also known as alcohol dehydrogenase, in maize plays a significant role in anaerobic metabolism, as it helps in the fermentation process when oxygen is limited (Gerlach et al., 1982). Multiplex PCR is a modification of PCR in which n target sequences are simultaneously amplified with n pairs of primers, where $n > 1$ (Green and Sambrook, 2019), simplifying and improving PCR analysis by reducing the time, amplifying multiple target regions at once (Singh et al., 2014). This study discusses the development and optimization of duplex PCR for the simultaneous detection of wheat and maize DNA in one mixed sample.

Material and methods

Plant material

The seed bulk sample was done according to the International seed testing association rules (ISTA, 2016, International Rules for Seed Testing). The working sample of the test portions consisted of 3000 seeds ground in a single grinding step. Following the binomial distribution, this guarantees a minimum 95% probability to detect one seed in 3000 with a probability level of a seed sample being contaminated as $p = 0.1\%$ (Bassani et al., 2015; Lane et al., 2018). Accordingly, the sample used in this study contained a seed mixture of 3000 maize grains with one wheat grain. The sample was homogenized using a batch mill, 200 mg of ground sample was transferred to a 2 mL Eppendorf tube in four repetitions and stored at $+4\text{ }^{\circ}\text{C}$ until DNA extraction.

DNA extraction

The genomic DNA extraction was carried out using the Cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1989). The concentration and purity of extracted DNA were assessed with an Eppendorf BioPhotometer™ D30. The extracted DNA samples were diluted with ultrapure water to a working concentration of $50\text{ ng }\mu\text{l}^{-1}$.

PCR reactions and product analysis

The primer set for the respective crop-specific markers was retrieved from the literature (Table 1).

Table 1. Primers pairs and amplicon size for the targeted gene in wheat and maize

Crop	Target gene	Primer name	Sequence (5'→3')	Length (bp)	Reference
Wheat	ACC-1 (acetil koenzim A karboksilaza)	F: Acc-1F SQ50542	F: GCCTACCCCCTTCAACAAGA	94	(Gagnon et al, 2024)
		R: Acc-1RSQ50543	R: ATGTACGCGCTTGAACCCTT		
Maize	ADH-1 (alcohol dehydrogenase 1)	F: ADH-F3	F: CGTCGTTTCCCATCTCTTCCTCC	135	(Hernandez et al., 2004; Paterno et al., 2009)
		R: ADH-R4	R: CCACTCCGAGACCCTCAGTC		

The duplex PCR reaction was carried out using Veriti® 96-Well thermal cycler. The PCR reaction mixture (Promega®) per sample was prepared in a final volume of $20\text{ }\mu\text{l}$ as follows: $14.5\text{ }\mu\text{l}$ ultrapure water, $2.5\text{ }\mu\text{l}$ PCR buffer, $0.5\text{ }\mu\text{l}$ dNTP, $1.25\text{ }\mu\text{l}$ Mg^{+2} , $0.125\text{ }\mu\text{l}$ of DNA polymerase, $0.3\text{ }\mu\text{l}$ wheat and $0.25\text{ }\mu\text{l}$ of maize-specific primer pairs. The thermal cycling reaction conditions were as follows: initial denaturation $95\text{ }^{\circ}\text{C}$ for 2 min, followed by $95\text{ }^{\circ}\text{C}$ for another 25 sec, $62\text{ }^{\circ}\text{C}$ for 30 sec, $68\text{ }^{\circ}\text{C}$ for 45 sec total of 40 cycles, than finally at $68\text{ }^{\circ}\text{C}$ for 10 min. The agarose gel electrophoresis was performed using a 2% agarose gel. The DNA fragments were separated through a gel based on their size and visualized using a G:BOX F3. For single-channel capillary electrophoresis (CGE), a Qsep1® BiOptic fragment analyzer for DNA was used to provide data on fragment sizes and concentrations. The PCR products were diluted to a 1/20 ratio with the provided dilution buffer, CGE was performed according to the provided Qsep1 protocol, and then analyzed using Q-Analyzer software.

Results and discussion

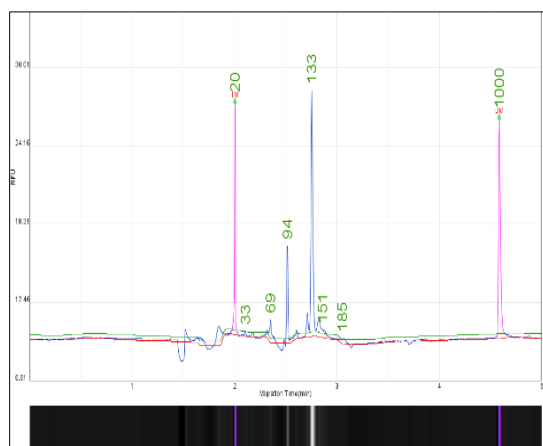
PCR analyses are nowadays a widely spread tool in agricultural and food research. It is a DNA-based technique that consists of a few basic steps: DNA extraction, quality and yield measurement by spectrophotometry or similar, PCR amplification, and at the end visualisation by agarose gel electrophoresis. Good quality DNA with the absence of any enzyme inhibitors is essential for all downstream applications (Abdel-Latif and Osman, 2017). The obtained

concentration of DNA extracted from samples ranged between 33.84 and 39.77 ng μl^{-1} , and the absorbance ratio A_{260}/A_{280} between 1.85 and 1.93. Those results indicated a high quality of isolated DNA suitable for PCR amplification.

Even though a single PCR reaction is suitable for the detection of both maize and wheat, duplex and multiplex systems, once optimised, allow us to reduce time and cost with the same accuracy. The results associated with *ACC 1* wheat primers gave the expected amplicon of size 94 bp. Also, the results associated with *ADH 1* maize primers gave the expected amplicon of size 135 bp (Picture 1). The duplex PCR applied to seed mixture materials showed only the expected amplification product of the specific size.



Picture 1. Agarose gel electrophoresis profile of duplex PCR products of *ACC 1* and *ADH 1*: line 1-DNA ladder, line 2-wheat, line 3-maize, line 4-seed mixture of maize and wheat, line 5-DNA ladder



Picture 2. Single-channel capillary electrophoresis profile of duplex PCR products

This indicates that reference materials contain wheat and maize DNA. In this case, the results of a single PCR with reference primers for the specific identification of maize and wheat were used as a positive control. Optimisation procedure was carried out by adjusting annealing temperature and the concentration of reagents (Puente-Lelievre and Eischeid, 2021). Weak or faint bands can occur for several reasons, such as a lack of the required number of cycles, short annealing, denaturation, extension time, an inappropriate annealing temperature, etc. Dimer formation (line 2, Picture 1) can also occur for several reasons, such as incorrect primer concentration or poor primer design, as well as inappropriate annealing or denaturation temperature (Asif et al., 2021). The annealing temperature is one of the most important parameters, they suggest that lowering the annealing temperature by 4–6 °C is necessary for the same loci to be co-amplified in multiplex mixtures.

Additionally, the primer concentration should be between 0.1–0.5 mM, MgCl_2 concentration needs to be proportional to the amount of dNTP, etc. Even though the results are fit for purpose for stronger bands, additional optimisation is needed. The development of single-channel capillary electrophoresis has led to several advantages, such as fast separation PCR amplification products. Additionally, it not only replaces the complicated and time-consuming gel preparation but also improves the accuracy of analysis, increases reproducibility, and reduces human error, making it an efficient technique for helping in the multiplex PCR optimization process (Butler et al., 2001). The CGE of duplex PCR analysis showed the expected amplicons of 94 bp at a concentration of 0.5 ng/ μl , and of 133 bp at a concentration of 1.6 ng μl^{-1} (Picture 2). Results again confirm that the duplex PCR sample contains wheat and maize DNA.

Several authors presented their optimisation of multiplex PCR systems for the detection of plant-specific genes in grain mixtures. Kutateladze et al. (2013) in their study developed and optimized a triplex PCR method for the simultaneous identification of maize, wheat, and soybean, which can be used for food analysis. The author highlights the importance of the method for food safety and quality assurance. From farm to fork, different crops are colliding at different levels of processing. In many cases, it is important to avoid mixing for commercial reasons as well as for safety. Indicating the presence of allergens in raw material as in the final product, poses a risk for both consumers and producers, so the interest in providing a fast and accurate detection method is crucial. Köppel et al. (2010) optimised two tetraplex real-time PCR assays for the detection and quantification of DNA from eight allergens in food. Optimisation of a large number of targets in a single tube with no adverse effect on method specificity and sensitivity significantly reduces time and cost and provides a promising approach. In the literature, possible applications of multiplexing employing plant-specific primers are numerous. Koh et. al. (2017) developed a multiplex PCR method for reliably distinguishing six *Brassica* species, which can be implemented in any basic molecular laboratory for the management of *Brassica* germplasm collections. A large number of researchers are developing fast and accurate methods for plant identification, food authentication, as well as food quality and safety monitoring. The method of choice for such applications is widely spread PCR as an effective and accurate technique with high specificity and sensitivity. To provide a cost and time-effective approach, multiplexing is the way to go.

Conclusions

There is a constant need to develop tools for the rapid and efficient detection of plant DNA in mixtures. Such a tool is needed to indicate quality assurance or detect possible allergens in the food chain. The multiplex PCR detection method enables simultaneous amplification of multiple target regions, saves time and reduces reagent consumption and cost, and allows broad applications. In this study, we successfully optimized duplex PCR for the simultaneous detection of wheat and maize DNA in a seed mixture with a limit of detection of 0.1%. The optimized multiplex PCR method can be used not only for detecting small amounts of foreign grains in the seed samples and for determining GM content in food products, but also for food authentication, quality control, and safety monitoring.

References

- Abdel-Latif A., and Osman G. (2017). Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant methods*. 13: 1-9.
- Asif S., Khan M., Arshad M. W., Shabbir M. I. (2021). PCR Optimization for Beginners: A Step by Step Guide. *Research in Molecular Medicine*. 9 (2): 81-102.
- Bassani N., Belter A., Grohmann L., Kreysa, J., De Loose M., Mazzara M., Villa D. (2015). European Network of GMO Laboratories Working Group" Seed Testing"(WG-ST): Working Group Report. In JRC technical reports (p. 44). Joint Research Centre.
- Butler J.M., Ruitberg C. M., Vallone P.M. (2001). Capillary electrophoresis as a tool for optimization of multiplex PCR reactions. *Fresenius J Anal Chem*. 369: 200–205
- Erenstein O., Jaleta M., Sonder K., Mottaleb K., Prasanna B. M. (2022). Global maize production, consumption and trade: trends and R&D implications. *Food Security*. 14: 1295–1319.
- Faris J., Sirikhachornkit A., Haselkorn R., Gill B., Gornicki P. (2001). Chromosome Mapping and Phylogenetic Analysis of the Cytosolic Acetyl-CoA Carboxylase Loci in Wheat. *Molecular Biology and Evolution*. 18 (9): 1720–1733.

- Gagnon M. C., Duceppe M. O., Colville A., Pope L., Cote M. J., Ogunremi D. (2024). An integrated strategy involving high-throughput sequencing to characterize an unknown GM wheat event in Canada. *Plant Biotechnology Journal*. 22: 904–914.
- Gerlach W. L., Pryor A. J., Dennis E. S., Ferl R. J., Sachs M. M., Peacock W. J. (1982): cDNA cloning and induction of the alcohol dehydrogenase gene (*Adhl*) of maize. *Proceedings of the National Academy of Sciences of the United States of America*, 79: 2981-2985.
- Green M.R., and Sambrook J. (2019). *Polymerase Chain Reaction*. Cold Spring Harbor Protocols (6)
- Hernández M., Duplan M. N., Berthier G., Vaitilingom M., Hauser W., Freyer R., Bertheau Y. (2004). Development and Comparison of Four Real-Time Polymerase Chain Reaction Systems for Specific Detection and Quantification of *Zea mays* L. *Journal of Agricultural and Food Chemistry*. 52 (15): 4632–4637.
- Igrejas G., Ikeda T. M., Guzman C. (2020). The Importance of Wheat. *Wheat Quality For Improving Processing And Human Health*. Igrejas G. and Branlard G., 1–7, Fukuyama, Japan: Springer
- ISTA, 2016. *International Rules for Seed Testing*. International Seed Testing Association. Bassersdorf, Switzerland.
- Koh J. C., Barbulescu, D. M., Norton S., Redden B., Salisbury P. A., Kaur S., Slater, A. T. (2017). A multiplex PCR for rapid identification of Brassica species in the triangle of U. *Plant Methods*. 13: 1-8.
- Kutateladze T., Gabriadze I., Vishnepolsky B., Karseladze M., Datukishvili N. (2013). Development of triplex PCR for simultaneous detection of maize, wheat and soybean. *Food Control*. 34 (2): 698–702.
- Lane S. E., Richards R. S., McDonald C., Robinson A. P. (2018). *Sample size calculations for phytosanitary testing of small lots of seed*. Centre of Excellence for Biosecurity Risk Analysis. The University of Melbourne.
- Lorenz, T. C. (2012). *Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies*. *Journal of Visualized Experiments*. 63: 3998.
- Nikolić Z., Milošević M., Vujaković M., Marinković D., Jevtić A., Balešević-Tubić S. (2008). Qualitative Triplex PCR for the Detection of Genetically Modified Soybean and Maize. *Biotechnology & Biotechnological Equipment*. 22 (3): 801-803.
- Paternò A., Marchesi U., Gatto F., Verginelli D., Quarchioni C., Fusco C., Zepparoni A., Amaddeo D., Ciabatti I. (2009). Finding the Joker among the Maize Endogenous Reference Genes for Genetically Modified Organism (GMO) Detection. *Journal of Agricultural and Food Chemistry*. 57 (23): 11086–11091.
- Puente-Lelievre C., and Eischeid A. C. (2021). Development and validation of a duplex real-time PCR assay with locked nucleic acid (LNA) probes for the specific detection of allergenic walnut in complex food matrices. *Food Control*. 121: 107644.
- Rogers S.O., and Bendich A. J. (1989). Extraction of DNA from plant tissues. *Plant molecular biology manual*, 73-83.
- Singh J., Birbian N., Sinha S., Goswami A. (2014). A critical review on PCR, its types and applications. *International Journal of Advanced Research in Biological Sciences* 1 (7): 65–80.
- Yanagi M. (2024). Understanding the ecological significance of wheat: Roles, climate change impacts, and biodiversity conservation strategies. *Geographical Research Bulletin*. 3: 6-9.

ORIGINAL SCIENTIFIC PAPER

Response of spring oat varieties to silicon and sulphur fertilization in heavy drought

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Summary

The aim of this study was to investigate the effects of silicon (Si), sulphur (S) and their combination, applied as foliar fertilizers at different phenophases, on the development and productivity of spring oats under severe drought conditions. Field trials conducted in 2022 in Debrecen, Hungary, revealed that S and Si+S treatments decreased the leaf area index, whereby Si+S treatments showing significantly the lowest values at 98 day after sowing (DAS). In contrast, S and Si+S fertilisation significantly increased yield, particularly in the genotypes Mv Pehely, Mv Szellő and Panni. Silicon fertilization alone proved ineffective. These results suggest that foliar fertilization is an effective strategy to mitigate drought stress and stabilize oat yields under extreme climatic conditions.

Key words: silicon, sulphur, LAI, yield, cereals

Introduction

Oats grains are extensively used as a concentrated and green feed for livestock. Oat flour is also consumed in human diet as a high nutritional food product (Loskutov and Khlestkina, 2021). Development of innovative strategies that improve nitrogen use efficiency (NUE), grain nutritional composition and grain yield for the sustainability of oat-based farming systems is crucial.

Agro-technical measures such as combined application of fertilizers and micronutrients containing fertilizers i.e silicon, have been frequently reported to enhance plant tolerance to various abiotic and biotic stresses, such as drought, salt, freezing, nutrient imbalance and radiation damage (Verma et al., 2021; Wang et al., 2021; Kovács et al., 2022). Improving plant nutrient use efficiency, particularly through the use of sulphur, is a widely accepted strategy for increasing resilience, yield and protein content in cereals (Shah et al., 2022, Barczak et al., 2018), which supports the need to incorporate these essential nutrients into oat cropping systems.

The objectives of the study were to evaluate the effects of drought stress on leaf area index (LAI) and grain yield-related traits of oat varieties and to assess the impact of foliarly applied silicon and sulphur fertilizers on grain yield and abiotic stress resistance in oats.

Material and methods

A field experiment was conducted in 2022 in the experimental garden of University of Debrecen (47°33'02" N; 21°35'56" E). The area has homogeneous, chernozem soil with good humus content (Hu% = 2.7–3.66), 80 cm humus layer and favourable water regime. The Arany yarn number (K_A) of the soil is 45, which classifies it to clayey loam (K_A : 42-50) soil texture class (Szolnoki and Farsang, 2013). Available phosphorus (P_2O_5 (AL) 1020-1538 mg kg⁻¹) and potassium (K_2O (AL) 366-638 mg kg⁻¹) supply is very good in the upper 0-60 cm layers (Stefanovits et al., 1999).

Sowing was carried out on 18 March 2022, using a seeding rate 550 seeds m^{-2} seed, and a sowing depth of 5 cm. The forecrop was winter wheat. The experiment was harvested on 11 July 2022. Fertilizers (NPK) were applied uniformly in the experiment: $\text{N}_5\text{P}_{10}\text{K}_{30}$ kg ha^{-1} during October 2021 and N_{55} kg ha^{-1} 10 March 2022.

Four different nutrient treatments (Control, Silicon, Sulphur, and S+ Si) and eight oat varieties (GK Kormorán, GK Pillangó, Lota, Panni, Mv Kengyel, Mv Ménes, Mv Pehely, Mv Szellő) were arranged in a split plot design with four replication. Nutrient application was assigned to the main plots, and oat varieties to the subplots. The subplot size was (3 m x 4 m = 12 m^2).

Nutrients were applied foliarly at three developmental stages, namely three leaves unfolded (BBCH13), flag leaf stage (BBCH39) and early milk stage (BBCH73). The applied foliar fertilizers were Jello Fluid liquid foliar fertilizer with high content sulphur (lignosul-fonate formulation, 1000g L^{-1} SO_3 , 30g L^{-1} N, 30g L^{-1} MgO, 27g L^{-1} B, 0.003g L^{-1} Mo) and Optysil with high content of Si (200 g SiO_2 L^{-1}).

The treatments were as follows:

1. Control, without foliar fertilization
2. Silicon fertilization (Si) 0.5 L ha^{-1}
3. Sulphur fertilization (S) 5.0 L ha^{-1}
4. Silicon+Sulphur fertilization (Si + S) 0.5 + 5.0 L ha^{-1}

Leaf area index (LAI) was measured using an LAI-2000 Plant Canopy Analyzer (LI-COR, USA) in one sensor mode. Both above-canopy and below-canopy readings were taken using the same LAI-2050 optical sensor.

Grain yield of each plot was harvested using a Wintersteiger 125 plot combine with 125-cm cutting width. Grain samples were collected from each plot to determine the grain moisture content and 1000 kernels weight. To classify drought, the Palfai Drought Index was calculated according to the method of Palfai and Herceg (2012). Genstat 18th edition software was used to analyse the collected data and the mean of leaf area index and yield were compared and evaluated using the least significance difference at 5% significance level.

Results and discussion

Characteristics of the experimental year

The 2021/2022 growing season in Debrecen exhibited significant deviations from the 30-year climatic average in both temperature and precipitation (Table 1). The spring-summer season was characterized by above-average temperatures, with a particularly pronounced heat anomaly in June (+3.3 °C). Precipitation was substantially below average and irregularly distributed, with long dry periods. In June only scattered rainfall events occurred, resulting in a 53.6 mm deficit compared to the multi-year average, followed by continued drought conditions until late July.

The Palfai Drought Index (PaDI) which quantifies drought severity for agricultural purposes and is particularly suited to the Southeast Europe region (Palfai and Herceg, 2012) was calculated with a PaDI value of 11.6 °C 100 mm^{-1} . According to Palfai's classification (10–15) this indicates a severe drought. These conditions coincided with the critical flowering and grain-filling stages of oat development, likely imposing significant stress on crop growth and yield formation. After a few mm of rainfall in early June, a complete absence of precipitation persisted until harvest, leading to premature maturation of spring oats and a substantially reduced average yield. Such environmental conditions are known to reduce photosynthetic activity, accelerate senescence and diminish yield potential (Fahad et al., 2017).

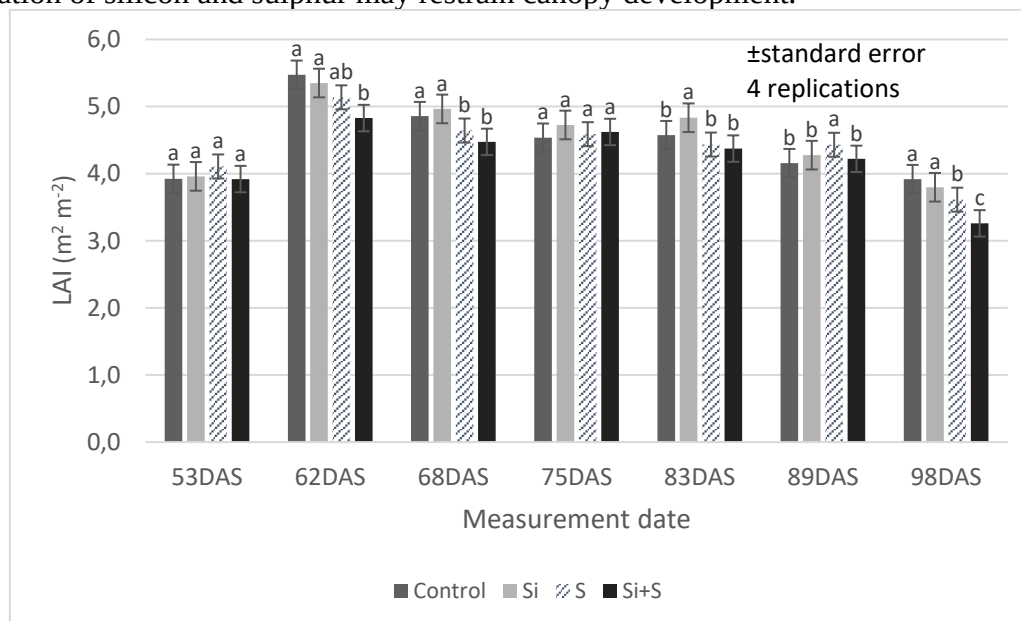
Table 1. Monthly temperature and precipitation (Debrecen, 2021/2022)

Year	Month	Average T_{month} °C			$\sum P_{\text{month}}$ mm		
		Monthly	30Y	Deviation	Monthly	30Y	Deviation
		average	average		average	average	
2021	Oct	9.5	10.4	-0.9	11.5	41.0	-29.5
	Nov	5.3	5.2	0.1	47.0	39.7	7.3
	Dec	1.3	0.1	1.2	34.5	43.2	-8.7
2022	Jan	-0.5	-1.0	0.5	1.5	29.2	-27.7
	Feb	4.1	0.7	3.4	17.5	35.2	-17.7
	Mar	5.2	5.6	-0.4	3.5	30.5	-27.0
	Apr	9.7	11.7	-2.0	55.4	44.0	11.4
	May	17.9	16.4	1.6	43.0	54.3	-11.3
	Jun	23.1	19.9	3.3	11.0	64.6	-53.6
	Jul	23.9	21.3	2.6	41.0	68.7	-27.7
	Aug	23.8	21.1	2.7	10.5	49.7	-39.2
	Sep	15.8	15.9	-0.1	161.0	48.5	112.5

$\sum P_{\text{month}}$: Monthly sum of precipitation; Average T_{month} : Monthly average temperature; 30Y average: 30 year (1991-2020) average temperature or precipitation in Debrecen; deviation: Deviation from the 30 year average temperature or precipitation.

Effect of silicon and sulphur foliar nutrition on the leaf area index of spring oats

As shown in Graph 1, foliar application of silicon (Si) had no significant effect on the leaf area index compared to untreated control plots. In contrast sulphur (S), and the combined with silicon (Si+S) significantly reduced LAI relative to the control at 98 day after sowing (DAS). The most pronounced reduction was observed in the Si+S treatment. These findings are consistent with our previous study conducted in 2021 (Kutasy et al., 2022), where the highest LAI values in winter oats were recorded in the untreated control, while the lowest values were observed in Si+S-treated plots. This suggests that under drought stress, combined foliar application of silicon and sulphur may restrain canopy development.



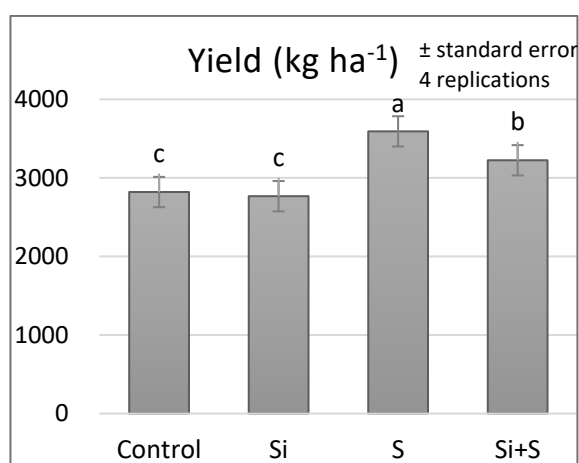
Graph 1. Effect of silicon and sulphur nutrition on the leaf area index of spring oat (average of genotypes)

Different letters mean statistically different values between treatments at the same measurement date ($p = 5\%$) DAS: day after sowing

Effect of silicon and sulphur foliar nutrition on the yield of spring oats

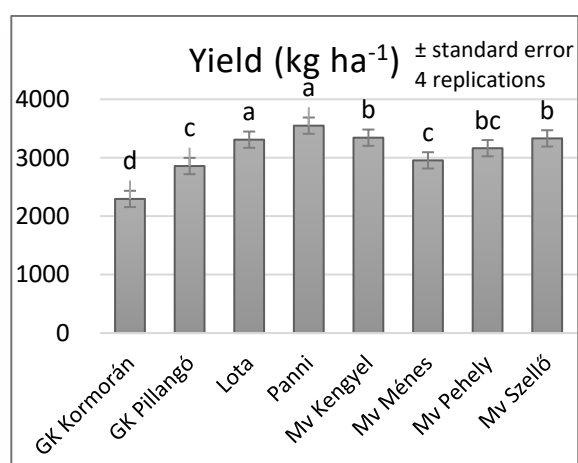
The effect of treatments on grain yield was inversely related to their impact on leaf area index (Graph 2). Sulphur applied individually resulted in the highest average yield, followed by the Si+S treatment. The control and Si treatments produced statistically similar yields. These results suggest a synergistic effect of combined silicon and sulphur application under water-limited conditions. Sulphur likely contributed directly to metabolic processes such as enzyme activation and nitrogen assimilation (Kopriva et al., 2019), while silicon may have alleviated drought-induced oxidative stress and enhanced water-use efficiency (Hattori et al., 2005).

Genotypic variation in yield performance was evident (Graph 3). The varieties Panni, Lota, Mv Kengyel and Mv Szellő exhibited significantly higher average grain yields compared to GK Kormorán, which had the lowest yield. These differences suggest that genetic background plays an important role in yield potential under the given environmental conditions. Further research would be needed to assess genotype-specific responses to individual foliar treatments. This observation aligns with previous findings on genotype $\times$ environment interactions in cereals (Sadras and Richards, 2014).



Graph 2. Effect of silicon and sulphur nutrition on the yield of spring oat (average of genotypes)

Different letters mean statistically different values between treatments ($p = 5\%$)



Graph 3. Effect of silicon and sulphur nutrition on the yield of spring oat (average of treatments)

Different letters mean statistically different values between treatments ($p = 5\%$)

Conclusions

The foliar application of sulphur and its combination with silicon restricted vegetative growth, resulting in reduced leaf area, but was associated with improved grain yield under drought conditions. This suggests that targeted foliar nutrient application may contribute to mitigating drought-induced stress in spring oats. Observed genotypic differences emphasize the importance of variety selection to optimize the effectiveness of such treatments. These findings support the potential integration of foliar nutrient strategies into oat production systems facing increasing climate variability. However, as these results are based on a single-year experiment, further validation across multiple seasons and a broader range of genotypes is essential.

Acknowledgement

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References

- Barczak B., Klikocka H., Kozera W., Knapowski T. (2018). Assessment of the effect of sulphur fertilisation on oat grain yield and micronutrient uptake. *J. Elem.*, 23(1): 45 - 56. DOI: 10.5601/jelem.2017.22.1.1318
- Fahad S., Bajwa A.A., Nazir U. et al. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science*, 8, 1147. <https://doi.org/10.3389/fpls.2017.01147>
- Hattori T., Inanaga S., Araki H. et al. (2005). Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *Physiologia Plantarum*, 123, 459–466. <https://doi.org/10.1111/j.1399-3054.2005.00481.x>
- Kopriva S., Malagoli M., Takahashi H. (2019). Sulfur nutrition: impacts on plant development, metabolism, and stress responses. *Journal of Experimental Botany*, 70, 4069–4073. <https://doi.org/10.1093/jxb/erz253>
- Kovács S., Kutasy E., Csajbók J. (2022). The Multiple Role of Silicon Nutrition in Alleviating Environmental Stresses in Sustainable Crop Production. *Plants*, 11(9), 1223. <https://doi.org/10.3390/plants11091223>
- Kutasy E., Buday-Bódi E., Virág I. C., Forgács F., Melash A. A., Zsombik L., Nagy A., Csajbók J. (2022). Mitigating the Negative Effect of Drought Stress in Oat (*Avena sativa* L.) with Silicon and Sulphur Foliar Fertilization. *Plants*, 11(1), 30. <https://doi.org/10.3390/plants11010030>
- Loskutov I.G., Khlestkina E.K. (2021). Wheat, Barley, and Oat Breeding for Health Benefit Components in Grain. *Plants*, vol. 10, no. 1, p. 86, Jan. 2021, doi: 10.3390/plants10010086.
- Palfai, I., Herceg, A. (2012) Palfai Drought Index (PaDI) – Expansion of applicability of Hungarian PAI for Southeast Europe (SEE) region Lower-Tisza District Water Directorate, Szeged – Hungary, Drought Management Centre for South-East Europe (DMCSEE)– Summary of the result of the project. Ljubljana, Slovenia: Slovenian Environmental Agency
- Sadras V.O., Richards R.A. (2014). Improvement of crop yield in dry environments: benchmarks, levels of organisation and the role of nitrogen. *Field Crops Research*, 168, 61–67. <https://doi.org/10.1016/j.fcr.2014.01.004>
- Shah S.H., Islam S., Mohammad F. (2022) Sulphur as a dynamic mineral element for plants: a review. *J Soil Sci Plant Nutr* 22, 2118–2143. <https://doi.org/10.1007/s42729-022-00798-9>
- Stefanovits P., Filep Gy., Fülek Gy. (1999). Talajtan. Budapest: Mezőgazda Kiadó. In Hungarian ISBN: 963-286-178-7
- Szolnoki Z., Farsang A. (2013). Evaluation of Metal Mobility and Bioaccessibility in Soils of Urban Vegetable Gardens Using Sequential Extraction. *Water Air Soil Pollut* 224, 1737 (2013). <https://doi.org/10.1007/s11270-013-1737-4>
- Verma K.K., Song X.-P., Tian D.-D., Guo D.-J., Chen Z.-L., Zhong C.-S., Nikpay A., Singh M., Rajput V.D., Singh R.K., et al. (2021). Influence of Silicon on Biocontrol Strategies to Manage Biotic Stress for Crop Protection, Performance, and Improvement. *Plants*, 10, 2163. <https://doi.org/10.3390/plants10102163>
- Wang M., Wang R., Mur L.A.J., Ruan J., Shen Q., Guo S. (2021). Functions of silicon in plant drought stress responses. *Horticulture Research*, Volume 8, 2021, 254, <https://doi.org/10.1038/s41438-021-00681-1>

ORIGINAL SCIENTIFIC PAPER

Application of UAV imagery to improve interpretability of soybean breeding experiments on heat stress

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Summary

In this study, unmanned aerial vehicle (UAV)-based multispectral imaging was performed on 40 soybean genotypes in an experimental field in a randomized block design with three replicates. The vegetation index NDVI was derived and sampled from images and statistically compared between soybean genotypes using ANOVA post-hoc test Bonferroni test ($p_{\text{corrected}}=0.00125$). The results indicate grouping of genotypes with higher and lower vegetation indices, which may be closely related to high air temperatures during the imaging and tolerance to the first stages of heat stress. This research has demonstrated the potential of using UAV and the ability to detect different genotypes of soybeans based on differences in NDVI values related to heat stress, which can be used in the future as a complement to plant breeding and selection.

Key words: soybean, NDVI, plant breeding experiment, UAV, imagery

Introduction

Modern agriculture has significantly advanced through plant breeding, which one of the missions is to modify a plant's genetics and achieve desired traits. Main efforts are put to answer challenges in climate changing world, ensuring food security and sustainability through enhancement of crop yields, disease resistance and improvement of nutritional value. Field trials play a central role in that process. Conducting such experiments with extensive and variable germplasm collections demand substantial resources and expertise to analyse and interpret the complexities of plant-environment interactions.

In recent years the rapid development of unmanned aerial systems (UAS) found applications in crop monitoring (Hafeez et al., 2023, Radoglou-Grammatikis et al., 2020, Gokool et al., 2023) introducing innovations in plant characterization. There are numerous examples in the literature reporting UAS phenotyping and monitoring based on spectral and textural properties of canopy to detect key agronomic traits related to yield, stress tolerance and growth patterns using sensors such as red-green-blue (RGB) cameras, multi- and hyperspectral cameras, Light Detection and Ranging (LiDAR), and thermal sensors (Xie and Yang, 2020).

Being aware of the possibilities of using unmanned aerial vehicles and analysing aerial photographs, we wanted to see if we could increase the interpretability of soybean plant breeding experiments. The hypothesis that was put forward is that it is possible to detect the properties of different genotypes of soybeans at the beginning of summer using aerial photographs to detect heat stress tolerance.

Material and methods

This research was conducted on Maksimir experimental field of the University of Zagreb Faculty of Agriculture, (N 45° 49' 41", E 16° 01' 59") during vegetation season 2024. Three soil types were recorded in the immediate vicinity of the research area: eutric cambisol, humofluvisol and pseudogley (Rubinić et al., 2015). An experimental field (45m×20m) with 40 soybean genotypes (Table 1) was established on May 1st 2024 in a randomized block design with three replicates.

Table 1. List of 40 soybean genotypes as experiment members. Superscript letters indicate maturity group (MG): ^a – MG 0, ^b – MG 0-1, ^c – MG 1

Nr.	Genotype	Nr.	Genotype	Nr.	Genotype	Nr.	Genotype
1	ZA 4-2 ^b	11	ASD 63 ^b	21	BM 49 ^b	31	Maksimirka - Control ^b
2	RB 31-6 ^a	12	ASD 70 ^b	22	BM 76 ^b	32	Ascasubi ^b
3	ZT 67-1 ^a	13	BM 10 ^b	23	BM 93 ^b	33	Bahia ^b
4	MB 58-2 ^a	14	BM 102 ^b	24	BM-110 ^b	34	ES-Advisor ^a
5	BM29-1-3 ^b	15	BM 104 ^b	25	BM-16 ^b	35	ES-Tribor ^a
6	BM67-2-1 ^a	16	BM 105 ^b	26	BM-27 ^b	36	ES-Pallador - Control ^c
7	ASD 31 ^b	17	BM 20 ^b	27	BM-28 ^b	37	ES-Conqueror ^c
8	ASD 36 ^b	18	BM 25 ^b	28	BM-31 ^b	38	KWS-Carlotta ^a
9	ASD 48 ^b	19	BM 34 ^b	29	BM-54 ^b	39	KWS-Annika ^c
10	ASD 61 ^b	20	BM 44 ^b	30	Pedro - Control ^b	40	Zagorka ^b

On the first day of summer June 21st, 2024 (8th week from sowing) the field was photographed with a DJI P4 MS drone equipped with a 5-channel multispectral camera. The vegetation index NDVI was calculated from the images and a spatial analysis was performed in which the average NDVI value at 10 randomly selected points and the standard deviation were sampled for each genotype. The average values were analysed using the ANOVA post hoc Bonferroni test to identify the differences between genotypes.

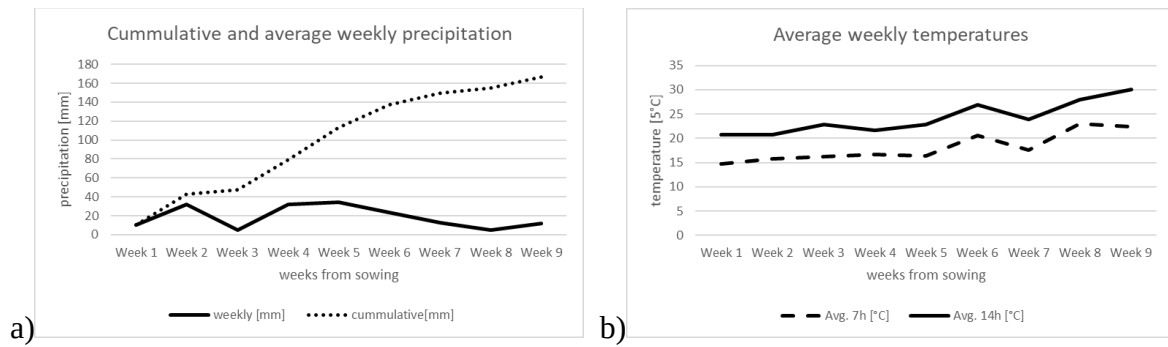
For the purposes of interpretation, we used daily meteorological data of air temperature, humidity and rainfall measured at the nearby official Weather station Zagreb-Maksimir provided by the Croatian Meteorological and Hydrological Service (DHMZ, 2024).

Results and discussion

Data on daily air temperatures and precipitation obtained from the Croatian Meteorological and Hydrological Service showed that field received around 160 mm of rain since sowing and also indicating that at the time of recording (00:45 pm) it was hot day (23.8 °C at 7:00 am and 33.1 °C at 02:00 pm) in a series of warm days (Table 2, Graph 1). Relative air humidity on June 21st, 2024 was measured at 7:00 am and 02:00 pm showing 86% and 44%, respectively. Although the mentioned temperatures are around and above the optimum of 32 °C (Poudel et al., 2023), it is possible that the temperature and humidity were significantly higher in the field itself next to the ground. All these values indicate that the plants on that day were exposed to conditions around the threshold of heat stress, that is expected to be detected by remote sensing techniques.

Table 2. Daily air temperatures (* indicates the recording date)

Date	Air temperature [°C]	
dd/mm/yyyy	07:00 am	02:00 pm
15/06/2024	17.3	25.9
16/06/2024	18.9	26.4
17/06/2024	20.5	27.7
18/06/2024	22.1	29.4
19/06/2024	23.5	32.8
20/06/2024	25.9	28
21/06/2024*	23.8	33.1
22/06/2024	26	26.4
23/06/2024	22.1	26.8



Graph 1. a) Cumulative and average weekly precipitation b) Average weekly temperatures

In total, 1200 NDVI data points, 10 per parcel, were used in statistical analysis. In general, it is noticeable that the values are in most cases without significant difference, but there are also genotypes that significantly differ as high or low (Figure 1). Thus, genotype 8 (ASD 36) with the lowest NDVI (0.54) was significantly lower than 11 genotypes. It is followed by genotype 21 (BM 49) with NDVI=0.57, which was significantly lower than six genotypes, indicating potential heat stress in plants. In contrast, genotype 3(ZT 67-1) had the highest NDVI (0.74), significantly differing from four genotypes and indicating more vigorous plants at high temperatures.

	GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9	GT10	GT11	GT12	GT13	GT14	GT15	GT16	GT17	GT18	GT19	GT20	GT21	GT22	GT23	GT24	GT25	GT26	GT27	GT28	GT29	GT30	GT31	GT32	GT33	GT34	GT35	GT36	GT37	GT38	GT39	GT40
GT1	1	0.033	0.003	0.407	0.673	0.809	0.526	0.087	0.048	0.282	0.423	0.294	0.626	0.369	0.451	0.457	0.332	0.632	0.395	0.419	0.28	0.079	0.779	0.25	0.312	0.448	0.053	0.114	0.148	0.046	0.619	0.532	0.154	0.092	0.474	0.27	0.124	0.202	0.029	0.064
GT2	0.033	1	0.416	0.177	0.005	0.033	0.092	1E-04	0.879	0.245	0.172	0.275	0.129	0.249	0.127	0.191	0.2	0.129	0.159	0.213	5E-04	0.594	0.074	0.369	0.161	0.004	0.595	0.592	0.471	0.812	0.114	0.07	0.447	0.608	0.118	0.268	0.479	0.397	0.969	0.705
GT3	0.003	0.416	1	0.028	1E-04	0.002	0.009	2E-06	0.336	0.042	0.027	0.055	0.023	0.053	0.015	0.037	0.03	0.023	0.022	0.043	8E-06	0.152	0.01	0.094	0.018	2E-04	0.125	0.179	0.12	0.269	0.018	0.005	0.108	0.173	0.014	0.049	0.114	0.097	0.438	0.213
GT4	0.407	0.177	0.028	1	0.172	0.501	0.802	0.01	0.235	0.817	0.981	0.811	0.776	0.906	0.91	0.968	0.906	0.772	0.995	0.981	0.043	0.363	0.605	0.707	0.918	0.11	0.301	0.43	0.525	0.24	0.764	0.762	0.543	0.385	0.88	0.788	0.484	0.634	0.164	0.303
GT5	0.673	0.005	1E-04	0.172	1	0.448	0.233	0.13	0.008	0.095	0.183	0.109	0.353	0.161	0.188	0.219	0.121	0.359	0.156	0.194	0.439	0.013	0.474	0.093	0.096	0.666	0.005	0.028	0.039	0.006	0.336	0.222	0.041	0.019	0.204	0.091	0.028	0.064	0.004	0.01
GT6	0.809	0.033	0.002	0.501	0.448	1	0.654	0.032	0.051	0.343	0.52	0.357	0.759	0.45	0.558	0.558	0.407	0.765	0.487	0.513	0.134	0.086	0.94	0.301	0.382	0.281	0.051	0.131	0.173	0.046	0.754	0.667	0.18	0.103	0.587	0.328	0.142	0.241	0.029	0.068
GT7	0.526	0.092	0.009	0.802	0.233	0.654	1	0.013	0.131	0.613	0.823	0.617	0.943	0.719	0.887	0.848	0.7	0.938	0.799	0.795	0.057	0.214	0.756	0.528	0.694	0.147	0.155	0.279	0.354	0.128	0.937	0.965	0.368	0.257	0.919	0.588	0.313	0.453	0.083	0.173
GT8	0.087	1E-04	2E-06	0.01	0.13	0.032	0.013	1	2E-04	0.004	0.011	0.006	0.036	0.011	0.01	0.017	0.008	0.008	0.004	0.014	2E-04	0.053	0.005	0.005	0.344	9E-05	0.001	0.001	1E-04	0.031	0.01	0.001	0.004	0.011	0.004	9E-04	0.003	1E-04	3E-04	
GT9	0.048	0.879	0.336	0.235	0.008	0.051	0.131	2E-04	1	0.32	0.228	0.35	0.17	0.316	0.175	0.247	0.265	0.171	0.216	0.274	0.001	0.715	0.103	0.454	0.224	0.006	0.728	0.702	0.574	0.94	0.154	0.104	0.549	0.724	0.164	0.346	0.588	0.489	0.848	0.829
GT10	0.282	0.245	0.042	0.817	0.095	0.343	0.613	0.004	0.32	1	0.799	0.986	0.615	0.923	0.719	0.796	0.906	0.612	0.805	0.847	0.019	0.488	0.454	0.867	0.88	0.063	0.418	0.558	0.673	0.33	0.598	0.566	0.695	0.51	0.96	0.97	0.63	0.792	0.228	0.411
GT11	0.423	0.172	0.027	0.981	0.183	0.52	0.823	0.011	0.228	0.799	1	0.794	0.794	0.889	0.93	0.986	0.888	0.79	0.985	0.963	0.047	0.353	0.623	0.692	0.897	0.117	0.291	0.419	0.512	0.233	0.783	0.783	0.53	0.375	0.9	0.771	0.471	0.619	0.159	0.295
GT12	0.294	0.275	0.055	0.811	0.109	0.357	0.617	0.006	0.35	0.986	0.794	1	0.616	0.913	0.718	0.791	0.986	0.614	0.8	0.84	0.025	0.522	0.623	0.685	0.871	0.071	0.458	0.586	0.699	0.364	0.601	0.573	0.721	0.541	0.69	0.983	0.659	0.814	0.257	0.444
GT13	0.626	0.129	0.023	0.776	0.353	0.759	0.943	0.036	0.17	0.615	0.794	0.616	1	0.704	0.848	0.815	0.689	0.995	0.774	0.77	0.125	0.261	0.835	0.536	0.687	0.231	0.214	0.314	0.386	0.173	0.996	0.97	0.399	0.279	0.876	0.593	0.352	0.472	0.119	0.219
GT14	0.369	0.249	0.053	0.906	0.161	0.45	0.719	0.011	0.316	0.923	0.889	0.913	0.704	1	0.819	0.881	0.991	0.701	0.899	0.929	0.044	0.468	0.547	0.807	0.974	0.104	0.41	0.529	0.632	0.328	0.691	0.68	0.651	0.487	0.791	0.894	0.593	0.738	0.234	0.4
GT15	0.451	0.127	0.015	0.91	0.188	0.558	0.887	0.01	0.175	0.719	0.93	0.718	0.848	0.819	1	0.949	0.81	0.844	0.911	0.895	0.044	0.281	0.666	0.619	0.813	0.12	0.218	0.349	0.436	0.175	0.838	0.847	0.453	0.305	0.968	0.692	0.393	0.545	0.116	0.23
GT16	0.457	0.191	0.037	0.968	0.219	0.558	0.848	0.017	0.247	0.796	0.986	0.791	0.815	0.881	0.949	1	0.879	0.812	0.971	0.951	0.065	0.373	0.651	0.694	0.889	0.141	0.317	0.433	0.524	0.254	0.806	0.812	0.541	0.392	0.92	0.769	0.486	0.625	0.178	0.315
GT17	0.322	0.2	0.03	0.906	0.121	0.407	0.7	0.006	0.265	0.906	0.889	0.896	0.689	0.991	0.81	0.879	1	0.686	0.898	0.932	0.026	0.412	0.52	0.702	0.981	0.078	0.343	0.483	0.59	0.277	0.674	0.654	0.61	0.435	0.779	0.874	0.546	0.706	0.185	0.344
GT18	0.632	0.129	0.023	0.772	0.359	0.765	0.938	0.038	0.171	0.612	0.79	0.614	0.995	0.701	0.844	0.812	0.686	1	0.77	0.766	0.129	0.261	0.84	0.534	0.684	0.235	0.214	0.314	0.385	0.173	0.999	0.965	0.398	0.279	0.871	0.591	0.353	0.471	0.12	0.219
GT19	0.395	0.159	0.022	0.995	0.156	0.487	0.799	0.008	0.216	0.805	0.985	0.8	0.774	0.899	0.911	0.971	0.898	0.77	1	0.976	0.036	0.34	0.598	0.694	0.909	0.1	0.274	0.41	0.507	0.219	0.761	0.756	0.525	0.364	0.88	0.766	0.643	0.618	0.147	0.282
GT20	0.419	0.213	0.043	0.981	0.194	0.513	0.795	0.014	0.274	0.847	0.963	0.84	0.77	0.929	0.895	0.951	0.932	0.766	0.976	1	0.566	0.41	0.608	0.739	0.944	0.125	0.353	0.471	0.566	0.282	0.759	0.758	0.584	0.023	0.049	0.019	0.004	0.013	5E-04	0.001
GT21	0.28	5E-04	9E-06	0.043	0.439	0.134	0.057	0.412	0.001	0.019	0.047	0.025	0.125	0.044	0.044	0.065	0.026	0.129	0.036	0.056	1	0.002	0.179	0.022	0.017	0.812	4E-04	0.005	0.007	7E-04	0.112	0.048	0.007	0.003	0.049	0.019	0.004	0.013	5E-04	0.001
GT22	0.079	0.594	0.152	0.363	0.013	0.086	0.214	3E-04	0.715	0.488	0.353	0.522	0.261	0.468	0.281	0.373	0.412	0.261	0.34	0.41	0.002	1	0.163	0.649	0.361	0.011	0.957	0.959	0.813	0.76	0.241	0.174	0.784	0.997	0.264	0.521	0.84	0.701	0.564	0.879
GT23	0.779	0.074	0.01	0.605	0.474	0.94	0.756	0.053	0.103	0.454	0.623	0.462	0.835	0.547	0.666	0.651	0.52	0.84	0.598	0.608	0.179	0.163	1	0.397	0.508	0.311	0.124	0.21	0.264	0.102	0.834	0.775	0.214	0.18	0.693	0.437	0.234	0.338	0.068	0.139
GT24	0.25	0.369	0.094	0.707	0.093	0.301	0.528	0.005	0.454	0.867	0.692	0.885	0.536	0.807	0.619	0.694	0.782	0.534	0.694	0.739	0.022	0.649	0.397	1	0.753	0.061	0.593	0.706	0.824	0.476	0.52	0.486	0.847	0.664	0.594	0.898	0.788	0.935	0.349	0.563
GT25	0.312	0.161	0.018	0.918	0.096	0.382	0.694	0.003	0.224	0.8	0.897	0.871	0.687	0.974	0.813	0.889	0.981	0.684	0.959	0.944	0.017	0.361	0.508	0.751	1	0.064	0.283	0.44	0.548	0.225	0.671	0.643	0.568	0.388	0.781	0.847	0.499	0.671	0.148	0.295
GT26	0.448	0.004	2E-04	0.11	0.666	0.281	0.147	0.344	0.006	0.063	0.117	0.071	0.231	0.104	0.12	0.141	0.078	0.235	0.1	0.125	0.812	0.011	0.311	0.063	0.064	1	0.005	0.027	0.095	0.217	0.139	0.028	0.014	0.129	0.06	0.02	0.043	0.003	0.008	
GT27	0.053	0.595	0.125	0.301	0.005	0.051	0.156	9E-05	0.728	0.418	0.291	0.458	0.214	0.41	0.218	0.317	0.343	0.214	0.274	0.353	4E-04	0.957	0.124	0.593	0.283	0.005	1	0.917	0.759	0.776	0.192	0.117	0.727	0.956	0.203	0.454	0.784	0.562	0.909	
GT28	0.114	0.592	0.179	0.43	0.028	0.131	0.279	0.001	0.702	0.558	0.419	0.586	0.314	0.529	0.349	0.433	0.483	0.314	0.41	0.471	0.005	0.959	0.21	0.706	0.44	0.02	0.917	1	0.866	0.743	0.296	0.241	0.839	0.964	0.331	0.589	0.893	0.759	0.565	0.851
GT29	0.148	0.471	0.12	0.525	0.099	0.173	0.354	0.001	0.574	0.673	0.512	0.699	0.386	0.632	0.646	0.524	0.59	0.385	0.507	0.566	0.007	0.813	0.264	0.824	0.548	0.001	0.927	0.759	0.861	1	0.605	0.367	0.311	0.974	0.824	0.415	0.706	0.99		

to later ones. However, this expectation was not supported by the results. Additional factors potentially contributing to the manifestation of heat stress included exceptionally warm nocturnal conditions, high morning humidity, and light winds below 2 m/s. Furthermore, soil uniformity was not assessed, suggesting the possibility of multiple soil types across the furrowed experimental plot. This heterogeneity could influence soil water capacity and water-air relations, thereby impacting the performance of the genotypes to maintain stomatal conductance and consequently canopy temperature and finally deceleration of crucial enzymatic functions and overall metabolic processes (Sofi et al., 2019). However, the significant differences in this case study show perspective for further investigations. This approach opens new possibilities in soybean selection and plant breeding by conducting a series of recordings at key stages of development in future research and strongly confirming the relationship of a specific trait with the recorded data, which enables precise intervention in time and space and, as a result, more efficient research.

Conclusions

This research has demonstrated the potential of using UAS and the ability to detect different genotypes of soybeans based on differences in NDVI values related to heat stress, which can be used in the future as a complement to plant breeding and selection.

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References

- DHMZ (2024). Croatian Meteorological and Hydrological Service. Source: https://meteo.hr/proizvodi.php?section=podaci&param=xml_korisnici, [Accessed: 01.05.2024 - 01.07.2024].
- Hafeez A., Husain M. A., Singh S. P., Chauhan A., Khan M. T., Kumar N., Chauhan A., Soni S. K. (2023). Implementation of drone technology for farm monitoring & pesticide spraying: A review. *Information Processing in Agriculture*. 10 (2): 192-203.
- Gokool S., Mahomed M., Kunz R., Clulow A., Sibanda M., Naiken V., Chetty K., Mabhaudhi T. (2023). Crop Monitoring in Smallholder Farms Using Unmanned Aerial Vehicles to Facilitate Precision Agriculture Practices: A Scoping Review and Bibliometric Analysis. *Sustainability*. 15 (4): 3557.
- Poudel S., Adhikari B., Dhillon J., Reddy K. R., Stetina S. R., Bheemanahalli R. (2023). Quantifying the physiological, yield, and quality plasticity of Southern USA soybeans under heat stress. *Plant Stress*. 9: 100195.
- Radoglou-Grammatikis P., Sarigiannidis P., Lagkas T., Moscholios I. (2020). A compilation of UAV applications for precision agriculture. *Computer Networks*. 172: 107148.
- Rubinić V., Pejić M., Vukoje I., Bensa A. (2015) Influence of Geomorphology and Land Use on Soil Formation – Case Study Maksimir (Zagreb, Croatia). *Agriculturae Conspectus Scientificus*. 80 (1): 1-8.
- Sofi P.A., Ara A., Gull M., Rehman K. (2019) Canopy temperature depression as an effective physiological trait for drought screening. *Drought—Detection and Solutions*, IntechOpen, 2019.
- Xie C., Yang C. (2020) A review on plant high-throughput phenotyping traits using UAV-based sensors. *Computers and Electronics in Agriculture*. 178: 105731.

ORIGINAL SCIENTIFIC PAPER

Influence of different olive (*Olea europaea* L. cv. Coratina) irrigation practices on fruit morphological characteristics in two consecutive years at the Žman locality

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Summary

The aim of the paper is to determine how different practices affect the morphological parameters of the olive fruit at harvest in the circumstances of different weather conditions of the growing season. Morphological measurements of the fruit were performed according to IOC. The field experiment was conducted on 24 olive trees of the variety 'Coratina' in 2020 and 2021 at the location Žman (Dugi otok, Zadar County). A two-way ANOVA (x-year treatments) was used for statistical data processing, and differences between mean values on morphological parameters of fruits were determined by the Tukey test. From the obtained results, irrigated treatments resulted with greater pulp mass and content. The year significantly influenced pulp mass. Acceptable loss in pulp content can be compensated by better water management.

Key words: irrigation rate, morphometrics, pulp mass and content, water management

Introduction

The olive tree (*Olea europaea* L.) is a typical evergreen fruit tree that has an important socioeconomic and cultural impact in the Mediterranean (Liphschitz et al., 1991; Loumou et al., 2003). According to National Bureau of Statistics (2020), olive trees in Croatia occupied about 20,282 ha of area, and 33,230 tons of fruit were harvested, from which 40,278 hl of olive oil were produced. Although olive tree is a xerophytic species, for regular fruiting it is necessary to ensure irrigation (Therios, 2009; Rallo et al., 2018). Lack of water in the soil during certain stages of olive development, such as flowering, fruit setting and oil accumulation, especially in shallow water and heterogeneous terrain, can affect yield decline (Cimato and Žužić, 1993; Bogunović et al., 2009). However, some studies have determined that there is no significant difference in yield and vegetative development between treatments that provided 100% of the water lost through evapotranspiration and those that added 66% (Tognetti et al., 2006; Fernandes-Silva et al., 2010; Valenčić et al., 2018;). Fernandes-Silva et al. (2010) found that irrigation increased fruit yield due to a higher number of fruits per tree and a higher mass per fruit at three irrigation levels. Following the above, the aim of the study is to determine the impact of different irrigation practises in two consecutive years on the morphological characteristics of the fruit and stone of the olive cultivar 'Coratina' on Dugi otok (Žman).

Materials and methods

The field experiment was conducted in a private olive grove at the location Žman (43.961932 N, 15.122649 E) on Dugi otok in Zadar County (Croatia) in two consecutive growing seasons,

2020 and 2021. The olive grove covers an area of 5 ha and includes 1,100 trees. It is located on a karst relief created by the corrosive action of water. The soil in the olive grove is defined as typical karst soil on limestone, whose formation has been under strong anthropogenic influence (Husnjak et al., 2019). The surrounding vegetation is dominated by pine and holm oak forests, macchia and garrigue, as well as a large number of olive groves and vineyards (Džaja, 2003). The climate of the Dugi otok area is Eu-Mediterranean, characterized by mild rainy winters and dry hot summers, which is favorable for olive trees (Šegota Filipčić et al., 2003; Perica, 2006). At the meteorological station within the olive grove on Dugi Otok (Figure 1.), total precipitation amounted to 804 mm in 2020 and 784.4 mm in 2021.

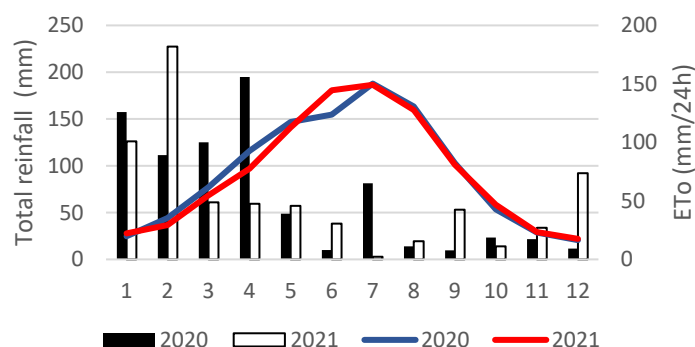


Figure 1. Monthly rainfall and monthly reference evapotranspiration in 2020 and 2021 from Žman, Zadar County. ETo, evapotranspiration (data collected from the meteorological station)

The field study was set on 24 olive trees of the 'Coratina' variety. The variety originated from southern Italy. The trees are of medium vigor, with thin and upright growth. The fruits are uniform and symmetrical, with a size of 23.9 mm, a width of 15.18 mm and a weight of 4.7 g (Barranco et al., 2000). Each experimental plot contained two trees of the same treatment in three replicates. Irrigation was done with a drip irrigation system. Perennial irrigation pipes (PE 16 mm (3/4")) with 30 self-compensating drippers were placed spirally around each tree and covered the width of the crown. The study was carried out in both consecutive years, respectively, from April 10th to August 18th in 2020, and from May 25th to September 13th in 2021, respectively. The treatments represented different quantity of water amount (WA) in irrigation events (IM) used per olive tree by tags: C (0%) - olive trees with rain feed (WA 0 L, IM: 0), T₁ (PP) - production practice, WA and IM determined by producers experience (in 2020 - WA: 1.393 L, IM: 11, and in 2021, WA: 7.62L, IM: 4), T₂ (SAN) - WA and IM determined based on evapotranspiration and the phenophase of olive development, with up to 80% field capacity (in 2020, WA: 1.261L, IM: 19 and in 2021, WA: 6.761L, IM: 10), T₃ (100%) - WA and IM determined based on 100% of the calculated evapotranspiration (in 2020, WM: 1.800 L, IM: 19 and in 2021, WA: 1.000L, IM: 10) by the tree. The irrigation requirement was calculated using the equation ((d'Andria et al., 2007; 1).

$$(1) \text{ IR} = \text{ETc} - \text{Ep} - \text{R}$$

(IR = irrigation requirement, ETc = olive evapotranspiration (crop water requirement), Ep = effective precipitation (all precipitation above 10 mm), R = field capacity (Serafini et al., 2007)). Olive evapotranspiration was calculated according to equation (d'Andria et al., 2007; 2).

$$(2) \text{ ETc} = \text{ETo} \times \text{Kc}$$

(Eto = evapotranspiration (PinovaTM), Kc = correlation factor for olives). Evapotranspiration was determined using the data from the PinovaTM weather station in the grove. The maximum water capacity and the start of irrigation was determined when the calculations confirmed the value of the management allowable depletion. The amount of water was indicated by flow meter (Conrad Electronic SE; 552059 digital water meter). The statistical analysis of the

morphological characteristics of the fruit (treatments and years) was performed using a two-way ANOVA. The difference between the mean values was determined by the backtest (Tukey). Statistical analysis of the data was performed using TIBCO Statistica Software Inc. v. 13.5.0 (2018).

Results and discussion:

The obtained results, shown in Table 1, correspond to the literature description. According to the main factor treatments, all morphological characteristics of the fruit and stone differed by treatments, except for the ratio of length to width of the fruit. Most of the monitored morphological parameters of the fruit increased with the irrigation T₃ (100%) rate. The results are in accordance with Nanos et al. (2008) who determined that deficit irrigation affects the size, firmness and weight of olive fruit. Nevertheless, fruits were larger in deficit irrigation compared to irrigation treatments with less added water. Also, Valenčić et al. (2018) had similar results with the variety 'Istarska bjelica'.

Except for the width of the fruit and the mass of the pulp, measurements differ significantly when comparing the two years. The results on morphological characteristics of the pit were significantly higher in 2020, when compared to 2021, as well as the pulp content (Table 1). Although the fruit in the second year was larger, the pit mass did not follow the growth of the fruit. Therios (2009) made the same point, stating that external factors affect the pulp more than the pit. The meteorological data (Figure 1.) indicate that the amount of precipitation in 2020 resulted in greater pit characteristics, and therefore the proportion and pulp content turned out to be significantly higher in 2021.

The interaction of treatment and year indicates that there are significant differences among all measurements. So, the most important measurement, the pulp content, is the highest in T₃ (75.87), and the lowest in K (68.98) and differed significantly compared to T₁ and T₂. Similarly, Patumi et al. (2001) reported that 66% irrigation treatment on Kalamata olives resulted in fruit growth suitable for olive oil production, and that 100% irrigation had the best effect on fruit characteristics, which differed significantly from the treatment without irrigation. However, although fertilization and irrigation can significantly affect the morphological parameters of olive fruit (Mele et al., 2018), our research has shown that under the same fertilization conditions, they can be reliable for assessing water management. Under conditions of optimal water use with a slight deficit (80% of water capacity) at T₂, the pulp content is acceptably lower compared to the consecutive years.

Conclusions

Irrigation and year significantly influenced fruit morphological characteristics. The fruit morphological characteristics were significantly higher in 2020, when compared to dry year 2021. Different irrigation rates and irrigation events compared to rainfed treatment increased fruit morphological characteristics. The pulp content was significantly higher in 2021, when compared to 2020. It can be concluded that irrigation with the SAN treatment (T₂) on the 'Coratina' olive tree provides a sufficient amount of water that is needed, as well as T₃, but with greater savings and better water management.

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References

Barranco D., Cimato A., Fiorini P., Rallo L., Touzani A., Castaned C., Serafini F., Trujillo, I. (International Olive Oil Council (COI) (2000). World catalogue of olive varieties. Madrid,

- Bogunović M., Bensa A., Husnjak S., Miloš B. (2009). Pogodnost tala Dalmacije za uzgoj maslina. *Agronomski glasnik* 71 (5-6): 367-404.
- d'Andria, R.; Lavini, A.; Tombesi, A.; Tombesi, S. (2007). Irrigation. In *Production Techniques in Olive Growing*; Artegraf, S.A., Ed.; International Olive Council: Madrid, Spain, pp. 169–209.
- Džaja K. (2003). Geomorfološke značajke Dugog otoka. *Geoadria* 8 (2): 5-44.
- Fernandes-Silva A.A., Ferreira T. C., Correia C.M., Malheiro M.C., Villalobos F. J. (2010). Influence of different irrigation regimes on crop yield and water use efficiency of olive. *Plant Soil* 333, 35–47.
- Husnjak S., Magdić I., Balog N. (2019). Elaborat; Značajke antropogenih tala maslinika na području Novigrada u ravnim kotarima i Žmana na Dugom otoku. Projekt SAN jn-68/2018 Zagreb: Sveučilište u Zagrebu Agronomski fakultet, Zavod za pedologiju.
- Liphshitz N., Gophna R., Hartman M., Bigger G. (1991). The beginning of olive (*Olea europaea* L.) cultivation in the old world: a reassessment. *Journal of Archaeological Science* 18 (4): 441-453.
- Loumou A., Giourga C. (2003). Olive groves: “The life and identity of the Mediterranean”. *Agriculture and Human Values* 20 (1): 87-95.
- Mele M.A., Islam M.Z., Kang H.M., Giuffrè A.M. (2018). Pre-and post-harvest factors and their impact on oil composition and quality of olive fruit. *Emirates Journal of Food and Agriculture* 592-603.
- Perica, M. (2006.). Masline - Klima, podizanje novih nasada. *Glasnik Zaštite Bilja* 29 (6): 26-29.
- Rallo L., Díez C. M., Morales-Sillero, A., Miho H., Priego-Capote F., Rallo, P. (2018). Quality of olives: A focus on agricultural preharvest factors. *Scientia Horticulturae* 233: 491–509.
- StatSoft Incorporated (2013) *Electronic statistics textbook*. Tulsa, UK: StatSoft. (Accessed on 2/5/20159, <http://www.statsoft.com/textbook>).
- Serafini F., Sbitri M. O., Tombesi A., Tombesi S., d'Andria R., Lavini A., Saavedra M., Jardak T., Fernández-Escobar R. (2007). *Production techniques in olive growing*; International Olive Council; Madrid Spain, 175-176.
- Šegota T., Filipčić A. (2003). Köppenova podjela klima i hrvatsko nazivlje. *Geoadria*, 8 (1), 17-37.
- Šikić Z., Marcelić Š., Brkić Bubola K., Jukić Špika M., Gašparović Pinto A., Zorica M., Kolega Š., Pasković I., Novoselić A., Klisović D., Kos, T. (2023). Modulation of the Irrigation Practices in Croatia for More Sustainable Olive Growing. *Agriculture*. 2023; 13(9):1854.
- Therios I. (2009). *Olives. Crop production science in horticulture* 18. CAB International, Oxfordshire, United Kingdom, p. 410.
- Tognetti R., Andria R., Lavini A., Morelli G. (2006). The effect of deficit irrigation on crop yield and vegetative development of *Olea europaea* L. (cvs. ‘Frantoio’ and ‘Leccino’) *European Journal of Agronomy* 25(4): 356-364.
- Valenčić V., Podgornik M., Bandelj D., Bučar-Miklavčič M., Bešter E., Miklavčič Višnjavec A., Pintar, M., Baruca Arbeiter A., Hladnik M., Butinar, B. (2018). Influence of irrigation treatments on the yield and quality of 'Istrska belica' olive oil. *Acta Horticulturae*. 1199: 471-476.
- Internet sources:
 National Bureau of Statistics (2020), (<https://dzs.gov.hr/>) (Accessed: 23.03.2022).
 Pinova-agrometeorological stations, (<https://pinova-meteo.com/>) (Accessed: 10.04.2022).

ORIGINAL SCIENTIFIC PAPER

Grapevine water management in the continental climate of Croatia – the example of the Đakovo vineyards

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Summary

This study utilized the CROPWAT 8.0 model to investigate the impact of varying climatic conditions on grapevine irrigation water requirements within the Đakovo vineyard region. Data from an experimental field at the Faculty of Agrobiotechnical Sciences in Mandićevac, spanning from an average year (2022) to extreme drought years (2000 and 2011), were analyzed. Results emphasize the necessity of tailored irrigation strategies, especially during prolonged dry periods. The study found that irrigation requirements ranged from 99 mm under extreme drought conditions to 125.3 mm during a severe drought, with an average year requiring approximately 112 mm. Data analysis highlights the necessity of irrigation for grapevines, especially considering the growing seasons and dry spells experienced during the specific year of the study.

Key words: grapevine, Đakovo vineyard region, irrigation water requirement

Introduction

Viticulture and winemaking in the Republic of Croatia have a long tradition mainly located in four regions: Dalmatia, Croatian Istria and Kvarner, Slavonia and the Croatian Danube Region, and Croatian Uplands where the agroecological, climatic, and pedological conditions of the respective regions display marked differences. Given the aforementioned regional variations, viticulture and winemaking represent the primary agricultural activity in the mentioned areas with many native (indigenous) varieties. A comprehensive survey conducted by the Ministry of Agriculture (2023) in cooperation with the Croatian Bureau of Statistics (CBS, 2022) revealed that the National Registry of Grapevine Cultivars in the Republic of Croatia encompasses 258 distinct varieties, whereby 134 are classified as autochthonous, while approximately 200 are deemed suitable for the production of wines eligible for a protected designation of origin. According to the data of the Agency for Payments in Agriculture, Fisheries and Rural Development, grape production in 2022 was 85,760.29 t and wine production was 560,789.04 hl. Area under grapevines as of December 31, 2023, were 17,277.67 ha. In the past 5 years, the area under grapevines has decreased by 11%, and the production of grapes and wine has decreased by 3% (APPRRR, 2023). From 2018 to 2023, in Croatia, the viticulture and winemaking sector contributed an average of 6.2% to the total value of agricultural production. In continental Croatia, the adverse consequences of climate change are manifested in increasingly frequent and prolonged periods of drought, as well as in extreme weather events characterized by intense, heavy rainfall events that cause significant damage to crop production. Vitali Čepo (?) reports that to date, the agricultural sector has been the most affected by climate change whereby between 1980 and 2013, losses attributed to climate change amounted to approximately 2.25 billion € (68 million € annually). In Europe, most vineyards are managed under rainfed conditions, where water deficit has become increasingly an issue (Yang et al., 2022). Although the grapevine adapts well to cultivation in semi-arid or arid climates and is therefore considered a water deficit-tolerant species (Chaves et al., 2007),

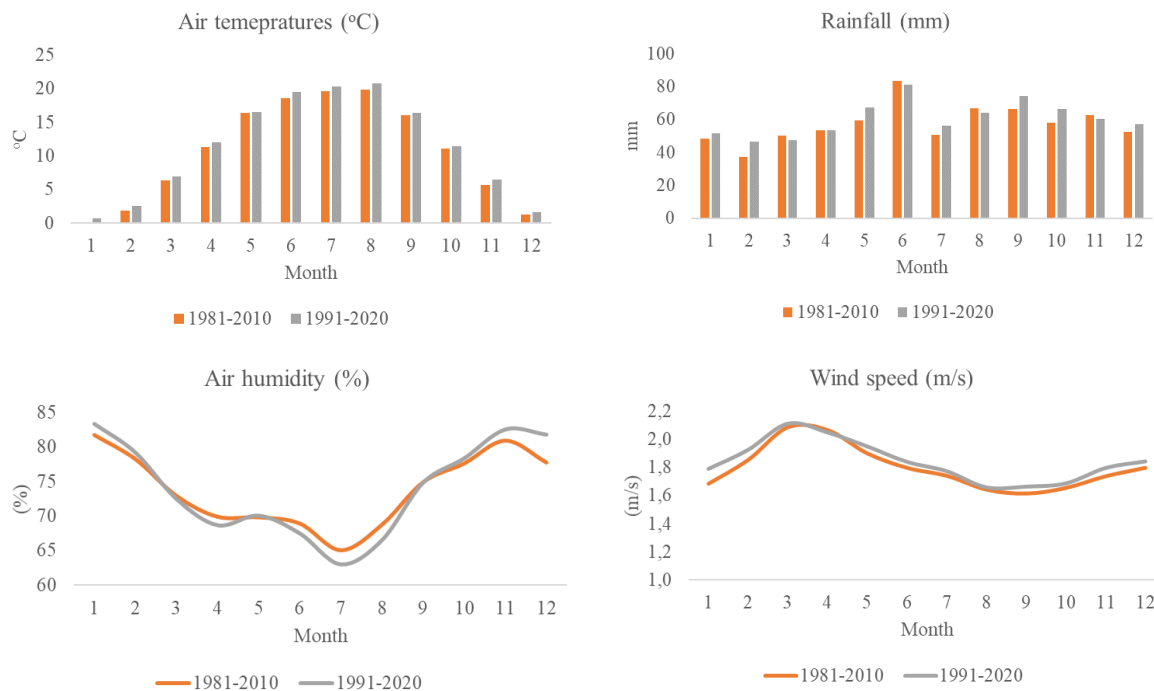
whereby moderate drought can improve grape and wine quality, it should be noted that severe water stress will have a negative impact on yield and quality (Chaves et al., 2010). Severe lack of water will result in a decrease of the total leaf area, the exposed leaf area, and the yield (Chacón-Vozmediano, 2020). Given the prominence of climate change, the study aims is to analyze the grapevine water requirements (GWR) in the Đakovo vineyard region for two 30-year periods (1981-2010 and 1991-2020). Furthermore, the study goal is to analyze the grapevine irrigation water requirements (IWR) for specific weather conditions in continental climate; average, dry, and extremely dry year.

Material and methods

A comparative analysis of meteorological data was conducted to evaluate climate change trends over two 30-year periods (long-term averages (LTA), 1981-2010 and 1991-2020). Data on rainfall (mm), air temperature (°C), and air humidity (%) were sourced from the Đakovo meteorological station, while insolation (h) and wind speed (m/s) data were acquired from the Osijek weather station. For a grape water requirement (GWR) and irrigation water requirement (IWR) analysis, the years 2000, and 2011 representing dry, extremely dry, and year 2022 representing average year, were chosen. A water balance analysis, including GWR and IWR, was conducted using the CROPWAT 8.0 computer program (Allen et al., 1998). The Empirical method was specifically chosen to calculate effective rainfall (Reff). Soil data derived from the pedological study at the Mandićevac experimental station, Faculty of the Agrobiotechnical Sciences Osijek, were used for analytical and modeling purposes. The following soil properties were used: silty loam soil texture, total available soil moisture: 140 mm m^{-1} , maximum rain infiltration rate: 120 mm day^{-1} , and maximum rooting depth: 60 cm. The reference (ET_o) and crop evapotranspiration (ET_c) were computed using the Penman-Monteith method, which is an integral component of the software. Crop-specific parameters, that is *kc*, critical depletion fraction, and yield response factor were obtained from Allen et al. (1998). The model is set up to irrigate at critical depletion (40% of management allowable depletion (MAD), and to refill soil water content to 100% of field capacity (FC).

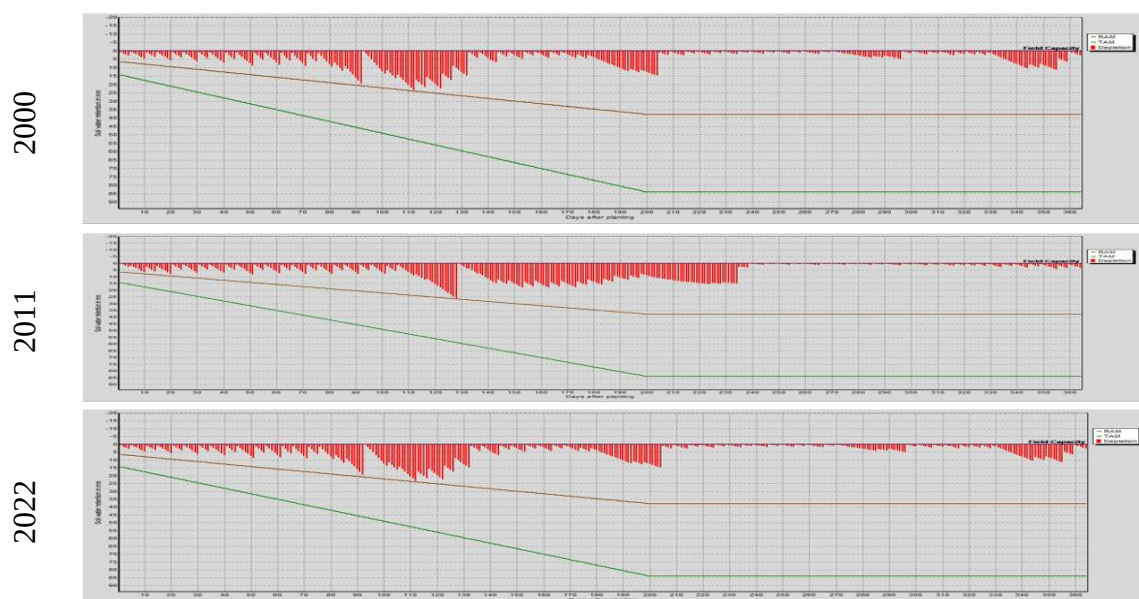
Results and discussion

The monthly values of rainfall (mm), wind speed (m/s), air temperatures (°C), and humidity for two 30-year periods (1981-2010 and 1991-2020) are presented in Graph 1. Two 30-year periods were given with the intention of highlighting differences in observed climatological characteristics in the context of climate change. When analyzing 30-year averages, the grapevine IWR was 6 mm for the 1981-2010 and 5 mm for the 1991-2020 period. Despite the increase in rainfall in second 30-year period (1981-2010=689.9 mm, 1991-2020=726 mm), the growing frequency, intensity, and duration of droughts cannot be overlooked. Furthermore, the observed increases in air temperatures (1981-2010=10.7 °C, 1991-2020=11.3 °C), wind speed (1981-2010=1.79 m s⁻¹, 1991-2020=1.83 m s⁻¹), and sunshine hours (1981-2010=4.9 h day⁻¹, 1991-2020=5.2 h day⁻¹) contribute to increased GWR, resulting in higher ET_o (1981-2010=1.61 mm/day, 1991-2020=2.13 mm day⁻¹), and ET_c (1981-2010=186.5 mm, 1991-2020=262.6) rates. In the average year (2022), the Đakovo area received 669 mm of rainfall, of which 534 mm was recorded as effective (Reff). Rainfall amount deviated -5% from the LTA for the year and -7% during the growing period. The annual air temperature was 2.3°C higher, and during the growing period 1.8°C higher, compared to the LTA. In the same year, ET_o was 2.36 mm day⁻¹, ET_c was 287.8 mm.



Graph 1. Average monthly air temperatures (°C), and humidity (%), wind speed (m/s), and monthly rainfall (mm) for two periods (1981-2010, and 1991-2020) in Đakovo

Consequently, IWR was 112 mm (Graph 2). During the dry year of 2011, the annual rainfall was 489.4 mm, 350.6 mm of Reff, which is 30% less than the LTA. Cindrić et al. (2016) claim that the 2011 and 2012 droughts are categorized as among the most severe in continental Croatia. The average annual air temperature was 1°C higher compared to the LTA and 1.8°C higher during the growing period. Consequently, ETo was 2.23 mm/day, and ETc was 269 mm. Consequently, the IWR was 99 mm (Graph 2). In the extremely dry year of 2000, the annual rainfall was recorded at 445 mm, while the Reff was 283.1 mm, which is 36.8% less than the LTA, and a decrease of 39.2% during the growing period. In addition to extremely low rainfall, there were high air temperatures, 2°C higher annually and 1.68°C higher during the growing period compared to LTA. Consequently, ETo was 2.31 mm/day, ETc 287.4 mm and IWR 125.3 mm (Graph 2). Given this comprehensive approach, it is essential to analyze IWR on a monthly timescale, with a particular focus on drought occurrences, as emphasized in the research findings of Perčec-Tadić et al. (2014). The authors state that in the Pannonian region, there is no rainfall deficit on the annual scale; the deficit rather occurs on a monthly basis. Furthermore, the authors noted that in the warm half of the year, there is a susceptibility to desertification in eastern Croatia (Slavonia). In this context, it is important to highlight the year 2000, when low rainfall was recorded during April and June, and almost no rainfall occurred in August (0.9 mm). An interesting observation is the higher grapes IWR during an average year (2022) compared to dry the growing season (2011), which is attributed to increased air temperatures, low rainfall amount during July (15.8 mm), and consequently, higher reference evapotranspiration (ETo) and crop evapotranspiration (ETc). The literature review shows that heat events delayed ripening and caused a significant reduction in berry quality (Greer and Weedon, 2013), earlier onset of many growth stages (Alikadic et al., 2019), a potential decrease in winegrape yields in a warmer climate due to flower abortion (Merrill et al., 2020), and that the interacting elevated temperature and drought on grape composition are genotype dependent (Pascual et al., 2024).



Graph 2. Water depletion in dry, extremely dry (2000 and 2011), and average year (2022)

Grapevine drought-sensitive stages have been discussed by Yang et al. (2022). The author claims that the flowering-veraison water deficit is critical for potential yield, which can have different impacts between Central and Southern European wine regions. The grape flowering stage in continental Croatia typically takes place in late May, followed by ripening in late July and early August. Considering a long-term analysis (1981-2020) for the Đakovo region, 17 years are recorded with a lack of rainfall during the month of May, 12 years during June, and 22 years with a lack of rainfall during August. The data indicates a concerning upward trend in air temperature during critical growing phases of grapevine development, coupled with a rainfall deficit. This has been discussed by de Odruna (2010) and Fraga et al. (2018). The authors highlight the issue of extremely high air temperatures in combination with drought, and the negative consequences manifested in reduced grapevine metabolism, higher sugar levels, lower acidity, and yield, particularly when such conditions occur during the ripening phase. In the Đakovo area, the increase in air temperature during the month of July has been particularly pronounced since 2009, with temperatures exceeding the long-term average (LTA) by 3°C to 5°C (2015: 5.39°C, 2021: 5.59°C).

Conclusions

A detailed analysis of the climatic conditions in the Đakovo vineyard region, with particular emphasis on monthly values, clearly reveals the need for irrigation of grapevines during average (112 mm), dry (99 mm), and extremely dry years (125 mm). It is important to highlight not only the deficiency and irregular distribution of precipitation but also the above-average air temperatures during the growing season.

Acknowledgement

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References

- Alikadic A., Pertot I., Eccel E., Dolcia C., Zarbo C., Caffarra A., De Filippi R., Furlanello C. (2019). The impact of climate change on grapevine phenology and the influence of altitude: A regional study. *Agric. For. Meteorol.* 271: 73–82.
- Allen R.G., Pereira L.S., Raes D., Smith M. (1998). Crop evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56. Food and agricultural organisation of United Nations. Rome, Italy.
- APPRRR, Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (2023). Registri. Available from: <https://www.apprrr.hr/registri/>
- Cindrić K., Mihajlović D., Telišman Prtenjak M., Herceg Bulić I., Pasarić Z. (2016). Analysis of the extraordinary 2011/2012 drought in Croatia. *Theoretical and Applied Climatology*. 123(3–4): 503–522.
- Chacón-Vozmediano J.L., Martínez-Gascueña J., García-Navarro F.J. (2020). Jiménez-Ballesta R. Effects of Water Stress on Vegetative Growth and ‘Merlot’ Grapevine Yield in a Semi-Arid Mediterranean Climate. *Horticulturae*. 6: 95.
- Chaves M., Santos T., Souza C., Ortuño M., Rodrigues M., Lopes C., Maroco J., Pereira J. (2007). Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Annals of Applied Biology*. 150: 237–252.
- Chaves M.M., Zarrouk O., Francisco R., Costa J.M., Santos T., Regalado A.P., Rodrigues M.L., Lopes C.M. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany*. 105: 661–676.
- Croatian Bureau of Statistics, CBS (2022). Proizvodnja povrća, voća i grožđa u 2021. Available from: <https://podaci.dzs.hr/2021/hr/10118>
- De Oruna, R.M. (2010). Climate Change Associated Effects on Grape and Wine Quality and Production. *Food Research International*. 43, 1844–1855.
- Fraga H., García de Cortázar Atauri I., Santos J.A. (2018). Viticultural irrigation demands under climate change scenarios in Portugal. *Agric. Water Manag.* 196: 66–74.
- Greer D.H., Weedon M.M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semillon grapevine performance and berry ripening. *Frontiers in plant science*, 4: 491.
- Merill N.K., de Cortázar-Atauri I. G., Parker A.K., Walker M.A., Wolkovich E.M. (2020). Exploring Grapevine Phenology and High Temperatures Response Under Controlled Conditions. *Front. Environ. Sci.* 8: 516527.
- Ministry of agriculture, Forestry and Fisheries (2023). Viticulture (winegrowing) and winemaking. Available on: <https://poljoprivreda.gov.hr/vinogradarstvo-i-vinarstvo/193>
- Pascual I., Martínez-Lüscher J., José Irigoyen J., Goicoechea N., Carmen Antolín M. (2024). Grapevine varieties show different sensitivities to flavonoid alterations caused by high temperatures under two irrigation conditions. *Food Research International*. 194: 114899.
- Perčec-Tadić, M. Gajić-Čapka, M., Zaninović, K., Cindrić, K. (2014). Drought Vulnerability in Croatia. *Agriculturae Conspectus Scientificus*. 79(1): 31–38.
- Vitali Čepo, D. (upisati godinu?). Klimatske promjene u Hrvatskoj. Available from: <https://www.znanost-klima.org/wp-content/uploads/2021/12/Klimatske-promjene-u-Hrvatskoj.pdf>
- Yang C., Menz C., Fraga H., Costafreda-Aumedes S., Leolini L., Concepción Ramos M., Molitor D., van Leeuwen, C., Santos J.A. (2022). Assessing the grapevine crop water stress indicator over the flowering-veraison phase and the potential yield lose rate in important European wine regions, *Agricultural Water Management*. 261: 107349.

ORIGINAL SCIENTIFIC PAPER

The SOYNUT Project: Initial Findings on Grain Oil and Protein in Soybeans

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Summary

Soybean is a valuable source of high-quality protein and oil. Improving grain quality is an important goal of soybean breeding programmes. As a result, an interdisciplinary team from the Agricultural Institute Osijek and the Faculty of Food Technology Osijek initiated the SOYNUT project. This manuscript introduces the project and presents the first-year findings on grain protein and oil contents for 40 soybean genotypes created and owned by the Agricultural Institute Osijek. Results revealed significant genotype-dependent variations in protein (41.62%–48.35%) and oil (21.68%–26.22%) contents. Environmental factors, particularly high temperatures and drought, influenced composition. Although these findings provide initial insights, they are not sufficient to draw specific conclusions. Therefore, further project activities including an additional two years of field trials, along with comprehensive analyses of protein and oil content, amino acid composition, and fatty acid composition will help validate our observations and provide a more complete understanding of soybean quality traits.

Key words: soybean, nutritional value, grain oil content, grain protein content, plant breeding

Introduction

Soybean (*Glycine max* L. Merr.) is a key source of high-quality protein and oil, essential for human and animal nutrition. Grain contains approximately 40% highly digestible proteins, nutritionally comparable to animal proteins (Vratarić and Sudarić, 2008) and 19% to 23% oil in dry matter (Panthee et al., 2004). Despite its nutritional benefits, soybean is primarily used as livestock feed. As soybean cultivation in Europe has been expanding (FAOSTAT, 2024), along with increasing concerns over climate change, there is a growing interest in plant-based diets and conventionally bred local, high grain quality soybeans.

To address market demands, researchers from the Agricultural Institute Osijek (AIO) and the Faculty of Food Technology Osijek launched the project in 2024, titled "The evaluation of soybean germplasm: Nutritional quality of grains under climate change conditions (SOYNUT)", funded by the EU under the National Recovery and Resilience Plan (2021–2026). The main goal of the project is creating a platform with soybean germplasm's nutritional quality data (protein, amino acids, oil, and fatty acid profiles) collected from the three-year field experiment with 55 genotypes. The platform will enable objective cultivar recommendations depending on the environmental conditions, identification of superior cultivars for food and feed industries and parental components for breeding programmes. It will intensify interdisciplinary scientific research and professional work with the existing infrastructure capacities of the AIO and ensure the development and strengthening of its scientific staff. The project ultimately aims to enhance local soybean production, supporting the long-term sustainability of soybean cultivation in Croatia and Europe, and contributing to European self-sufficiency in GMO-free plant proteins and oils. This manuscript presents the first-year protein

and oil content results of the SOYNUT project, focusing on 40 AIO soybean genotypes divided into three groups representing three breeding cycles.

Material and methods

The 2024 field experiment was set up at the Agricultural Institute Osijek (AIO, 45°32' N, 18°44' E, Osijek, Croatia) in a randomized complete block design with three replicates. The individual plots sown by hand consisted of two 500 cm long rows, with an inter-row distance of 50 cm.

The plant material comprises 40 genotypes, including: (a) archived AIO varieties presently not in commercial production (AV; G1–G13); (b) the commercial AIO varieties (CV; G14–G26); (c) elite lines (EL; G27–G40). The investigated genotypes differing in morphological and qualitative characteristics cover all maturity groups (MGs) grown in Croatia and areas with similar environmental conditions, which ensures the divergence of the tested germplasm.

The first year of the field experiment was characterized by extremely unfavorable weather conditions for soybean cultivation (Figure 1). According to the data of the Croatian Meteorological and Hydrological Service, the spring was extremely hot with below-average rainfall in April and above-average rainfall in May. The summer was extremely hot as well with below-average rainfall. On the other hand, both temperatures and rainfall were above-average in September.

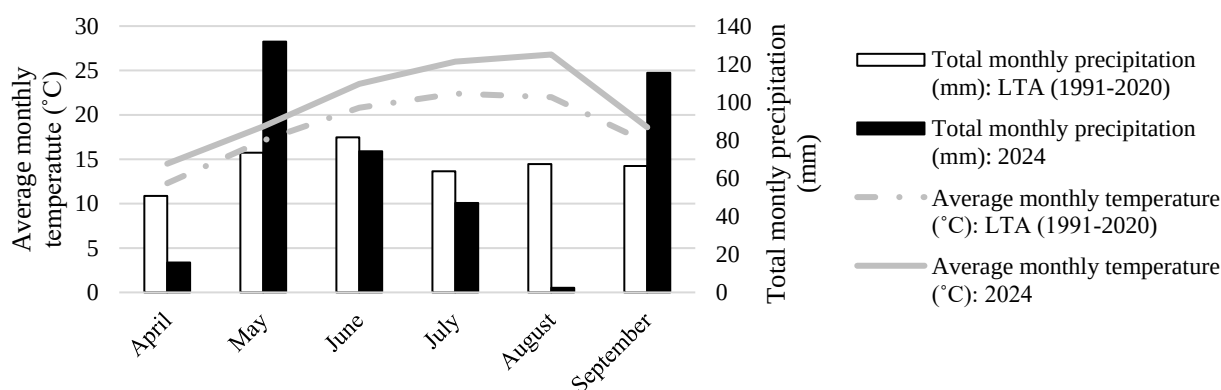


Figure 1. The long-term average (LTA) monthly temperatures and average monthly temperatures (°C), total monthly precipitation LTA, and total monthly precipitation (mm) in 2024 for the soybean growing season (April–September) in Osijek, Croatia (Croatian Meteorological and Hydrological Service)

During the field trial, all currently accepted agricultural management practices were applied. The experiment plots were harvested at full maturity. Grain protein and oil contents were determined on the Infratec 1241 (Foss, Sweden) device from one kg of grain per plot. One-way ANOVA with Tukey post-hoc comparison ($p < 0.001$) was conducted in Jasp 0.18.1.0.

Results and discussion

The three different genotype sets (AV, CV, EL) significantly ($p < 0.001$) differed in grain protein and oil contents (Figure 2). The average grain protein content determined for EL (44.74%) was significantly ($p < 0.001$) above the average grain protein content of AV (43.78%) and CV (43.57%; Figure 2a). On the other hand, grain oil content was significantly ($p < 0.001$) less in EL (24.08%), but only compared to AV (24.67%; Figure 2b). This was expected as the relationship between protein and oil contents is almost always significantly negative (Vollman et al., 2000, Kurasch et al., 2017, Pannecoucq et al., 2018). According to some studies,

weather conditions during the growing season in 2024 (Figure 1) were more beneficial for protein accumulation than oil accumulation. For example, Gibson and Mullen (1996) reported a positive linear relationship between the average temperatures and protein content during high daily and night temperatures from seed-filling to maturity. Dornbos and Mullen (1992), Vollmann et al. (2000) and Matoša Kočar et al. (2017) confirmed a significant protein content rise during high temperatures in combination with drought in the seed-filling period. On the other hand, negative impacts of drought stress were reported by Rotundo and Westgate (2009), while Vollmann et al. (2000) reported that low precipitation rates and high temperatures were unfavourable for oil synthesis in genotypes of later maturity.

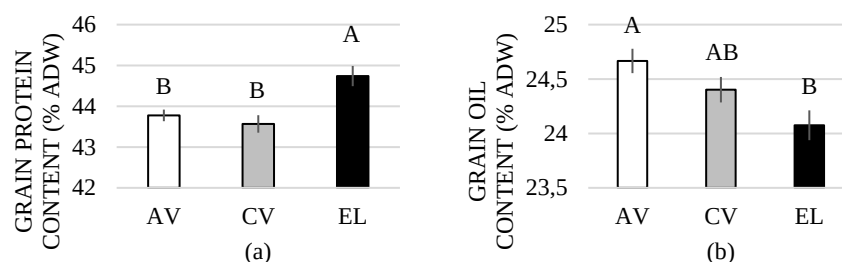
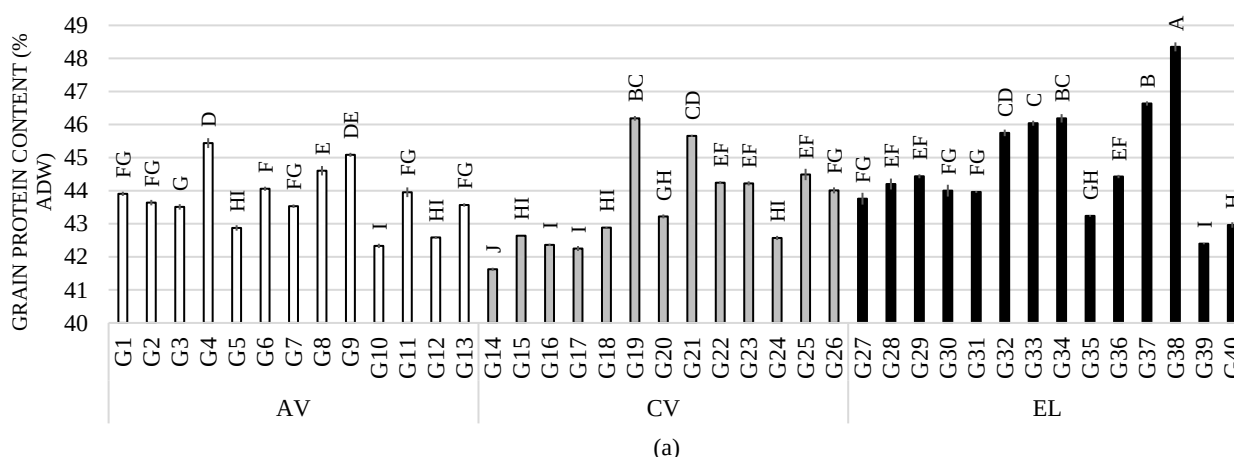


Figure 2. Average grain protein (a) and oil (b) contents (% absolute dry weight; ADW) $\pm$ the standard error for three sets of soybean genotypes: archived varieties (AV), commercial varieties (CV) and elite lines (EL) measured on grain samples collected from 2024 season field trial. Values with the same letter in the superscript are not significantly different (ANOVA, Tukey test, $P < 0.001$).

Individual genotypes varied significantly ($p < 0.001$) in grain protein and oil content (Figure 3). Grain protein content (Figure 3a) varied between 41.62% (G14) and 48.35% (G38). In AV it ranged between 42.33% (G10) and 45.44% (G4), in CV between 41.62% (G14) and 46.19% (G19), and in EL between 42.4% (G39) and 48.35% (G38). Grain oil content varied between 21.68% (G38) and 26.22% (G10). In AV it ranged between 23.77% (G9) and 26.22% (G10), in CV between 22.77% (G21) and 25.40% (G16), and in EL between 21.68% (G38) and 25.17% (G31).



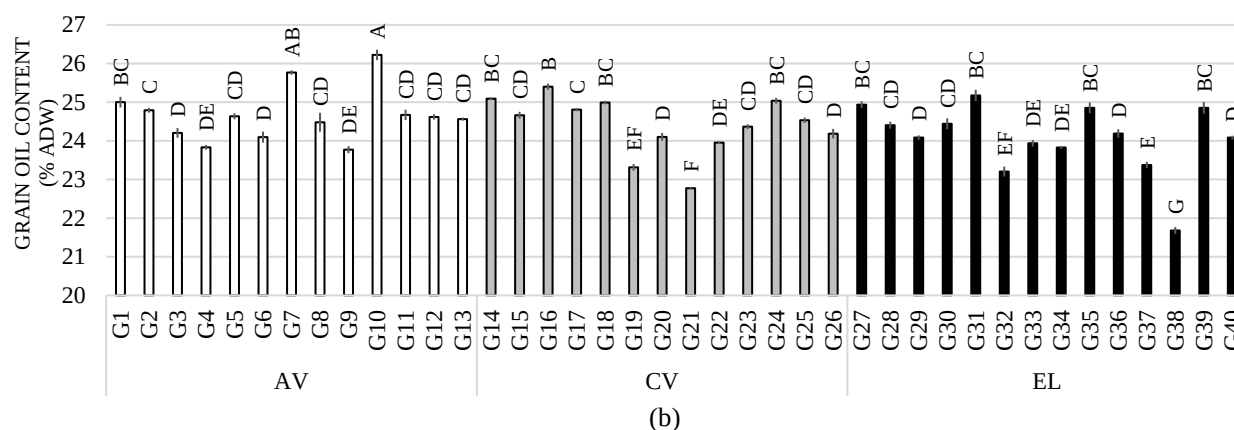


Figure 3. Average grain protein (a) and oil (b) content (% absolute dry weight; ADW) $\pm$ the standard error for 40 soybean genotypes (G1-G40) divided into three sets of genotypes: archived varieties (AV), commercial varieties (CV) and elite lines (EL) measured on grain samples collected from 2024 season field trial. Values with the same letter in the superscript are not significantly different (ANOVA, Tukey test, $P < 0.001$).

Wide ranges of protein and oil content variations determined in this experiment are consistent with those from some other studies. Fogelberg and Recknagel (2017) reported average protein content ranging 37–43% in seven short-season, well-adapted soybean cultivars evaluated at three sites in Germany during 3 years. In a two-year, two-location study in Belgium, Pannecouque et al. (2018) reported average protein and oil contents in 14 early maturing soybean varieties ranging 35.5–43.3% and 20.3–24.1% respectively. Sharma et al. (2014) reported average protein and oil contents in eight genotypes in India ranging 39.40–44.40% and 14–18.7% respectively. Furthermore, average protein and oil contents of the whole US Department of Agriculture (USDA) soybean germplasm collection ranged from 34.1 to 56.8%, and 8.1 to 27.9% respectively (Wilson, 2004).

Conclusions

The preliminary results of the SOYNUT project on oil and protein content in 40 AIO soybean genotypes indicated significant genotype and environment effects with wide ranges of trait variability. While these findings provide initial insights, they are not sufficient to draw specific conclusions. Therefore, further steps in this project will include an additional two years of field trials, along with comprehensive analyses of protein and oil content, amino acid composition, and fatty acid composition. These efforts will help validate our observations and provide a more complete understanding of soybean quality traits.

Acknowledgement

The results presented in the paper are an output from research project "The evaluation of soybean germplasm: Nutritional quality of grains under climate change conditions (SOYNUT)". financed by the European Union from the National Recovery and Resilience Plan 2021-2026 within the framework of the Agricultural Institute Osijek's Program Agreement with the Croatian Ministry of Science, Education and Youth.

References

- Dornbos D. L. Jr, Mullen R. E. (1992). Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. *Journal of the American Oil Chemists' Society*. 69: 228-231.
- FAOSTAT Database (2019). Available from: <http://faostat3.fao.org/home/E>
- Fogelberg F., Recknagel J. (2017). Developing soy production in central and northern Europe. In: Murphy-Bokern D., Stoddard F. L., Watson C. A., editors. *Legumes in Cropping Systems*. CAB International; pp. 109-124.
- Gibson L. R., Mullen R. E. (1996). Soybean seed composition under high day and night growth temperatures. *Journal of the American Oil Chemists' Society*. 73: 733-737.
- Kurasch A. K., Hahn V., Leiser W.L., Starck N., Würschum T. (2017). Phenotypic analysis of major agronomic traits in 1008 RILs from a diallel of early European soybean varieties. *Crop Science*. 57: 726-738.
- Matoša Kočar M., Sudarić A., Vila S., Petrović S., Rebekić A., Josipović A., et al. (2017). Varijabilnost fenotipske ekspresije svojstava kvalitete zrna elitnih linija soje. *Poljoprivreda*. 23: 40-48.
- Pannecoucq J., Goormachtigh S., Heungens K., Vleugels T., Ceusters J., Van Waes C., et al. (2018). Screening for soybean varieties suited to Belgian growing conditions based on maturity, yield components and resistance to *Sclerotinia sclerotiorum* and *Rhizoctonia solani* anastomosis group 2-2IIIB. *The Journal of Agricultural Science*. 156: 342-349.
- Panthee D., Kwanyuen P., Sams C., West D., Saxton A., Pantalone V. (2004). Quantitative trait loci for β -conglycinin (7S) and glycinin (11S) fractions of soybean storage protein. *Journal of the American Oil Chemists' Society*. 81: 1005-1012.
- Rotundo J. L., Westgate M. E. (2009). Meta-analysis of environmental effects on soybean seed composition. *Field Crops Research*. 110: 147-156.
- Sharma S., Kaur M., Goyal R., Gill B. S. (2014). Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. *Journal of Food Science and Technology*. 51: 551-557.
- Vollmann J., Fritz C. N., Wagentristl H., Ruckebauer P. (2000). Environmental and genetic variation of soybean seed protein content under central European growing conditions. *Journal of the Science of Food and Agriculture*. 80: 1300-1306.
- Vratarić M., Sudarić A. (2008). Soja. *Poljoprivredni institut Osijek, Osijek*: 1-459.
- Wilson R. F. (2004). Seed composition. In: Boerma H. R., Specht J. E., editors. *Soybeans: Improvement, Production and Uses*, Ed 3. Madison, Wisconsin, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. pp. 621-677.

ORIGINAL SCIENTIFIC PAPER

‘Divna’ and ‘Petra’ – new late-season plum cultivars from the Fruit Research Institute, Čačak

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Summary

This study evaluated the key characteristics of tree vigor, yield, and fruit physical and chemical properties of two new late-season plum cultivars, ‘Divna’ and ‘Petra’ released in the Fruit Research Institute, Čačak, and compared them to the standard cultivar ‘Stanley’. The new cultivars exhibited slightly lower tree vigor but higher yield compared to ‘Stanley’ although their fruits were generally smaller in size. ‘Petra’ exhibited the highest levels of soluble solids and total sugars, while ‘Divna’ had the highest total acid and reducing sugar content. In contrast, ‘Stanley’ had the highest values for sucrose content, juice pH, and ripening index. The late ripening time, good fruit quality, and high cropping potential of ‘Divna’ and ‘Petra’ make them promising candidates for cultivation in new orchards, as well as valuable genetic resources for future plum breeding programs.

Key words: plum, new cultivar, late-season, fruit quality, yield

Introduction

Although more than 6000 plum cultivars have been recognized globally many breeding programs worldwide are actively developing new cultivars (Milošević and Milošević, 2018). In European plum (*Prunus domestica* L.), which is commonly cultivated in temperate regions across Europe, including Serbia, the primary breeding objectives are largely similar across programs. These objectives include medium to large, high-quality fruits with a dark blue color, high yield performance and tolerance or resistance to Sharka disease (Plum pox virus) (Milošević and Milošević, 2018; Neumüller et al., 2021). Additionally, some programs have unique breeding goals tailored to specific needs.

One of the oldest and most successful plum breeding programs in Europe has been conducted at the Fruit Research Institute in Čačak. This program has aligned with the demands and needs of producers, focusing on common objectives as well as specific ones such as very early and late ripening times, self-fertility, medium tree size, climate change tolerance, and resistance to major diseases (Milošević et al., 2021). As a result of this long-term breeding effort, 18 plum cultivars have been released. Some of these cultivars, such as ‘Čačanska Lepotica’, ‘Čačanska Rodna’ and ‘Čačanska Najbolja’ are widely known and utilized for both production and further breeding globally (Jacob, 2002; Hartman and Neumüller, 2006; Milošević et al., 2021). Among the most recently recognized cultivars are ‘Divna’ (‘Stanley’ × ‘Čačanska Rana’) and ‘Petra’ (‘Stanley’ × ‘Opal’), late-season cultivars, released in 2018.

The aim of this study was to evaluate and present the key characteristics of tree vigor, yield, and the physical and chemical properties of the fruits of these new cultivars compared to the standard cultivar ‘Stanley’.

Material and methods

The characteristics of tree vigor, yield, and fruit of the new late-season plum cultivars ‘Divna’ and ‘Petra’ which ripen in mid-September in the Čačak region, were evaluated alongside the standard cultivar ‘Stanley’.

The study was carried out in a private orchard located in the village of Teočin (western Serbia). The orchard was established in 2011, arranged at a planting density of 667 trees per hectare, with a spacing of 5.0 m × 3.0 m. The experimental design followed a randomized block layout, including five trees for each rootstock, replicated four times ($n = 20$). The trees were trained and pruned to a central leader crown, following standard practices, except for irrigation.

Trunk diameter was measured 10 cm above the grafting union at the end of the vegetative cycle each year, and the trunk cross-sectional area (TCSA, cm^2) was calculated. Yield per tree (Y , g) and cumulative yield (kg) were recorded at harvest using an ACS electronic scale (Zhejiang, China). The yield efficiency (YE , kg cm^{-2}) was determined by dividing the yield per tree by the TCSA value.

Twenty-five fruits from both new cultivars and standard cultivar were collected from two replicates per combination. Fruit weight (FW) and stone weight (SW), (both in g) were measured with an Ohaus Adventurer technical scale (Parsippany, NJ, USA), and flesh percentage (FP, %) was calculated as the ratio of the edible part to the total fruit weight. The height (H), width (W), and thickness (T) of the fruits (all in mm) were measured using a Kronen digital caliper (Kronen GmbH, Kehl am Rhein, Germany). The sphericity (ϕ) was calculated using the formula $\phi = (\text{HWT})^{1/3}/H$.

Chemical analysis of the fruits was conducted at the stage of commercial maturity. Soluble solids content (SSC, %) was determined with a Carl Zeiss binocular refractometer (Germany) at 20°C. Total sugars (TS, %) and reducing sugars (RS, %) were quantified using the Luff-Schoorl method, with sucrose content (SU, %) calculated from the formula $\text{SU} = (\text{TS} - \text{RS}) \times 0.95$. The pH of the fruit juice was measured with a CyberScan 510 pH meter (Nijkerk, Netherlands). Total acids (TA, %) were expressed as malic acid, determined through titration with 0.1 N NaOH up to pH 8.1, using phenolphthalein as an indicator. The ripening index was calculated by dividing the soluble solids content by the total acids content.

The results were statistically evaluated using analysis of variance (ANOVA) with Microsoft Office Excel software (Microsoft Corp., Redmond, WA, USA). The means were compared using the LSD test, with significance set at $P \leq 0.05$.

Results and discussion

Tree vigor in plum is affected by various factors, including genotype, rootstock, environmental conditions, training system, orchard management, and yield (Milošević et al. 2013; Reig et al. 2018). In this study, tree vigor was assessed through TCSA. The cultivars ‘Stanley’ and ‘Divna’ exhibited similar vigor which was slightly greater than that of ‘Petra’ (Table 1).

Table 1. Tree vigor and yield parameters of new plum cultivars ‘Divna’ and ‘Petra’ compared to control cultivar ‘Stanley’

Parameter	TCSA (cm^2)	Yield per tree (kg)	Cumulative yield (kg)	Yield efficiency (kg cm^{-2})
Divna	77.11±1.56 ab	31.93±0.48 a	31.93±1.15 a	0.42±0.01 a
Petra	74.74±1.85 b	29.39±0.69 b	29.39±1.33 b	0.40±0.01 a
Stanley	78.71±1.56 a	31.64±0.64 ab	24.19±0.98 c	0.31±0.01 b

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Previous findings (Glišić et al., 2018; Milošević et al., 2019) demonstrated comparable trends among these cultivars, supporting the classification of the new cultivars as moderately vigorous, given that ‘Stanley’ is considered a cultivar with moderate vigor (Milatović, 2019). The highest Y and CY were observed in ‘Divna’, whereas ‘Petra’ had the lowest Y and ‘Stanley’ the lowest

CY (Table 1). Moreover, 'Divna' and 'Petra' demonstrated significantly higher YE compared to 'Stanley' (Table 1). These results surpass those reported by Glišić et al. (2018) and Milošević et al. (2019) for the same cultivars, likely due to more favorable weather conditions and precipitation during the study period, as well as differences in orchard management and fertilization practices. Overall, YE was high across all cultivars, which can be attributed to their substantial yields and moderate tree vigor.

Table 2. Fruit and stone weight and flesh percentage of new plum cultivars 'Divna' and 'Petra' compared to control cultivar 'Stanley'

Parameter	Fruit weight (g)	Stone weight (g)	Flesh percentage (%)
Divna	29.23±0.92 b	1.36±0.05 b	95.28±0.20 a
Petra	29.70±0.93 b	1.44±0.03 c	95.09±0.16 a
Stanley	39.54±0.92 a	2.54±0.05 a	93.50±0.20 b

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

FW is one of the most important fruit properties influencing various physical attributes of the fruit, either directly or indirectly (Walkowiak-Tomczak et al., 2008). In this study, significant variations in FW, SW, and FP were observed among the examined cultivars (Table 2). 'Stanley' produced the largest FW and SW but had the lowest FP. Conversely, 'Divna' and 'Petra' exhibited similar and significantly smaller FW and SW but significantly higher FP. However, the differences between 'Divna' and 'Petra' in these parameters, except for SW, were not significant. 'Stanley' having the largest fruit size, also exhibited the greatest linear dimensions among the examined cultivars (Table 3).

Table 3. Linear dimensions and sphericity of fruit of new plum cultivars 'Divna' and 'Petra' compared to control cultivar 'Stanley'

Parameter	Fruit height (mm)	Fruit width (mm)	Fruit thickness (mm)	Sphericity
Divna	48.38±0.62 b	36.21±0.93 b	34.44±0.88 b	0.81±0.01 ab
Petra	51.13±1.35 b	35.48±0.53 b	33.19±0.45 c	0.77±0.01 b
Stanley	58.72±1.41 a	48.12±0.93 a	41.47±0.88 a	0.83±0.01 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Similar to FW, 'Divna' and 'Petra' displayed comparable linear dimensions, with no significant differences observed in H and W. However, 'Petra' had the smallest T, attributed to its highly elongated shape. This elongation was further confirmed by the sphericity index, which was lowest in 'Petra', intermediate in 'Divna' and highest in 'Stanley' (Table 3). Notably, a sphericity index closer to 1 indicates a more rounded fruit shape, while lower values reflect greater elongation (Jannatizadeh et al., 2008). The results of this study regarding physical properties are consistent with the findings of Glišić et al. (2018) and Milošević et al. (2019), yet higher than those reported by Molnar et al. (2016) and Ivanova et al. (2018). These discrepancies are likely attributable to differences in tree age, soil characteristics, fertilization regimes, and cultural practices.

The chemical properties of the evaluated cultivars varied significantly as shown in Tables 4 and 5. 'Petra' exhibited the highest levels of SSC and TS, while 'Divna' demonstrated the highest levels of TA and RS. In contrast, 'Stanley' had the highest SU, juice pH and ripening index. Conversely, 'Divna' recorded the lowest levels of SSC, SU, juice pH, and ripening index, whereas 'Stanley' had the lowest TA, TS and RS.

Table 4. Fruit chemical parameters of new plum cultivars ‘Divna’ and ‘Petra’ compared to control cultivar ‘Stanley’

Parameter	Soluble solids (%)	Total acids (%)	pH	Ripening index
Divna	21.11±0.30 b	1.35±0.01 a	3.46±0.03 c	15.67±0.35 c
Petra	23.25±0.22 a	1.12±0.01 b	3.79±0.01 b	20.82±0.24 b
Stanley	21.50±0.30 b	0.98±0.01 c	4.26±0.03 a	22.04±0.35 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

In this study, the examined cultivars displayed slightly higher levels of SSC, RS and TA but lower levels of TS and SU compared to the findings of Glišić et al. (2018) and Milošević et al., 2019. Generally, genotype, as well as soil and climatic conditions, plays a crucial role in determining the composition of primary metabolites in fruits, including plums (Popski et al., 2018; Ceccarelli et al., 2021). Plums with a SSC $\geq 12.0\%$ tend to achieve approximately 75% consumer acceptance, regardless of their total acids levels (Crisosto et al., 2004).

Table 5. Content of sugars of new plum cultivars ‘Divna’ and ‘Petra’ compared to control cultivar ‘Stanley’

Parameter	Total sugars (%)	Reducing sugars (%)	Sucrose (%)
Divna	11.99±0.02 ab	7.72±0.06 a	4.06±0.05 b
Petra	12.12±0.02 a	7.21±0.01 b	4.66±0.02 b
Stanley	11.95±0.02 b	6.25±0.06 c	5.41±0.05 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Moreover, the perception of sweetness is significantly influenced by different organic acids and their relative concentrations, which affect the balance between sugars and acids, thereby playing a vital role in taste and overall fruit quality (Colarič et al., 2005). Based on these parameters, it is reasonable to suggest that ‘Divna’ and ‘Petra’ possess the potential to satisfy key consumer preferences.

Conclusions

The evaluation of key tree growth and yield parameters, as well as the most important physical and chemical properties of the new plum cultivars ‘Divna’ and ‘Petra’ named and released at the Fruit Research Institute, Čačak, alongside the standard cultivar ‘Stanley’ demonstrated that the new cultivars exhibit excellent productivity and fruit quality traits. These attributes make them highly promising for cultivation in new orchards. A particularly notable advantage of ‘Divna’ and ‘Petra’ is their very late ripening time, an important trait given the limited number of cultivars that mature during the same period. Additionally, their desirable characteristics position them as valuable parental genotypes for future breeding programs at the Fruit Research Institute, Čačak, or within other plum breeding initiatives.

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References

- Ceccarelli, D., Antonucci, F., Talento C., Ciccoritti, R. (2012). Chemical characterization in the selection of Italian autochthonous genotypes of plum. *Sci. Hortic.* 281, 109922. <https://doi.org/10.1016/j.scienta.2021.109922>
- Colarič M., Veberič R., Štampar F., Hudina M. (2005). Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *Journal of the Science of Food and Agriculture*. 85: 2611–2616. <https://doi.org/10.1002/jsfa.2316>
- Crisosto C.H., Garner D., Crisosto G.M., Bowerman E. (2004). Increasing ‘Blackamber’ plum (*Prunus salicina* Lindley) consumer acceptance. *Postharvest Biology and Technology*. 34: 237–244. <https://doi.org/10.1016/j.postharvbio.2004.06.003>
- Glišić I., Milošević N., Karaklajić-Stajić Ž., Đorđević M., Lukić M. (2018). ‘Divna’ – new plum (*Prunus domestica* L.) cultivar developed at Fruit Research Institute, Čačak. *Journal of Pomology*. 52 (201): 7–13.
- Hartmann W., Neumüller M. (2006). Breeding for resistance: breeding for Plum pox virus resistant plums (*Prunus domestica* L.) in Germany. *EPPO Bulletin*. 36: 332–336.
- Jacob H.B. (2002). New plum and mirabelles varieties out of the breeding work and development in Geisenheim. *Acta Horticulturae*. 577: 173–176.
- Ivanova D., Dimkova S., Todorova S., Marinova N. (2018). Morphological characteristics and chemical composition of fruit of plum hybrids of *Prunus domestica* L. *Journal of Mountain Agriculture on the Balkans*. 21 (3): 257–265
- Jannatizadeh A., Naderi-Boldaji M., Fatahi R., Ghasemi-Varnamkhasti M., Tabatabaeefar A. (2008). Some postharvest physical properties of Iranian apricot (*Prunus armeniaca* L.) fruit. *International Agrophysics*. 22 (2): 125–131.
- Milatović D. (2019). In *Plum*, Milatović D. (ed.), 1–531. Čačak, Republic of Serbia: Scientific Pomological Society of Serbia.
- Milošević N., Đorđević M., Glišić I., Karaklajić-Stajić Ž., Lukić M., Radičević S., Marić S. (2019). ‘Petra’ – new plum (*Prunus domestica* L.) cultivar from Fruit Research Institute, Čačak. *Journal of Pomology*. 53 (205/206): 29–36.
- Milošević T., Milošević N., Glišić I. (2013). Agronomic properties and nutritional status of plum trees (*Prunus domestica* L.) influenced by different cultivars. *Journal of soil science and plant nutrition*. 13 (3): 706–714. <http://dx.doi.org/10.4067/S0718-95162013005000056>
- Molnár A.M., Ladányi M., Kovács S. (2016). Evaluation of the production traits and fruit quality of German plum cultivars. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*. 64 (11): 109–114.
- Neumüller M., Dittrich F., Holzapfel C. (2021). Recent achievements and future challenges in breeding for European plum cultivars: finding the suitable genetic resources for important traits. *Acta Horticulturae*. 1322: 1–6.
- Popski., Stefanova B., Minkov P. 2021. Impact of soil management systems on the chemical composition of fresh plum fruit of ‘Katinka’ cultivar. *Sci. Papers Ser. B Hortic.* 45, 225–229.
- Reig G., Font i Forcada C., Mestre L., Jiménez S., Betrán J.A., Moreno M.Á. (2018). Horticultural, leaf mineral and fruit quality traits of two ‘Greengage’ plum cultivars budded on plum based rootstocks in Mediterranean conditions. *Scientia Horticulturae*. 232: 84–91. <https://doi.org/10.1016/j.scienta.2017.12.052>
- Walkowiak-Tomeczak D., Reguła J., Łysiak G. (2008). Physico-chemical properties and antioxidant activity of selected plum cultivars fruit. *Acta Scientiarum Polonorum Technologia Alimentaria*. 7 (4): 15–22.

ORIGINAL SCIENTIFIC PAPER

Morphological Comparison of Arugula (*Eruca sativa* Mill.) and Spinach (*Spinacia oleracea* L.) in Floating Aquaponic and Hydroponic system

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Summary

Hydroponic and aquaponic systems are methods of growing plants in water enriched with nutrients. The research, conducted from March 27 to May 31, 2023, at the Faculty of Agrobiotechnical Sciences in Osijek, aimed to determine morphological differences in arugula (*Eruca sativa* Mill.) and spinach (*Spinacia oleracea* L.) grown in a floating aquaponic system compared to a floating hydroponics system. Parameters such as the number of leaves, root length, fresh and dry mass of leaves and roots were analyzed. The results revealed differences in the morphological characteristics of arugula and spinach grown in the two systems. Generally, the values of the examined parameters were higher in the floating hydroponic system compared to the aquaponic system. Therefore, it can be concluded that, in this study, the floating hydroponics system proved to be more effective for growing arugula and spinach, as plants in the aquaponic system exhibited lower values of morphological growth indicators and ultimately had lower yields.

Key words: baby leaf, vegetables, soilless cultivation, fish

Introduction

The need for continuous development of alternative agricultural methods has always been important, especially in response to the numerous problems faced by conventional agriculture (Rajalakshmi et al., 2022). Aquaponics and hydroponics are modern innovative techniques that depart from conventional soil-based farming methods (Hadeed et al., 2024, Tetreault et al., 2023). Aquaponics represent a combination of hydroponics and aquaculture, where aquatic animals create metabolic products that plants use as nutrients for growth. With their growth, plants extract substances from the water, thus cleaning and reducing the need for water changes (Love et al., 2014). Fish produce amniotic fluid, which bacteria convert into nitrite and from nitrite into nitrate. While ammonia and nitrites are toxic to fish, nitrates are desirable for plant growth (Rakocy et al., 2006). Hydroponics, a method of cultivating plants in nutrient-rich water, existed for over a century. This system utilizes closed-loop mechanisms where plant roots are immersed in a solution containing essential minerals and dissolved oxygen. By bypassing the limitations of soil absorption, this direct nutrient delivery approach leads to faster growth rates and higher yields compared to traditional farming methods (Tarun, 2024). The aim of this study was to compare the morphological indicators of arugula and spinach in floating aquaponics compared to floating hydroponics (FH).

Material and methods

The research was conducted at the Faculty of Agrobiotechnical Sciences in Osijek from March 27 to May 31, 2023. The materials used in the experiment were arugula seeds (Tricia, Enza Zaden, Netherlands) and spinach seeds (Rembour F1, Bejo Zaden, Netherlands), which were sown in polystyrene containers with 40 sowing places. After germination (15 days), thinning

was performed, leaving two plants per sowing unit. As a result, each container contained 80 plants, with a total of 160 plants in two containers per experimental variant. The polystyrene containers were previously filled with commercial substrate (Klassmann Potgrond P) and vermiculite in a volume ratio of 1:4. After the appearance of four to five leaves, the containers with plants were moved to pools filled with nutrient solution (FH), or to pools filled with water enriched with substances from the metabolism of fish (FH). Due to the location of the experiment, the amount of light was not sufficient, so additional lighting of 6×18 W was used throughout the day (24 hours).

FH method was applied in aquaponic and hydroponic systems. In the FH system, containers with plants were floating on the nutrient solution, where roots were completely submerged and aerated by an air pump (Oase 37125 Aqua Oxy CWS 1000). The tank dimensions were 80 × 120 × 30 cm, and 200 L of nutrient solution was maintained at 20 cm. The solution was made following Hoagland's recipe with 50% decrease in concentrations of essential elements as advised for leafy crops (Hoagland and Snyder, 1933). Salts were obtained from Gram-mol d.o.o. (Croatia), TTT d.o.o. (Croatia), and Haifa Chemicals (Israel). The solution pH averaged 6.6 and EC 2.1 mS/cm, which was monitored daily using a pH meter (pH3310, WTW®) and EC meter (Eutech Cond 6+, Thermo Scientific®). The temperature of the solution averaged 21.6 °C. The aquaponic system also employed the FH method, with two pools (80 × 120 × 30 cm) and two modified filter buckets (16 L). Five goldfish (*Carassius auratus* L.) were maintained in one pool and fed daily with koi food (Dajana Pond Sticks Extra), while the second pool was employed for plant growth. A pump (JQL-1000) recirculated water from the first tank (radial flow settler) and the second (biological and mechanical filter), packed with synthetic fiber wadding and broken brick pieces for filtration. The aquaponic solution averaged pH 8.1, EC 0.72 mS/cm (three times less than that in the hydroponic system), and an average temperature of 21.7 °C. Upon completion of the study, when the plants reached the technological maturity stage (baby leaf vegetables), plant material was sampled. Plants were harvested once, on May 31, 2023. Whole plants were collected, and morphological parameters—including the number of leaves per plant, root length and mass, and leaf mass—were recorded for each plant individually. The root and aboveground biomass were then weighed, placed in labeled paper bags and dried in an oven to determine dry mass. Statistical analysis was performed using analysis of variance (ANOVA) for all measured traits. The SAS 9.4 statistical software package (SAS Institute Inc., Cary, NC) was used, and the least significant difference (LSD) was calculated at a significance level of $p < 0.05$ according to Fisher's test. Significant differences between means in all tables are indicated using Duncan's multiple range test, where means not significantly different from each other share the same letter designation.

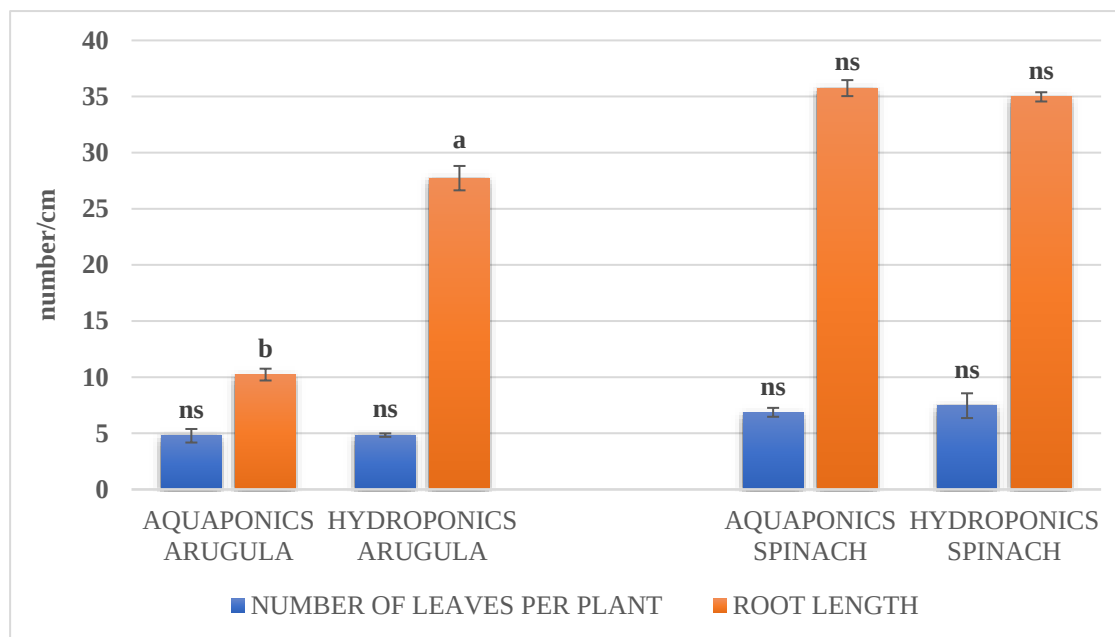
Results and discussion

The average number of arugula leaves per plant across the tested treatments was 4.8. A slightly lower number of leaves was observed in plants grown in aquaponics (4.8) compared to those grown in hydroponics (4.8); however, no statistically significant difference was found (Graph 1). In spinach, the average number of leaves per plant across both cultivation methods was 7.16. A slightly lower average number of leaves was observed in the aquaponic system (6.9) compared to the hydroponic system (7.5). However, no statistically significant difference was found in the number of leaves between the two cultivation methods. In the study by Modarelli et al. (2023), no significant difference was reported in the number of leaves of basil (*Ocimum basilicum* L.) grown in hydroponics and aquaponics, which aligns with the findings of our study. Similarly, Yang and Kim (2020) investigated the influence of different cultivation methods on the growth and development of lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), and basil (*Ocimum basilicum* L.). Their results indicated that plants grown in

hydroponics exhibited a higher number of leaves compared to those grown in aquaponics across all tested species.

The average root length per arugula plant was 18.98 cm. Significantly greater root length was observed in plants grown in hydroponics (27.73 cm) compared to those grown in aquaponics (10.24 cm) (Graph 1). In contrast, no significant difference was observed for spinach. The average root length in the aquaponic system was 35.75 cm, while in the hydroponic system, a slightly smaller root length of 34.97 cm was recorded. These results indicate that the cultivation method did not significantly affect the root length of spinach.

In a study by Atique et al. (2022), significantly greater root length was found in spinach plants grown in aquaponics compared to plants grown in hydroponics.



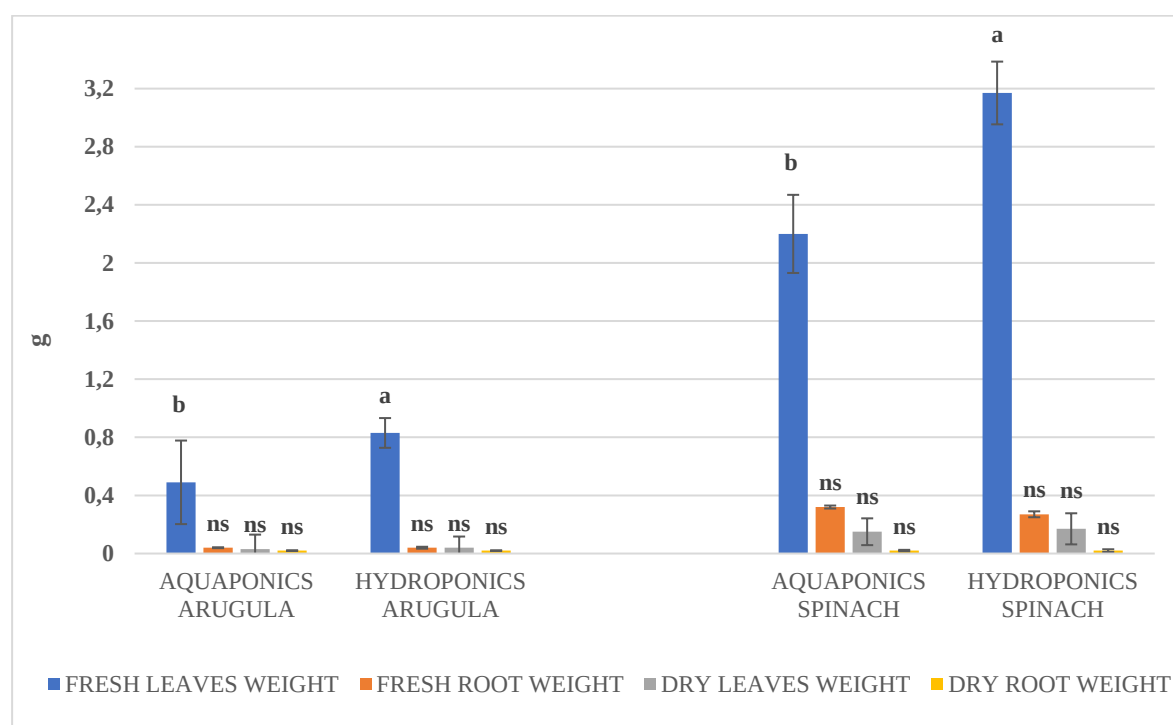
Graph 1. The influence of arugula and spinach growing system on leaf number and root length

In terms of average fresh leaves mass per plant, a statistically significant difference was observed between the mass of arugula and spinach leaves in hydroponic cultivation compared to aquaponic cultivation. Significantly higher fresh biomass values were achieved in the hydroponic system. The average fresh mass of arugula leaves in aquaponic cultivation was 0.49 g, whereas in hydroponic cultivation, the fresh mass reached 0.83 g. For spinach, the average fresh leaf mass per plant in aquaponic cultivation was 2.2 g, while in hydroponic cultivation, a significantly higher fresh leaf mass of 3.17 g was recorded (Graph 2). These results suggest that hydroponic systems may be more effective in increasing plant biomass than aquaponic systems, which aligns with previous studies highlighting differences in nutrient availability between the two cultivation methods. Similar results were found in their study by Wang et al. (2023) where they examined the influence of growth and development of Swiss chard (*Beta vulgaris* L.), kale (*Brassica oleracea* L.), mustard (*Brassica juncea* L.), coriander (*Coriandrum sativum* L.), lettuce (*Lactuca sativa* L.) and rocket (*Eruca vesicaria* L.) depending on the cultivation method, i.e. hydroponic and aquaponic plant cultivation. The results obtained determined a significantly higher fresh leaf mass in all tested species in hydroponic cultivation compared to aquaponic plant cultivation, which is in line with the results of our study.

In contrast to the mass of fresh leaves, the average fresh root mass of arugula and spinach did not show statistically significant differences between hydroponic and aquaponic cultivation. These results suggest that cultivation conditions may have a greater impact on the aboveground parts of the plants than on the root system.

The average dry mass of arugula leaves in aquaponic cultivation was 0.03 g, while in hydroponic cultivation a higher dry mass of 0.04 g was found, but no statistically significant difference was found. Also, there was no statistically significant difference in the average dry mass of leaves of spinach in hydroponic cultivation compared to aquaponic cultivation. The average dry mass of leaves per spinach plant in aquaponic was 0.15 g compared to hydroponic cultivation where the dry mass of leaves was 0.17 g (Graph 2). Similar results were found in their study by Wang et al. (2023), where all tested species had higher leaf dry mass in plants grown in hydroponics compared to plants grown in aquaponics.

Unlike leaf dry mass, the average root dry mass per plant is identical in both cultivation forms for both plant species. In the study by Atique et al. (2022), significant differences in root dry mass were found in hydroponic and aquaponic cultivation, which is not consistent with the results of our study.



Graph 2. The influence of arugula and spinach growing system on fresh and dry weight of root and leaves

Conclusions

Based on the research conducted on the cultivation of spinach (*Spinacia oleracea* L.) and arugula (*Eruca sativa* Mill.) in hydroponic and aquaponic systems, several key conclusions can be drawn. Significant differences were observed in the morphological properties of both spinach and arugula with respect to the cultivation form. However, the cultivation method did not significantly affect certain growth parameters, such as the number of leaves, dry mass of leaves and roots, and fresh mass of roots for both crops.

In the case of spinach, higher values for dry and fresh root masses and root length were observed in aquaponic cultivation, though these differences were not statistically significant. Conversely, fresh leaf mass in hydroponic cultivation was significantly higher compared to aquaponic cultivation. Similarly, for arugula, the fresh mass of leaves and root length were significantly higher in hydroponic cultivation. These findings suggest that while both hydroponic and aquaponic systems are viable for growing leafy vegetables, hydroponic cultivation generally results in better growth performance for spinach and arugula. However, the results also indicate that aquaponic systems may offer certain advantages in root development. The research

provides a preliminary foundation for further investigation into the potential of growing leafy vegetables in floating aquaponic systems, highlighting the need for more comprehensive studies to optimize cultivation practices and improve yield outcomes in both systems.

Acknowledgement

The data presented in this paper represent the results of research conducted in the final papers of students Bruno Tutić, titled Cultivation of Arugula (*Eruca sativa* Mill.) in a Floating Aquaponics System, and Dario Vavra, titled Cultivation of Spinach (*Spinacia oleracea* L.) in a Floating Aquaponics System.

References

- Atique F., Lindholm-Lehto P., Pirhonen J. (2022). Is Aquaponics Beneficial in Terms of Fish and Plant Growth and Water Quality in Comparison to Separate Recirculating Aquaculture and Hydroponic Systems? *Water* 14:9: 1447.
- Hadeed M. Z., Muhammad M., Safdar M., Raza A., Shahzad S., Malik A., Ahmed M., Raheem A. (2024). Role of Aquaponics and Hydroponics in Modern Agriculture. In J. Mabrouki & M. Azrour (Eds.), *Advancements in Climate and Smart Environment Technology*, 133-151.
- Hoagland D. R., Snyder W.C. (1933). Nutrition of strawberry plants undercontrolled conditions. *Proc. Am. Soc. Hort. Sci.* 30:288–296
- Love D.C., Fry J.P., Genello L., Hill E.S., Frederick A.J., Li X., Semmens K. (2014). An International Survey of Aquaponics Practitioners. *PLoS ONE* 9(7): e102662.
- Modarelli G.C., Vanacore L., Rouphael Y., Langellotti A.L., Masi P., De Pascale S., Cirillo C. (2023). Hydroponic and Aquaponic Floating Raft Systems Elicit Differential Growth and Quality Responses to Consecutive Cuts of Basil Crop. *Plants*, 12, 1355.
- Rajalakshmi M., Manoj V.R., Manoj H. (2022). Comprehensive Review of Aquaponic, Hydroponic, and Recirculating Aquaculture Systems, *Journal of Experimental Biology and Agricultural Sciences*, 10 (6): 1266-1289.
- Rakocy J.E., Masser M.P., Losordo T.M. (2006). Recirculating Aquaculture Tank Production Systems: Aquaponics — Integrating Fish and Plant Culture.
- Tarun K.P. (2024). Growing Beyond Soil: The Future of Farming with Hydroponics. *NewBioWorld A Journal of Alumni Association of Biotechnology*, 6(1):07-20.
- Tetreault J, Fogle RL, Fogarty S and Guerdat T (2023). Coupled aquaponics: Optimizing hydraulic retention times using a parallel unit process water treatment approach. *Front. Hortic*, 2:1140998.
- Wang Y.J., Yang T., Kim H.J. (2023). pH Dynamics in Aquaponic Systems: Implications for Plant and Fish Crop Productivity and Yield. *Sustainability*, 15(9):7137.
- Yang T., Kim H.J. (2020). Characterizing Nutrient Composition and Concentration in Tomato-, Basil-, and Lettuce-Based Aquaponic and Hydroponic Systems. *Water* 12, 1259.

ORIGINAL SCIENTIFIC PAPER

In vivo* evaluation of the antifungal activity of quaternary pyridinium salts against *Sclerotinia sclerotiorum

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Summary

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is a major plant pathogen with over 500 hosts, including carrot (*Daucus carota* L.), where it can cause losses of up to 50%, particularly during storage. Its control is challenging due to its ability to produce sclerotia resistant to degradation and the emergence of fungicide resistance. Given the environmental concerns associated with synthetic fungicides application, alternative antifungal compounds are being explored. Among them, quaternary pyridinium salts, particularly nicotinamide and isonicotinamide derivatives, have shown promising antifungal potential and could be viable alternatives to conventional fungicides. This study evaluated the efficacy of isonicotinamide derivatives against *S. sclerotiorum* under *in vivo* conditions, using carrot as a natural substrate. The selected quaternary pyridinium salt compounds had previously demonstrated the strongest inhibitory effects in *in vitro* tests. The results showed that 1-(3-bromopropyl)-4-carbamoylpyridinium bromide (12) at 10 µg/mL completely inhibited mycelial growth, while 4-carbamoyl-1-[2-(4-nitrophenyl)-2-oxoethyl]pyridinium bromide (10) was effective up to 120 hours, after which a slight increase in growth was observed. Unlike *in vitro* tests, where higher concentrations enhanced inhibition, this trend was inconsistent under *in vivo* conditions. These findings highlight the importance of *in vivo* testing for a more realistic assessment of antifungal efficacy. They also underscore the need for further optimization of formulation strategies to improve the stability and practical application of quaternary pyridinium salts in plant protection.

Key words: alternative antifungal compounds, mycelium growth, isonicotinamide derivatives, *Sclerotinia sclerotiorum*

Introduction

The species *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most virulent representatives of the genus *Sclerotinia*, with a wide ecological distribution and more than 500 known plant hosts (Lehner et al., 2017), including carrot (*Daucus carota* L.). The pathogen causes various symptoms depending on the plant species and the affected organ, but its white, cottony mycelium, known as white mold, is easily recognizable on all hosts. Carrot infection can lead to significant losses, especially during storage, where damages can reach up to 50%, making this crop particularly vulnerable to this pathogen (Kora et al., 2003; Elsherbiny & Taher, 2018). The control of these diseases is challenging due to their ability to produce sclerotia that are resistant to chemical and biological degradation and can survive in the soil for extended periods. The lack of resistant cultivars, the pathogen's genetic variability, and the increasing occurrence of fungicide resistance further complicate its management (Smolińska and Kowalska, 2018). The most commonly used

methods include crop rotation, sowing resistant cultivars, and applying biological and chemical control measures. In Croatia, certain fungicides containing active ingredients such as difenoconazole, tebuconazole, and boscalid are currently approved for the control of *S. sclerotiorum* (<https://fis.mps.hr/fis/>). Growing concerns about the environmental impact of synthetic fungicides have driven the need for the development of alternative antifungal compounds that are more effective, environmentally friendly, and less toxic to humans and the environment. Quaternary pyridinium salts, particularly nicotinamide and isonicotinamide derivatives, exhibit significant antifungal potential and represent a promising alternative to conventional fungicides (Ye et al., 2014; Bušić et al., 2022; Wang et al., 2022). The applied compounds were synthesized using environmentally friendly methods, including the use of deep eutectic solvents (DES) as an alternative to toxic organic solvents and microwave-assisted synthesis, which enhances reaction efficiency and reduces the environmental footprint (Bušić et al., 2020; Bušić et al., 2022). For the *in vivo* testing, compounds that exhibited the most potent inhibition of *S. sclerotiorum* mycelial growth in previous *in vitro* studies (Bušić et al., 2019) were selected. Since *in vitro* tests do not fully reflect natural conditions, this study aimed to evaluate the antifungal efficacy of selected quaternary pyridinium salts against *S. sclerotiorum* under *in vivo* conditions, using carrots as a natural substrate. The obtained results will contribute to a more accurate evaluation of their potential application in integrated plant protection and support the development of new antifungal agents.

Material and methods

Synthesis and characterization of the tested isonicotinamide derivatives

Quaternary pyridinium salts of isonicotinamide were synthesized via quaternization reactions using microwave-assisted synthesis and deep eutectic solvents (DES) as described by Bušić et al. (2019; 2020). The reactions were carried out with alkyl halides (methyl iodide), alkyl dihalides (dibromopropane, diiodopropane), and substituted 2-bromoacetophenones. Table 1 presents the selected compounds and their chemical designations. These methods enable a more environmentally friendly and safer synthesis, reducing toxic solvents while improving reaction efficiency.

Table 1. The list of selected isonicotinamide derivatives: chemical names and designations

<i>Isonicotinamide derivatives</i>	<i>Label</i>
4-carbamoyl-1-methylpyridinium iodide	(1)
4-carbamoyl-1-(2-phenyl-2-oxomethyl)pyridinium bromide	(4)
4-carbamoyl-1-[2-(4-methylphenyl)-2-oxoethyl]pyridinium bromide	(5)
4-carbamoyl-1-[2-(4-fluorophenyl)-2-oxoethyl]pyridinium bromide	(6)
4-carbamoyl-1-[2-(4-methoxyphenyl)-2-oxomethyl]pyridinium bromide	(7)
4-carbamoyl-1-[2-(4-nitrophenyl)-2-oxoethyl]pyridinium bromide	(10)
1-(3-Bromopropyl)-4-carbamoylpyridinium bromide	(12)

In vivo testing of the effect of synthesized compounds on the mycelial growth of Sclerotinia Sclerotiorum on carrot

The pathogen *S. sclerotiorum* was isolated from a tomato stem and cultivated on a PDA medium. Identification was confirmed by morphological analysis of mycelia and sclerotia and polymerase chain reaction (PCR) of the ITS region using ITS1/ITS4 primers (White et al., 1990). PCR was performed according to Karlsson et al. (2016), and the products were visualized on an agarose gel, purified, and sequenced at Macrogen Europe (Amsterdam, Netherlands). The sequences that were obtained were analyzed by comparing them with data in

the GenBank® database. After the isolation and identification of *S. sclerotiorum*, the pure culture was preserved by three methods: lyophilization (freeze-drying), storage at -80°C in 1.5 mL microtubes with a glycerol–water mixture, and storage at 4°C in 10 mL tubes containing sterile distilled water. The culture maintained in sterile water is renewed every two years.

The *S. sclerotiorum* isolate was first cultivated on potato dextrose agar (PDA, Biolife Italiana S.r.l., Milan, Italy) supplemented with streptomycin at a concentration of 1.30 g L^{-1} (Sigma-Aldrich, St. Louis, MO, USA), and incubated for seven days under controlled conditions (22°C , 12 h light / 12 h dark) to obtain actively growing mycelium for inoculation. The *in vivo* test was conducted using a modified method based on Sarven et al. (2020). Before infection, whole carrots were surface-sterilized with a 1% sodium hypochlorite (NaClO) solution for two minutes, rinsed with distilled water, and air-dried at room temperature.

Artificial infection

Sterile scalpels were used to make wounds approximately 4 mm in diameter on the surface of whole carrots. A pipette was used to apply a specific concentration of the synthesized compound into each wound. Each treatment group and control consisted of 16 carrots (4 carrots $\times$ 4 replicates). The control treatments included a negative control (1% DMSO), a positive control (commercial fungicide, $700\text{ }\mu\text{g mL}^{-1}$: 200 μg difenoconazole + 500 μg tebuconazole), and a distilled water control. All controls were applied in the same manner as the test compounds. Three hours after compound application, a 4 mm diameter mycelial plug, cut from a seven-day-old *S. sclerotiorum* culture grown on PDA, was placed into each wound.

Incubation conditions and mycelial growth monitoring

The carrots were placed on moist absorbent paper inside plastic containers measuring $26 \times 19 \times 6\text{ cm}$, with sterile cotton soaked in water to maintain humidity. Each container held four carrots, and each treatment was represented by four containers. The containers were placed in plastic bags and kept at room temperature ($22 \pm 2^{\circ}\text{C}$) under natural day/night conditions. The diameter of mycelial growth was measured after 48, 120, and 168 hours.

Statistical Data Analysis

The *in vivo* statistical analyses were performed using SAS software (SAS 9.4 for Windows, 2017, and SAS Enterprise Guide 7.1). Data were presented as the arithmetic mean (M) and standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used for comparisons between groups, followed by Tukey's multiple comparison test as a post-hoc analysis (Rowe, 2015). All applied tests were two-tailed, and p values ≤ 0.05 were considered statistically significant.

Results and discussion

In vivo testing was conducted on carrots using selected isonicotinamide derivatives, chosen based on *in vitro* results. The effectiveness of the compounds was tested at concentrations of $10\text{ }\mu\text{g mL}^{-1}$ and $100\text{ }\mu\text{g mL}^{-1}$, and the mycelial growth of *S. sclerotiorum* was monitored over an extended incubation period every 24 hours, from the appearance of mycelium to the formation of sclerotia, allowing for a more precise assessment of the compounds' effects. The results are presented separately according to concentration and incubation time.

Descriptive statistics and a comparison of the compounds at a concentration of $10\text{ }\mu\text{g mL}^{-1}$ are presented in Table 2. Statistical analysis using Tukey's test ($p \leq 0.05$) revealed significant differences among the compounds, allowing them to be ranked according to their antifungal activity. These rankings are shown in Table 2 and form the basis for the interpretation of compound effectiveness discussed below.

A comparison of the antifungal activity among the derivatives revealed that compounds (10) and (12) exhibited significantly stronger inhibitory effects on the mycelial growth of *S. sclerotiorum* compared to compounds (4), (5), (6), and (7). Although compounds (4), (5), (6), and (7) showed notable antifungal activity in *in vitro* assays, they did not exhibit significant inhibitory effects under *in vivo* conditions. In some cases, mycelial growth was even slightly higher than in the control, suggesting a possible stimulatory effect at the tested concentration. Compound (12) completely inhibited mycelial growth throughout the entire incubation period, while compound (10) was highly effective up to 120 hours, after which a slight increase in mycelial growth was observed.

Table 2. Mycelial growth of *Sclerotinia sclerotiorum* (mm) at 10 µg mL⁻¹ concentration of isonicotinamide derivatives

Isonicotinamide derivatives	Time after inoculation (h)		
	120 h M ± SEM	144 h M ± SEM	168 h M ± SEM
(1)	18.25 ± 1.81 A	29.88 ± 5.08 A	39.69 ± 4.99 A
(4)	29.88 ± 6.21 A	41.81 ± 7.51 A	50.88 ± 7.29 A
(5)	26.06 ± 3.03 A	37.13 ± 2.24 A	49.13 ± 5.43 A
(6)	23.63 ± 1.92 A	35.75 ± 3.50 A	49.06 ± 4.36 A
(7)	23.81 ± 4.32 A	34.13 ± 6.08 A	44.44 ± 7.89 A
(10)	0.00 ± 0.00 a	12.75 ± 4.21 Aa	14.38 ± 4.76 Aa
(12)	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Control (distilled water) a	25.63 ± 2.69	37.69 ± 3.71	50.25 ± 4.57
Positive control (difenoconazole and tebuconazole) A	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Comparison of compounds (Tukey's test)		4, 5, 6 < 10 1, 4, 5, 6 < 12	1, 4, 5, 6, 7 < 10 1, 4, 5, 6, 7 < 12

The table presents the mean values of mycelial growth (mm) for four replicates and the standard error of the mean. Significant differences between the compounds and the positive control are indicated by the letter A ($p \leq 0.05$), while significant differences between the compounds and the control (distilled water) are marked with the letter a ($p \leq 0.05$). According to Tukey's test ($p \leq 0.05$), the numbers assigned to the corresponding compounds indicate significant differences among compounds.

Wang et al. (2022) investigated the effect of nicotinamide derivatives on leaves of oilseed rape (*Brassica napus* L.) infested with *S. sclerotiorum*. They found that the compound 6-chloro-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-5-yl)nicotinamide reduced *S. sclerotiorum* mycelial growth by 73.5% but with lower efficacy compared to the commercial fungicide boscalid (94.1% inhibition). Compound (12) in this study exhibited an even more substantial effect under *in vivo* conditions, further confirming its antifungal potential. Although the results demonstrate the effectiveness of isonicotinamide derivatives, differences between *in vitro* and *in vivo* conditions were observed. Thus, compound (10) demonstrated strong antifungal activity in *in vitro* testing (Bušić et al., 2019), but its efficacy was lower under *in vivo* conditions, with mycelial regrowth observed after 120 hours. Similar findings were reported by Yan et al. (2022), where pyridine carboxamide derivatives showed moderate to good antifungal activity against *S. sclerotiorum* *in vitro*. However, they indicated that only 6-chloro-N-(2-(phenylamino)phenyl)nicotinamide exhibited a more substantial effect *in vivo* conditions. These findings emphasize the importance of *in vivo* testing in evaluating compounds' antifungal efficacy and highlight the need for further optimization of formulation and concentration to ensure their long-term effectiveness in plant protection.

Isonicotinamide derivatives (1), (4), (5), (7), (8), and (10), applied at a concentration of 100 $\mu\text{g mL}^{-1}$, exhibited weaker or comparable effects on the mycelial growth of *S. sclerotiorum* compared to the control (distilled water) throughout the entire incubation period (Table 3). In most cases, the measured growth was slightly higher than in the control, indicating a potential stimulatory effect, except for compound (12), which showed partial inhibitory activity. All tested compounds showed significantly lower efficacy compared to the commercial fungicide. Increasing the compound concentration (12) to 100 $\mu\text{g mL}^{-1}$ did not enhance its antifungal effect; its effectiveness decreased over time.

Table 3. Mycelial growth of *Sclerotinia sclerotiorum* (mm) at a concentration of 100 $\mu\text{g mL}^{-1}$ of isonicotinamide derivatives

Isonicotinamide derivatives	Time after inoculation (h)		
	120 h M $\pm$ SEM	144 h M $\pm$ SEM	168 h M $\pm$ SEM
(1)	30.19 $\pm$ 2.64 A	40.56 $\pm$ 2.70 A	40.56 $\pm$ 2.70 A
(4)	29.00 $\pm$ 1.82 A	37.94 $\pm$ 2.44 A	37.94 $\pm$ 2.44 A
(5)	31.63 $\pm$ 1.52 A	44.19 $\pm$ 2.31 A	44.19 $\pm$ 2.31 A
(6)	35.81 $\pm$ 1.67 Aa	47.56 $\pm$ 2.27 Aa	47.56 $\pm$ 2.27 A
(7)	29.56 $\pm$ 1.57 A	45.00 $\pm$ 3.15 A	45.00 $\pm$ 3.15 A
(10)	25.88 $\pm$ 2.94 A	34.81 $\pm$ 3.93 A	34.81 $\pm$ 3.93 A
(12)	12.38 $\pm$ 3.21 Aa	17.81 $\pm$ 5.03 Aa	26.75 $\pm$ 7.47 Aa
Control (distilled water) a	25.63 $\pm$ 2.69	37.69 $\pm$ 3.71	50.25 $\pm$ 4.57
Positive control (difenoconazole and tebuconazole) A	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Comparison of compounds (Tukey's test)	1, 4, 5, 6, 7, 9, 10 < 12 6, 9 < 8	1, 4, 5, 6, 7, 9, 10 < 12 6, 9 < 8	4, 5, 6, 7, 9, < 12 6, 9 < 8

The table presents the mean values of mycelial growth (mm) for four replicates and the standard error of the mean. Significant differences between the compounds and the positive control are indicated by the letter A ($p \leq 0.05$), while significant differences between the compounds and the control (distilled water) are marked with the letter a ($p \leq 0.05$). According to Tukey's test ($p \leq 0.05$), the numbers assigned to the corresponding compounds indicate significant differences among compounds.

In contrast, previous *in vitro* studies (Bušić et al., 2019) demonstrated that the same compounds exhibited a more substantial inhibitory effect on *S. sclerotiorum* mycelial growth with increasing concentration. This discrepancy may be attributed to simplified *in vivo* conditions, including interactions between the compounds and the plant tissue. This discrepancy may be attributed to different ambient factors, interactions between the compounds and plant tissues, or reduced compound stability or interactions with the natural substrate under *in vivo* conditions. Similar findings have been reported for other nicotinamide derivatives, indicating that antifungal activity is not solely dependent on concentration but also the specific characteristics of the compound (Siber, 2025).

Conclusions

The study demonstrated that isonicotinamide derivatives exhibit antifungal activity against *S. sclerotiorum* under *in vivo* conditions. Compound (12) at 10 $\mu\text{g mL}^{-1}$ completely inhibited mycelial growth, while compound (10) was effective for up to 120 hours, after which a slight increase in growth was observed. At a higher concentration (100 $\mu\text{g mL}^{-1}$), the efficacy of some compounds decreased, suggesting possible interactions with plant tissues or reduced stability under *in vivo* conditions. Unlike *in vitro* results, where increasing the concentration enhanced

antifungal activity, this trend was not consistently observed *in vivo*. These findings highlight the importance of *in vivo* testing for a more realistic evaluation of compound efficacy and underscore the need for further formulation optimization to improve stability and applicability in plant protection.

References

- Bušić, V., Roca, S., Vikić-Topić, D., Vrandečić, K., Čosić, J., Molnar, M., Gašo-Sokač, D. (2020). Eco-friendly quaternization of nicotinamide and 2-bromoacetophenones in deep eutectic solvents. Antifungal activity of the products. *Environmental Chemistry Letters*, 1-6. <https://doi.org/10.1007/s10311-020-00973-3>
- Bušić, V., Vrandečić, K., Siber, T., Roca, S., Gašo Sokač, D. (2022). Novel Synthetic Routes to Quaternary Pyridinium Salts and their Antifungal Activity. *Croatica Chemica Acta*, 95(1), 1-8. <https://doi.org/10.5562/cca3903>
- Bušić, V., Vrandečić, K., Siber, T., Roca, S., Vikić Topić, D., Gašo Sokač, D. (2019). A rapid microwave induced synthesis of isonicotinamide derivatives and their antifungal activity. *Croatica chemica acta*, 92(1), 125-135. <https://doi.org/10.5562/cca3527>
- Elshehry, E. A., Taher, M. A. (2018). Silicon induces resistance to postharvest rot of carrot caused by *Sclerotinia sclerotiorum* and the possible of defense mechanisms. *Postharvest Biology and Technology*, 140, 11-17. <https://doi.org/10.1016/j.postharvbio.2018.02.004>
- Karlsson, I., Edel-Hermann, V., Gautheron, N., Durling, M. B., Kolseth, A. K., Steinberg, C., Friberg, H. (2016). Genus-specific primers for study of *Fusarium* communities in field samples. *Applied and environmental microbiology*, 82(2), 491-501. <https://doi.org/10.1128/AEM.02748-15>
- Kora, C., McDonald, M. R., Boland, G. J. (2003). Sclerotinia rot of carrot: an example of phenological adaptation and bicyclic development by *Sclerotinia sclerotiorum*. *Plant Disease*, 87(5), 456-470.
- Lehner, M. S., de Paula Júnior, T. J., Del Ponte, E. M., Mizubuti, E. S., Pethybridge, S. J. (2017). Independently founded populations of *Sclerotinia sclerotiorum* from a tropical and a temperate region have similar genetic structure. *PLoS One*, 12(3). <https://doi.org/10.1371/journal.pone.0173915>
- Rowe, P. (2015). *Essential statistics for the pharmaceutical sciences*. John Wiley & Sons. <https://doi.org/10.1002/9781119109075>
- Sarven, M. S., Hao, Q., Deng, J., Yang, F., Wang, G., Xiao, Y., Xiao, X. (2020). Biological control of tomato gray mold caused by *Botrytis cinerea* with the entomopathogenic fungus *Metarhizium anisopliae*. *Pathogens*, 9(3), 213. <https://doi.org/10.3390/pathogens9030213>
- Siber, T. (2025). *Antifungalno djelovanje kvaternih piridinijevih spojeva na fitopatogene polifagne vrste gljiva rodova Fusarium, Botryotinia i Sclerotinia* (Doktorska disertacija). Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Hrvatska.
- Smolińska, U., Kowalska, B. (2018). Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum*—a review. *Journal of Plant Pathology*, 100, 1-12. <https://doi.org/10.1007/s42161-018-0023-0>
- Wang, W., Liu, X. J., Lin, G. T., Wu, J. P., Xu, G., Xu, D. (2022). Novel N-(1H-Pyrazol-5-yl) nicotinamide Derivatives: Design, Synthesis and Antifungal Activity. *Chemistry & Biodiversity*. <https://doi.org/10.1002/cbdv.202101032>
- White, T. J., Bruns, T., Lee, S. J. W. T., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18(1), 315-322.
- Yan, Z., Yang, Z., Qiu, L., Chen, Y., Li, A., Chang, T., Jin, F. (2022). Discovery of novel pyridine carboxamides with antifungal activity as potential succinate dehydrogenase inhibitors. *Journal of Pesticide Science*, 47(3), 118-124. <https://doi.org/10.1584/jpestics.D22-017>
- Ye, Y. H., Ma, L., Dai, Z. C., Xiao, Y., Zhang, Y. Y., Li, D. D., Zhu, H. L. (2014). Synthesis and antifungal activity of nicotinamide derivatives as succinate dehydrogenase inhibitors. *Journal of agricultural and food chemistry*, 62(18), 4063-4071. <https://doi.org/10.1021/jf405437k>
- <https://fis.mps.hr/fis/> (Accessed: 7 March 2025)

ORIGINAL SCIENTIFIC PAPER

Agronomic traits of 'Pinot Gris' and 'Riesling' grapevine varieties in the "Guyot" and "double Guyot" training systems

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Summary

In northwestern Croatia, varieties like 'Riesling' and 'Pinot gris' are frequently found in the vineyards, where the most common training systems are "Guyot" and "double Guyot". This study aims to compare these training systems in the new context of global warming and the possibility of extreme weather conditions. Research results show a significantly higher number of clusters at variety 'Riesling' on the „double Guyot“ training system. Yield per vine was also greater for both varieties at the „double Guyot“ training system. A significantly higher sugar concentration was observed for the “double Guyot” training system for both varieties. This research emphasized the importance of training system selection regarding a particular variety. Moreover, it was confirmed that for the above mentioned varieties, the training system with a higher yield do not necessarily reduce the must quality.

Key words: Training system, Guyot, double Guyot, 'Riesling', 'Pinot gris'

Introduction

The grapevine training system accomplishes many objectives. The most important, perennial wood and canes can be disposed in such a way as to manipulate the exposure of leaf area to maximize the interception of light, leading to higher yield potential, optimization of the leaf area to fruit ratio, higher quality of grape, and better disease control (Raynolds and Huves, 2009). One of the roles of training systems in cool climates is to maximize fruit exposure to optimize berry growth and composition. Fruit in exposed portions of the canopy generally exhibit higher concentrations of sugars, anthocyanins, and total phenolics, as well as lower levels of malic acid, potassium, and juice pH compared with shaded fruits (Smart and Robinson, 1991). Training systems can have a significant impact on vine yield, although results are very site- and cultivar-dependent. Increases in yield were simply due to the addition of more shoots per vine and per meter. The result of this is an increased number of clusters per wine (Raynolds and Huves, 2009). In ‘Shiraz’, minimally pruned vines produced substantially greater yields through an increase in shoot numbers and hence cluster number per meter of canopy (Wolf et al., 2003). In research by Vanden Heuvel et al. (2004), the yield of ‘Chardonnay’ increased on the dual cane system due to an increase in berry number per cluster, leading to an increase in cluster weight. In a comparison of four training systems (simple “Guyot”, “double Guyot”, “horizontal spurred cordon”, “vertical spurred cordon”), yields ranged among systems from 7.5 to 9.7 tons per hectare, although the training system had little or no impact on grape or wine composition, with sensory analysis showing no difference among systems (Peterlunger et al., 2002). Research has shown that in some varieties, lower yield contributes to larger clusters with better berry and must quality, but at the same time, in some varieties, higher loading does not mean a reduction in berry and must quality (Jackson et al., 2008)

The “double Guyot” training system was widely applied in winegrape production in northwestern Croatia during the 1990s and early 2000s. This system became prevalent due to its ability to reduce the number of vines per hectare. The most varieties were grafted onto the

vigorous Kober 5BB rootstock, which favoured higher yields per hectare. Additionally, it facilitated mechanization and cultivation practices of this period. Nowadays, the “Guyot” training system and the SO4 rootstock are the most commonly used. Moreover, improved mechanization and cultivation possibilities have led to changes in the expectations for final yield and grape quality in contemporary wine production. Detailed research comparing the two training systems in the continental part of Croatia is rare. Additionally, the impact of global warming, including extreme temperatures, high radiation index and water deficits in August and early September may cause different levels of stress on plants, depending on the training system employed. The objective of this study is to compare the two most prevalent grapevine training systems in the northwestern region of Croatia within the context of global warming and the potential for extreme weather conditions.

Material and methods

The experimental vineyard is located in the village of Grtovec, at the base of Ivanščica mountain at 350 meters above sea level, with a southeastern exposition. The experiment was conducted in a vineyard planting of the varieties ‘Riesling’ and ‘Pinot gris’. ‘Riesling’ is a high-quality late-ripening wine variety, while ‘Pinot gris’ is a high-quality early-ripening variety. Both are commonly grown in the vineyards of northwestern Croatia. The soil in the experimental vineyard is anthropogenic pseudogley. The vineyard was planted in 2001 on previously prepared soil. The vines were grafted onto Kober 5BB rootstock. The planting distance between rows is 1.6 meters, and the distance between vines is 0.8 meters. The training systems are „Guyot“ and „double Guyot“. The „Guyot“ training system was formed by one spur with two buds and one cane with 12 buds. The „double Guyot“ was formed by two spurs with two buds and two canes with 12 buds, each positioned on opposite sides of the trunk. The experiment was conducted during season 2023. The annual temperature average was 12.4°C (Zlatar area). The total annual precipitation was 1034 mm of rain, of which only 459 mm fell during the growing season. In July and August there was 250 mm of rain, which is a lot for two summer months that are usually dry (Croatian Meteorological and Hydrological Service).

For each variety, ten vines were selected for each training system, resulting in a total of 40 vines in the experiment. At harvest, the number of clusters per vine was determined, and the yield per vine was weighed (kilograms, kg). Average cluster mass was determined by dividing the yield per vine by the number of bunches per vine. Ten clusters per variety and training system were randomly sampled. Laboratory analysis was conducted to determine the following parameters: average mass of 30 berries (grams, g). Additionally, three must samples, each derived from ten clusters, were analysed for sugar concentration (degree Oechsle, °Oe), total acidity (gram per liter, g/L) and pH value according the “Compendium of international methods of wine and must analysis”, official document given by International Organisation of Vine and Wine.

The data obtained from the research were statistically processed using the analysis of variance (ANOVA), with testing the difference in mean values according to the Duncan multiple range test. The statistical analysis was performed using the XLSTAT Statistical and Data Analysis Solution software (Addinsoft, 2024).

Results and discussion

The yield results indicate a significantly higher number of clusters in the 'Riesling' variety when grown on the „double Guyot“ training system (Table 1). For the 'Pinot gris' variety, the number of clusters was also higher with the „double Guyot“ training system, although the difference was not significant. Yield per vine was greater at „double Guyot“ training system which implies greater shoots per vine, for both varieties. However, this difference was not significant. These findings can be attributed to the significantly higher number of buds per vine left by pruning

for both varieties. This result supports the conclusion of Raynolds and Huves (2009) that increases in yield were simply due to the addition of more shoots per vine.

Cluster characteristics show that there was no significant difference in average cluster weight between two training system. The average cluster weight was slightly higher on the „Guyot“ training system, but not significant. No significant difference in the weight of 30 berries was found. These results suggest that attention should be paid to the choice of training system when cultivating the ‘Riesling’ variety because number of clusters vary significantly depending on the which training system used.

Table 1. Results for yield and cluster characteristics

Variety	Training system	Number of clusters per vine	Yield per vine (kg)	Cluster average weight (g)	30 berry average mass (g)
‘Pinot gris’	Guyot	14,6a*	2,24a	166,82a	42,86a
	Double Guyot	18,5a	2,55a	145,25a	41,47a
	Pr>F**	0,1	0,37	0,468	0,593
‘Riesling’	Guyot	17,30b	2,14a	138,83a	45,18a
	Double Guyot	28,20a	2,94a	104,45a	47,00a
	Pr>F	0,005	0,081	0,136	0,435

*Mean values marked with different letters are statistically different at $p < 0.05$.

**The difference is significant for $(Pr > F) < 0.05$ with 95% probability.

The results of must analysis show significant differences in sugar concentration between two training systems for both varieties (Table 2). A significantly higher sugar concentration was observed for “double Guyot”. This finding is unusual in practice because it is usually considered that higher yield correlates with lower must quality. However, this result supports the conclusion of Jackson et al. (2008) that in some varieties the yield does not necessarily affect the quality of the berry content. This is especially evident in the ‘Riesling’ variety, where a significant increase in number of clusters and increase in yield did not negatively affect the quality of the must, but rather enhanced it. For the ‘Pinot Gris’ variety, although there was no significant increase in yield, cluster number and average grape mass in the “double Guyot” training system, there was an increase in must quality. Both training systems also affect titrable acidity and pH value of the must (Table 2). Titrable acidity was higher in the “double Guyot” training system for both varieties, and for ‘Pinot gris’ significantly. The pH value was significantly higher in the ‘Guyot’ training system for the ‘Riesling’ variety.

Table 2. Results of must analysis

Variety	Training system	Sugar concentration ($^{\circ}\text{Oe}$)	Titrable acidity (g/L)	pH value
‘Pinot gris’	Guyot	88,66 b*	6,38 b	3,21 a
	Double Guyot	100,00 a	7,33 a	3,25 a
	Pr > F **	0,013	0,011	0,123
‘Riesling’	Guyot	74,66 b	7,18 a	2,98 a
	Double Guyot	87,66 a	7,91 a	2,93 b
	Pr > F	0,004	0,131	0,011

*Mean values marked with different letters are statistically different at $p < 0.05$.

**The difference is significant for $(Pr > F) < 0.05$ with 95% probability

Conclusions

This research confirmed the importance of selecting an appropriate training system when planning the cultivation of a particular grape variety. It was also confirmed that for some varieties, training system with a higher yield does not necessarily have a negative impact on must quality. To confirm these results, especially in different climatic conditions, multi-year research should be conducted.

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References

- Croatian Meteorological and Hydrological Service- meteorological and hydrological data for Zlatar area in 2023.
- Jackson R. S. (2008.) Wine science, principles and applications, Academic press, San Diego
- Compendium of international methods of wine and must analysis (2024) International organization of Vine and Wine. ISBN Volume I : 978-2-85038-096-9
- Peterlunger E., Celotti E., Da Dalt G., Stefanelli S., Gollino G., Zironi R. (2002) Effect of training system on Pinot noir grape and wine composition. American. Journal of Enology and Viticulture 53:14-18.
- Reynolds A. G., Vanden Heuvel J. E. (2009) Influence of Grapevine Training Systems on Vine Growth and Fruit Composition. American Society for Enology and Viticulture: 251 – 259
- Smart R.E., Robinson M. (1991) Sunlight into Wine: A Handbook for Winegrape Canopy Management. Winetitles, Underdale, Australia.
- Vanden Heuvel J.E., Proctor J.T.A., Sullivan J.A., Fisher K.H. (2004) Influence of training/trellising system and rootstock selection on productivity and fruit composition of Chardonnay and Cabernet franc grapevines in Ontario, Canada. American Journal of Enology and Viticulture 55:253-264.
- Wolf T.K., Dry P.R., Iland P.G., Botting D., Dick J., Kennedy U., Ristic R. (2003) Response of Shiraz grapevines to five different training systems in the Barossa Valley. Australian. Journal Grape Wine Research. 9:82-95.

ORIGINAL SCIENTIFIC PAPER

Can the stinging nettle stem open up new possibilities as a phytochemical source?

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Summary

The stinging nettle stem is often discarded as biowaste despite its potential as a valuable by-product. The aim of this research was to determine its phytochemical content and evaluate its potential as a potential functional source. A comparative analysis of wild (WSN) and hydroponically grown nettle (HSN) assessed differences in phytochemicals, antioxidant capacity, and photosynthetic pigments. WSN had higher polyphenols (TPC, TNFC) and antioxidant capacity, while HSN had more flavonoids (TFC) and chlorophyll. Ascorbic acid levels were similar. These findings support the reuse of nettle stems as high-value products for functional foods within the circular bioeconomy concept.

Key words: polyphenols, hydroponic cultivation, re-use, bioactive compounds, antioxidant capacity

Introduction

As part of the new EU circular economy action plan (CEAP) a series of measures and plans are being adopted to conserve natural resources and reduce greenhouse gas emissions and waste. One of the main priorities is to create added value by changing how we consume, produce and dispose of raw materials. Therefore, any process of production, processing and transformation of raw materials should primarily support practices that prevent the generation of waste or provide opportunities for its sustainable recovery and reuse into high value-added products. The stinging nettle (*Urtica dioica* L.) is a plant species that offers many possibilities for use in food, but also in the cosmetics, pharmaceutical and natural dye industries due to its unique and complex chemical profile (Krajewska and Metlińska, 2022; Opačić et al., 2022). Nettle leaves, roots or seeds are mainly used as food or for processing into value-added products, while the stem is usually removed and represents biowaste. The stem accounts for about 15-35% of the above-ground biomass of the plant, and the proportion depends mainly on specific pedoclimatic and agrotechnical measures during cultivation (Opačić et al., 2023). According to the available literature, the stem is an important source of lignocellulosic fibres, especially cellulose and hemicellulose, and to a lesser extent lignin (Di Virgilio et al., 2015), then proteins (especially amino acids such as alanine, glycine, serine and glutamic acid) (Bhusal et al., 2022). It is also rich in minerals such as calcium, magnesium, potassium and silica (Rafajlovska et al., 2013), as well as numerous specialized metabolites, especially polyphenols, which are also characterized by their antioxidant potential (Devkota et al., 2022). Based on all this, the stinging nettle stem is potentially a valuable source of nutrients that could be used in the production of new sustainable products. Accordingly, the aim of this research was to determine the content of specialized metabolites of the nettle stem and, based on the data obtained, to evaluate the possibilities of its utilization to obtain value-added products.

Material and methods

The study was conducted in 2022. using wild and cultivated nettle grown in a greenhouse of the Department of Vegetable Crops of the University of Zagreb Faculty of Agriculture (FAZ). The cultivated nettle samples were grown using the floating hydroponic technique as part of a Croatian Science Foundation project (IP-2019-04-3325). The experiment was conducted in the fall-winter growing season. The nettles were sown in polystyrene plates filled with perlite; 50 seeds (B&T World Seeds, France) were sown per row opening, corresponding to 1.2 g m⁻². After 6 days of germination at 20-25°C and a relative humidity of 60-70%, the plates were placed in basins. The plates were arranged in a randomised block design with three replicates. A nutrient solution was prepared according to the Johnson recipe (Opačić et al., 2024), and the basin was initially filled to a column height of 25 cm. During the growing period, the nutrient solution was adjusted as needed. The nettle was manually mowed with scissors in March 2022. After mowing, the stems were separated from the leaves. The samples of wild nettle were collected in April 2022 in the area of the city of Zagreb, near the Sava dam and Lake Jarun (45°47'2.37 "N 15°56'29.08 "E). The analysis of phytochemical content was conducted in the Laboratory for Quality Analysis of Plant-Based Agricultural Products (FAZ) and included the determination of: ascorbic acid (AsA, mg 100 g⁻¹ FW) following the standard AOAC method (2002); total polyphenolic compounds (flavonoids and non-flavonoids, mg GAE 100 g⁻¹ FW) spectrophotometrically (UV-1900i, Shimadzu, Japan) at 750 nm, according to the method of Ough and Amerine (1988); photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls, and total carotenoids, mg g⁻¹) spectrophotometrically at wavelengths of 662, 644, and 440 nm, following the method of Holm (1954); and antioxidant capacity using two assays: ABTS, according to Miller et al. (1993), and FRAP, according to Benzie and Strain (1996). The research data were statistically analyzed using the SAS software system, version 9.3 (SAS/STAT, 2010). The results were subjected to analysis of variance (ANOVA), and all laboratory analyses were conducted in triplicate. Mean values were compared using the t-test (LSD) and were considered significantly different at $p \leq 0.0001$.

Results and discussion

The bioactive compounds content in the stem of wild (WSN) and hydroponically grown nettle (HSN) are presented in Table 1. Statistically significant differences were recorded only for polyphenolic compounds (TPC, TNFC and TFC), while the ascorbic acid content (AsA) did not differ significantly between the WSN and HSN. The average AsA content was 24.07 mg 100 g⁻¹ FW. Furthermore, TPC and total non-flavonoids (TNFC) content was significantly higher in WSN compared to HSN. For instance, the TPC content was approximately 16% higher in WSN than in HSN, while the TNFC content was 82% higher in WSN compared to HSN. However, an opposite trend was observed for total flavonoids (TFC), with significantly higher TFC values recorded in HSN compared to WSN, approximately 58% higher. According to the results, the significantly higher TPC and TNFC values in WSN compared to HSN, can be explained by the specific origin of the plant material. WSN samples represent nettle collected from natural habitats, while HSN samples are hydroponically grown in a controlled environment. Pedoclimatic factors, particularly in the case of WSN, significantly impact TPC levels, as these compounds are synthesized in the plant in response to stress factors such as high temperatures, water scarcity, and light exposure. Therefore, it is expected that WSN samples will accumulate more polyphenolic compounds compared to HSN, which, during their growth in controlled environments, are exposed to optimized abiotic factors (e.g., adequate temperature, nutrient availability), reducing their exposure to stress and consequently lowering the need for polyphenolic compound synthesis as a defensive response (Opačić et al., 2024). Notably, numerous literature sources (Radman et al., 2021; Opačić et al., 2022; Đurović et al., 2024; Opačić et al., 2024;) emphasize the exceptionally high nutritional value of nettle,

particularly its high levels of ascorbic acid (even around 117 mg, Dujmović et al., 2023) and polyphenolic compounds (Devkota et al., 2022; Đurović et al., 2024). However, most studies focus on nettle leaves or the entire above-ground part of the plant, while data on bioactive compounds in the stem remain largely absent from the literature.

Table 1. Bioactive Compound Content of Wild and Hydroponically Grown Nettle Stem

Sample	AsA	TPC	TNFC	TFC
WSN	23.78±1.35	56.46±1.45 ^a	39.39±0.58 ^a	17.07±1.45 ^b
HSN	24.35±1.46	48.58±6.48 ^b	21.64±0.45 ^b	26.94±±6.41 ^a
ANOVA	NS	p≤0.1571	p≤0.0001	p≤0.0001

WSN- stem from wild nettle; HST- stem from hydroponically grown nettle; AsA- ascorbic acid content (mg 100 g⁻¹ FW); TPC- total polyphenolic content (mg GAE 100 g⁻¹ FW); TNFC- total non-flavonoid content (mg GAE 100 g⁻¹ FW); TFC- total flavonoid content (mg GAE 100 g⁻¹ FW); NS- not significant. The results are expressed as the mean value with ±standard deviation. Different letters indicate significant differences between mean values at p≤0.01.

Statistically significant differences in the content of photosynthetic pigments between WSN and HSN (Table 2) were recorded for all analyzed pigments, except for total carotenoids (TCa), where no significant difference was found depending on the plant material origin. The content of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (TCh) was significantly higher in HSN stem samples compared to WSN, with TCh being approximately 33% higher in HSN compared to WSN. This result is expected, considering that the synthesis and accumulation of chlorophyll pigments are significantly influenced by factors such as light and nutrient availability, specifically nitrogen. Chlorophyll accumulation is significantly stimulated under conditions of higher nitrogen availability, which promotes plant growth and development, consequently affecting the increase in chlorophyll pigments (Noor et al., 2023). Furthermore, higher light intensity can negatively affect photosynthetic pigments if plants are exposed to excessive light (Vrkić et al., 2024). Therefore, HSN samples grown in a controlled environment with adequate nutrient supply were expected to accumulate more photosynthetic pigments than WSN. In general, the values of photosynthetic pigments in nettle stems are not high, especially when compared to the leaves (Dujmović et al., 2023).

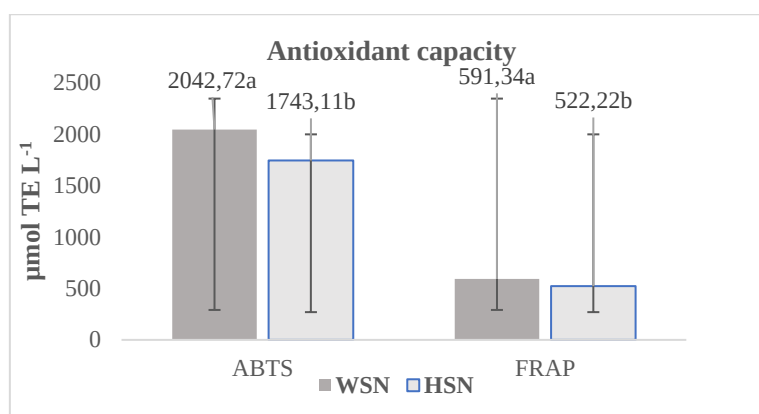
Table 2. Photosynthetic Pigments Content in the Stem of Wild and Hydroponically Grown Nettle

Sample	Chl_a	Chl_b	TCh	TCa
WSN	0.05±0.01 ^b	0.04±0.01 ^b	0.09±0.01 ^b	0.02±0.01
HSN	0.07±0.01 ^a	0.05±0.01 ^a	0.12±0.01 ^a	0.02±0.01
Of measurementANOVA	p≤0.0502	p≤0.0419	p≤0.0032	NS

WSN- stem from wild nettle; HST- stem from hydroponically grown nettle; Chl_a- chlorophyll a content (mg g⁻¹); Chl_b- chlorophyll b content (mg g⁻¹); TCh- total chlorophyll content (mg g⁻¹); TCa- total carotenoid content (mg g⁻¹); NS- not significant. The results are expressed as the mean value with ±standard deviation. Different letters indicate significant differences between mean values at p ≤ 0.01.

The antioxidant capacity of nettle stems was assessed using two assays, ABTS and FRAP (Graph 1). Significant statistical differences in antioxidant capacity were observed between the analyzed samples depending on the ABTS and FRAP methods. Notably, both methods indicated significantly higher antioxidant capacity values for the WSN sample compared to the HSN sample. For instance, the ABTS method showed approximately 18% higher antioxidant capacity in WSN compared to HSN, while the FRAP method indicated an increase of approximately 13%. Regardless of the method used, WSN samples exhibited significantly

higher antioxidant capacity compared to HSN, which is expected given their higher levels of polyphenolic compounds (TPC and TNFC). Polyphenols are potent antioxidants that significantly contribute to the overall antioxidant capacity. Once again, since wild plant material in its natural habitat is more exposed to abiotic factors that can stimulate plant defense mechanisms, the accumulation of molecules with strong antioxidant activity serves as a protective strategy, supporting the plant's growth, development, and survival. Other literature sources (Otlés and Yalcin, 2012) also suggest a high antioxidant capacity of nettle stems, with even higher values reported depending on the collection location compared to those found in the present study.



WSN- stem from wild nettle; HSN- stem from hydroponically grown nettle. The results are expressed as the mean value with $\pm$ standard deviation. Different letters indicate significant differences between mean values at $p \leq 0.01$.

Graph 1. Antioxidant capacity of the Stem of Wild and Hydroponically Grown Nettle

Conclusions

This study highlights the potential of stinging nettle stems as a valuable phytochemical source. Significant differences were observed between the stems of wild (WSN) and hydroponically grown nettle (HSN). WSN exhibited higher levels of total polyphenols (TPC, TNFC), particularly non-flavonoids, and greater antioxidant capacity (ABTS, FRAP). In contrast, HSN had higher flavonoid (TFC) and chlorophyll content, influenced by controlled nutrient availability. Ascorbic acid levels remained consistent. While most studies focus on nettle leaves, these findings underscore the bioactive potential of stems, including polyphenols, chlorophyll, and vitamins, for nutraceutical and functional food applications. Instead of discarding nettle stems, they can be repurposed as valuable by-products, contributing to the circular bioeconomy. Further research should explore their application in functional foods and other products, promoting the sustainable use of this underutilized plant part.

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References

- AOAC (2002). Official methods of Analysis (17th ed.). Association of Official Analytical Chemists. Washington, USA.
- Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1):70-76.

- Bhusal, K. K., Magar, S. K., Thapa, R., Lamsal, A., Bhandari, S., Maharjan, R., Shrestha, S. Shrestha, J. (2022). Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. *Heliyon*, 8(6): e09717.
- Devkota, H. P., Paudel, K.R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., Jha, N. K., Das, N., Singh, S.K., Chellappan, D. K., Dua, K. (2022). Stinging nettle (*Urtica dioica* L.): Nutritional composition, bioactive compounds, and food functional properties. *Molecules*, 27(16): p.5219.
- Di Virgilio, N., Papazoglou, E. G., Jankauskiene, Z., Di Lonardo, S., Praczyk, M., Wielgusz, K. (2015). The potential of stinging nettle (*Urtica dioica* L.) as a crop with multiple uses. *Industrial Crops and Products*, 68: 42-49.
- Dujmović, M., Opačić, N., Radman, S., Fabek Uher, S., Čoga, L., Petek, M., Voća, S., Šic Žlabur, J. (2023). How to Increase the Nutritional Quality of Stinging Nettle Through Controlled Plant Nutrition. *Food technology and biotechnology*, 61(4): 451-464.
- Đurović, S., Kojić, I., Radić, D., Smyatskaya, Y.A., Bazarnova, J. G., Filip, S., Tosti, T., (2024). Chemical Constituents of Stinging Nettle (*Urtica dioica* L.): A Comprehensive Review on Phenolic and Polyphenolic Compounds and Their Bioactivity. *International Journal of Molecular Sciences*, 25(6): p.3430.
- Holm, G. (1954). Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica*, 4: 457–471.
- Krajewska, A. and Mietlińska, K. (2022). Determining the parameters of the Stinging Nettle (*Urtica dioica* L.) hydrolate distillation process. *Molecules*, 27(12): p.3912.
- Miller N.J., Diplock A.T., Rice-Evans C., Davies M.J., Gopinathan V., Milner A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, 84(4): 407–412.
- Noor, H., Ding, P., Ren, A., Sun, M. and Gao, Z. (2023). Effects of nitrogen fertilizer on photosynthetic characteristics and yield. *Agronomy*, 13(6): p.1550.
- Opačić, N., Radman, S., Fabek Uher, S., Benko, B., Voća, S. and Šic Žlabur, J. (2022). Nettle cultivation practices- From open field to modern hydroponics: A case study of specialized metabolites. *Plants*, 11(4): p.483.
- Opačić, N., Benko, B., Toth, N., Petek, M., Čoga, L., Dujmović, M., Šic Žlabur, J., Radman, S. (2023). Agronomic traits of hydroponically grown nettle under conditions of two irrigation intervals. In: *58th Croatian & 18th International Symposium on Agriculture Proceeding*, Carović Stanko, K., Širić, I. (ed.), 169-174. Zagreb, Croatia: University of Zagreb Faculty of Agriculture.
- Opačić, N., Radman, S., Dujmović, M., Fabek Uher, S., Benko, B., Toth, N., Petek, M., Čoga, L., Voća, S., Šic Žlabur, J. (2024). Boosting nutritional quality of *Urtica dioica* L. to resist climate change. *Frontiers in plant science*, 15: p.1331327.
- Otles, S., Yalcin, B. (2012). Phenolic Compounds Analysis of Root, Stalk, and Leaves of Nettle. *The Scientific World Journal*, 2012: p. 564367.
- Ough, C.S., Amerine, M.A. (1988). *Methods for Analysis of Musts and Wines*, 2nd ed.; John Wiley & Sons: New York, NY, USA.
- Radman, S., Javornik, M., Žutić, I., Opačić, N., Benko, B. (2021). Impact of different nutrient solution composition on stinging nettle growth and mineral content. *Acta horticulturae*, 1320: 157-166.
- Rafajlovska, V., Kavrovski, Z., Simonovska, J. Srbinoska, M. (2013). Determination of protein and mineral contents in stinging nettle. *Quality of Life*, 7(1-2): 26-30.
- Vrkić, R., Šic Žlabur, J., Dujmović, M. Benko, B. (2024). Can LED Lighting Be a Sustainable Solution for Producing Nutritionally Valuable Microgreens?. *Horticulturae*, 10(3): p.249.

Examination of the effects of composted and non-composted sewage sludge on maize's physiological and antioxidative responses

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Summary

The aim of this study was to demonstrate that composted sewage sludge (CSS) has more favorable, positive effects on the measured parameters compared to non-composted sewage sludge (NCSS). In addition, the study hypothesized that the measured biochemical parameters increase parallel with the applied concentration of the test materials. The results indicated that NCSS treatment led to higher measured parameter values compared to CSS. Chlorophyll-a and, carotenoids, and Fv/Fo values were significantly higher at all CSS treatments relative to the control. While the activity of antioxidant enzymes was higher at NCSS compared to the CSS treatments.

Key words: alternative nutrients, chlorophyll, maize, oxidative stress, soil amendment

Introduction

The sustainable management of agricultural practices often requires innovative approaches to optimize crop growth and environmental health. Among these, the use of sewage sludge as an organic amendment has been given attention for its potential to improve soil fertility and support plant growth. Sewage sludge, a by-product of wastewater treatment, is rich in organic matter and provides an immediate source of nutrients, promoting rapid growth during early plant development (Marin and Rusănescu, 2023). However, its potential for heavy metal accumulation and inconsistent nutrient release can lead to physiological stress, negatively affecting chlorophyll content, leaf area, and overall biomass (Namdari et al., 2024). These conditions increase reactive oxygen species (ROS) levels, prompting plants to enhance antioxidative enzyme activity to counteract ROS. While this helps protect cells, prolonged exposure can overwhelm the antioxidative system, lowering metabolic efficiency and slowing growth.

Composting is a biological process that stabilizes organic waste, decreases pathogens, and increases the nutrient profile of sludge. It also increases the availability of beneficial microbes and organic compounds, making it a safer and more effective soil amendment (Pajura 2024). Despite these benefits, it is crucial to investigate the physiological and antioxidative responses of crops to both composted (CSS) and non-composted sludge (NCSS) to evaluate their viability and safety. CSS-treated plants exhibit lower ROS accumulation and a more balanced antioxidative response. This reduced stress is due to the enhanced stability and quality of composted sludge, which limits the bioavailability of heavy metals and other harmful compounds. As a result, CSS-treated plants demonstrate better growth resilience and reduced oxidative damage compared to NCSS-treated plants. In addition, CSS provides a more balanced and gradual nutrient release, supporting better growth rates, higher leaf chlorophyll content,

and improved photosynthetic efficiency (Pajura 2024). Prates et al. (2022) showed that CSS-treated maize induced significantly high plant height, stem diameter, and yield parameters. In addition, stronger root systems and enhanced tolerance to abiotic stresses, such as drought or salinity, were also reported in maize.

Although CSS and NCSS can be used as alternative nutrient materials, their effects on plants should be clarified before agricultural use. The goal of this study was to compare the effects of the CSS and NCSS on maize and to establish a more favorable product for agricultural use. The measured parameters included chlorophyll and carotenoid content, photochemical efficiency PSII system), and antioxidative parameters (ascorbate oxidase, guaiacol peroxidase, and superoxide dismutase). The study hypothesized that the measured biochemical parameters increase in proportion to the applied concentration of the test materials (0, as control, 25, 50, and 75% m/m).

Material and methods

The experiment was conducted in the glasshouse of the Institute of Horticulture, Faculty of Agriculture, Food and Environmental Sciences, University of Debrecen. The test plant was maize (*Zea mays* L. cv. Armagnac). Four treatments of non-composted and composted SS (0%, 25%, 50%, and 75% m/m) originated from a sewage treatment plant in Kecskemét, Hungary. The soil used in a pot experiment was poor-quality saline soil. The photosynthetic pigments were determined by the method of Moran and Porath (1980) and Wellburn (1994), and the photochemical efficiency PSII system was measured using the pulse-modulated chlorophyll fluorescence induction procedure (Murchie and Lawson, 2013) using an OS5p+ pulse-modulated portable chlorophyll fluorometer (Opti-Sciences, Hudson, USA). The leaves were dark-adapted for 20 min before the measurements. The enzyme extracts for the determination of antioxidant enzyme activity were prepared by the method of Pukacka and Ratajczak (2005). The activity of ascorbate peroxidase was carried out by the method of Mishra et al. (1993), and the guaiacol peroxidase activity was measured according to Zieslin and Ben-Zaken (1991). The superoxide dismutase activity was determined based on Giannopolities and Ries (1977), and Beyer and Fridovich (1987). The data were analyzed for normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The Mann–Whitney U nonparametric test ($p < 0.05$) was used to compare the mean values. Significant differences were denoted by lowercase letters. The statistical analysis was performed using IBM SPSS Statistics 25 software (Armonk, NY, USA).

Results and discussion

Leaf chlorophyll content provides an understanding of the physiological status of plants. Measuring chlorophyll (chl)-a and chl-b levels, as well as chl-a fluorescence, provides basic information about the photosynthetic status of plants. Chl-a fluorescence is used to detect changes in photosystem II (PSII) that occur under stress conditions. Typically, stressed plants experience a decrease in total chlorophyll content, accompanied by an increase in the chl-a/b ratio (Fang et al., 1998). While chlorophyll-a is involved in the reaction centers of photosynthesis, chlorophyll-b primarily functions in light harvesting.

In this study, the photosynthetic pigment content significantly increased at all treatments compared to the control (Table 1). The highest chl-a and chl-b contents accumulated under 25% NCSS treatment (chl-a was 2.4-fold and chl-b was 2.5-fold higher compared to the control). Chlorophyll-b is more susceptible to photodamage under stress conditions compared to chl-a. Therefore, plants may preferentially degrade chl-b to minimize photodamage and maintain photosynthetic efficiency. This selective degradation would result in a higher chl-a/b ratio. Increasing the chl-a/b ratio may be part of the plant's adaptive response to stress.

Carotenoids content in plants can change in response to stress, with an impact on photosynthesis and plant health. In addition, they act as antioxidants to help plants cope with stress. Carotenoids accumulate in plants in response to various biotic and abiotic stresses, making their elevated content a useful indicator of stress (Swapnil et al., 2021). In plants, carotenoids serve two key functions. First, they enhance light absorption in the blue spectral range (420–500 nm). Second, they protect the photosynthetic apparatus from reactive oxygen species (ROS), particularly singlet oxygen ($^1O_2$) generated by triplet-excited chlorophylls ($3Chl^*$). Both sludge treatments increased carotenoid content compared to the control, with NCSS inducing the highest levels. However, no significant difference was found between the NCSS and CSS treatments. A correlation was found between carotenoid content and F_o under CSS treatments. Both these two parameters were lower at CSS treatments compared to NCSS treatments.

Table 1. Effect of non-composted and composted sewage sludge on physiological parameters (chlorophyll content ($mg\ g^{-1}\ FW$) and photochemical activity of photosystem II) of 35-day-old maize.

Treatments	Chl-a	Chl-b	Car	Chl-a/b	Total Chl/Car
Control	9.51 a	3.94 a	6.61 a	2.41 a	2.57 a
25% NCSS	22.46 c	9.96 c	12.61 b	2.25 a	2.54 b
50% NCSS	19.76 bc	7.60 b	12.04 b	2.60 ab	2.13 a
75% NCSS	18.11 b	5.71 ab	11.25 b	3.17 b	2.19 a
25% CSS	19.40 b	9.13 b	9.47 b	2.12 b	3.03 b
50% CSS	18.52 b	7.48 b	8.72 b	2.47 b	2.98 b
75% CSS	20.09 b	8.30 b	9.05 b	2.42 b	3.06 b
Treatments	F_o	F_m	F_v	F_v/F_m	F_v/F_o
Control	165.0 a	815.8 ns	650.8 ns	0.80 ns	3.95 a
25% NCSS	217.8 b	787.8 ns	570.0 ns	0.71 ns	2.62 a
50% NCSS	218.2 b	735.0 ns	507.4 ns	0.70 ns	2.78 a
75% NCSS	240.8 b	782.0 ns	541.2 ns	0.70 ns	2.25 a
25% CSS	159.3 a	840.0 ns	680.8 ns	0.81 ns	4.28 b
50% CSS	154.2 a	809.4 ns	655.2 ns	0.81 ns	4.26 ab
75% CSS	149.0 a	782.5 ns	633.5 ns	0.81 ns	4.24 ab

Data presented as mean, $n = 5$, lower case letters indicate the significant difference among the treatments, FW = fresh weight, ns = not significant, NCSS = non-composted sewage sludge, CSS = composted sewage sludge

Studies have shown that under stress conditions, the photochemical activity of photosystem II (PSII) decreases (Murata et al., 2007; Didaran et al., 2024). The maximum quantum efficiency of photosystem II (F_v/F_m) did not decrease at any of the applied treatments.

Typically, metabolic processes, including photosynthesis, respiration, and photorespiration, result in the generation of ROS. However, stress usually overproduces ROS, such as hydrogen peroxide, singlet oxygen, and hydroxyl radicals. Antioxidants and ROS-detoxifying enzymes help in reducing high ROS levels. Numerous subcellular compartments contain antioxidant enzymes that lower increased intracellular ROS levels, including glutathione peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) (Didaran et al., 2024). In this study, the activity of APX was the highest at 75% NCSS treatment, 2.6-fold higher compared to the control value. The activities of APX and POD increased linearly with the increased concentration of the applied NCSS. However, there was no correlation between the activity of APX and POD and the concentration of the applied

CSS treatment. The activity of POD did not change significantly relative to the control. However, the SOD activity was significantly higher under all NCSS treatments compared to the control (Table 2). Due to its role in converting superoxide radicals into less toxic hydrogen peroxide, plants with higher SOD levels are better able to resist oxidative stress by neutralizing harmful reactive oxygen species; in other words, a higher SOD level indicates that the plant is more resilient to environmental stressors.

Table 2. Effect of composted and non-composted sewage sludge on biomarkers (APX, POD, and SOD) of 35-day-old maize.

Treatments	APX	POD	SOD
Control	0.201 a	0.2537 ns	0.105 a
25% NCSS	0.347 ab	0.3762 ns	0.156 b
50% NCSS	0.475 ab	0.4574 ns	0.150 ab
75% NCSS	0.530 b	0.5221 ns	0.162 b
25% CSS	0.192 a	0.0987 ns	0.130 a
50% CSS	0.250 ab	0.1118 ns	0.168 a
75% CSS	0.210 a	0.1269 ns	0.151 a

Data presented as mean, n = 5, lower case letters indicate the significant difference among the treatments, ns = not significant, NCSS = non-composted sewage sludge, CSS = composted sewage sludge, APX = ascorbate peroxidase ($\mu\text{mol ascorbate peroxidase min}^{-1} \text{mg prot}^{-1}$), POD = guaiacol peroxidase ($\mu\text{mol tetraguaiacol min}^{-1} \text{mg prot}^{-1}$), SOD = superoxide dismutase ($\text{U g}^{-1} \text{FW}$), FW = fresh weight

Conclusions

A higher chlorophyll a/b ratio at NCSS may be part of the plant's adaptive response to stress. NCSS treatments indicated higher APX, POD, and SOD activity compared to the control and CSS. Although CSS had a lower impact on the biochemical responses than NCSS, it still has a positive effect of enhancing chlorophyll.

While NCSS can provide short-term growth benefits, its long-term risks to plant health and the environment cannot be overlooked. Future research should focus on optimizing the composting process, exploring cost-effective methods for large-scale implementation, and conducting long-term field studies to assess the cumulative effects of sewage sludge amendments on soil and crop health. By balancing agricultural productivity with environmental sustainability, CSS has the potential to play a vital role in sustainable farming systems.

References

- Beyer W.F., Fridovich I. (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry*. 161: 559–566.
- Didaran F., Kordrostami M., Ghasemi-Soloklui A. A., Pashkovskiy P., Kreslavski V., Kuznetsov V., Allakhversiev S. (2024). The mechanisms of photoinhibition and repair in plants under high light conditions and interplay with abiotic stressors. *Journal of Photochemistry and Photobiology B: Biology*. 259: 113004
- Giannopolities C.H., Ries S.K. (1977). Superoxide Dismutase I. Occurrence in Higher Plant. *Plant Physiology*. 59: 309–314.
- Heath R.L., Packer L. (1968). Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics*. 125: 189–198.
- Marin E., Rusănescu C.O. (2023). Agricultural Use of Urban Sewage Sludge from the Wastewater Station in the Municipality of Alexandria in Romania. *Water*. 15: 458.

- Mishra N.P., Mishra R.K., Singhal G.S. (1993). Changes in the activities of antioxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant Physiology*. 102: 903–910.
- Moran R., Porath D. (1980). Chlorophyll determination in intact tissues using N, N-dimethylformamide. *Plant Physiology*. 65: 478–479.
- Murchie E. H., Lawson T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*. 64 (13): 3983–3998.
- Murata N., Takahashi S., Nishiyama Y., Allakhverdiev S. I. (2007). Photoinhibition of photosystem II under environmental stress. *Biochemica et Biophysica Acta (BBA) – Bioenergetics*. 1767 (6): 414–21
- Namdari M., Soleimani M., Mirghaffari N., Kharrazi S. M. (2024). Effect of biological sewage sludge and its derived biochar on accumulation of potentially toxic elements by corn (*Zea mays* L.) Scientific Report. 14:5985.
- Pajura R. (2024). Composting municipal solid waste and animal manure in response to the current fertilizer crises – a recent review. *Science of The Total Environment*. 912: 169221.
- Prates A.R., Kawakami K.C., Coscione A.R., Filho M.C.M.T., Arf O., Abreu-Junior C.H., Oliveira F.C., Moreira A., Galindo F.S., He Z., et al. (2022). Composted sewage sludge sustains high maize productivity on an infertile oxisol in the Brazilian Cerrado. *Land*. 11: 1246.
- Pukacka S., Ratajczak E. (2005). Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. *Journal of Plant Physiology*. 162: 873–885
- Swapnil P., Meena M., Singh S. K., Dhuldhaj U. P., Harish, Marwal A. (2021). Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Current Plant Biology*. 26: 100203
- Wellburn R.A. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvent with spectrophotometers of different resolution. *Journal of Plant Physiology*. 144: 307–313.
- Zeislin N., Ben-Zaken R. (1991). Peroxidases, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers. *Plant Physiology and Biochemistry*. 29: 147–151.

EXPERT PAPER

Remote sensing-based detection of drought effects in maize (*Zea mays* L.) on loess sandy soils (2022-2024)

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Summary

The effects of drought on maize (*Zea mays* L.) were analysed from 2022 to 2024 using NDVI, NDMI, and PRI indices on loess sandy soils in Eastern Hungary. Strong winds and buffer zones affected fields within a 2 km radius. In 2022, less protected areas exhibited earlier vegetative decline, with NDMI dropping from 0.2 to 0.0 and yields only 1.02 t ha⁻¹, compared to over 2.7 t ha⁻¹ in protected fields. In 2023, NDMI increased from 0.16 to 0.44, but PRI (down to -0.16) indicated early stress. In 2024, the NDVI stabilised at 0.6-0.7, and yield losses were less severe. A PRI value below 0.1 was consistently predictive of yield loss in the years studied and proved to be an early indicator of drought.

Key words: maize, water stress prediction, NDVI, NDMI, PRI

Introduction

Maize (*Zea mays* L.) is a major crop, fundamental to food, feed, industrial, and bioenergy sectors. Nevertheless, yield stability is increasingly threatened by climatic extremes, especially droughts and water deficits. The global average maize yield is 5.8 t ha⁻¹, which has decreased to 5.7 t ha⁻¹. In contrast, Hungary's average yield in 2022 was 3.39 t ha⁻¹, significantly lower than the typical average of 7 t ha⁻¹.

As Laiskhanov et al. (2022), Mohammed et al. (2022), and Belmahi et al. (2023) demonstrate, remote sensing-based indices, including the Normalized Difference Vegetation Index (NDVI), the Normalized Difference Moisture Index (NDMI), and the Photochemical Reflectance Index (PRI), offer valuable insights into crop status and stress responses. A substantial body of research has demonstrated the existence of strong correlations between NDVI and yield outcomes across a range of environments (Nagy et al., 2018). The NDMI, a tool developed for the assessment of vegetation moisture content, exhibits a high degree of sensitivity to variations in plant water status and soil moisture. It has been demonstrated to be particularly effective in the early detection of drought stress and in informing irrigation scheduling (Lopes, 2022; Ibrahim et al., 2023). As a proxy for photosynthetic efficiency and xanthophyll cycle activity, PRI (Gitelson et al., 2006; Tamás et al., 2023) enables the early detection of physiological stress before the occurrence of visible symptoms.

In order to achieve sustainable maize production, it is essential to adopt drought-tolerant hybrids, optimise sowing dates, and diversify cropping systems (Sárvári and Boros, 2009). The integration of agronomic practices with advanced remote sensing technologies has been identified as a potential strategy to enhance the stability of crop yield and climate resilience in maize cultivation.

A severe drought in 2022 caused extensive damage, thus highlighting the increasing impact of extreme weather events in eastern Hungary. The present study was initiated in 2022 with the objective of developing an early drought detection system and assessing intervention possibilities using remote sensing indices.

Materials and Methods

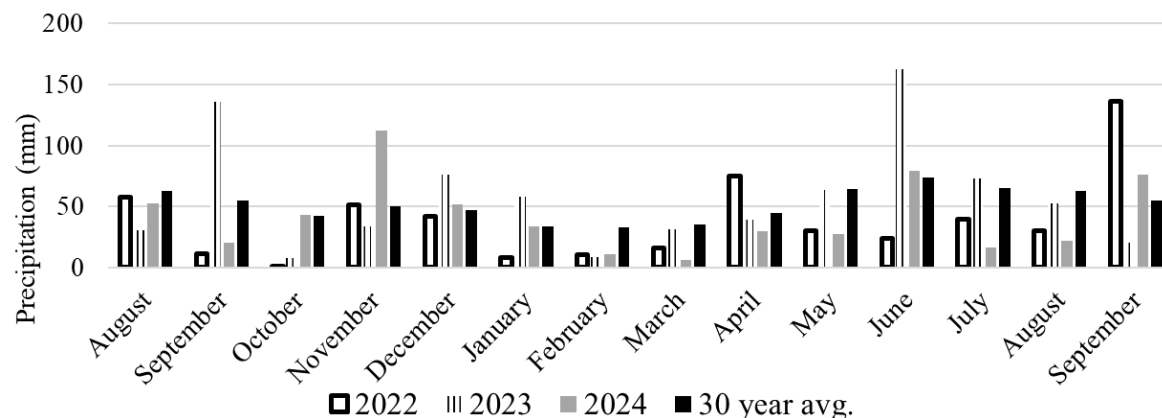
The experiment was conducted from 2022 to 2024 under large field conditions on loose-structured loess sandy soils. The three study fields, covering 5.55 ha, 1.87 ha, and 13.82 ha respectively, were situated within a 2 km radius near Újfehértó and Hajdúdorog, in the area of the former settlement of Kisszegegyháza in Hungary. The soils, classified as loess sandy upland and loess chernozem types, are fertile and conducive to cultivation.

Coordinates of the experimental sites and forecrop of maize:

- Újfehértó - 47.813283, 21.607106 (Iskola): 2022-triticale, 2023-maize
- Újfehértó - 47.825738, 21.600621 (Gencsi): 2022-maize, 2024-triticale
- Újfehértó - 47.839394, 21.589907 (Csajkos): 2023-triticale, 2024-maize

Weather conditions during the study years are presented in Graph 1.

Except April, in 2022 the precipitation levels were significantly below the long-term mean (LTM) (44.4 mm). In the period spanning from October 2021 to September 2022, the total precipitation recorded was 325.7 mm, and during the maize growing season, only 198 mm of rain were received. The summer months were marked by particularly high temperatures, with daily maximum temperatures averaging at 28.9°C in June, 30.6 °C in July, and 30.4 °C in August. In 2023 proved to be more favourable. From March to May 2023, precipitation levels remained consistent with the mean value, and in June, a remarkable 162.2 mm of rainfall was recorded. The maximum temperatures recorded were lower, averaging 25.1°C in June, 28.2°C in July, and 29.3 °C in August. In 2024, from February to August, received rainfall was below LTM (32,8 mm-63 mm) with the exception of June (78.8 mm). The summer months were characterised by elevated temperatures in comparison with the preceding year, with mean values of 27.1°C in June, 32.2°C in July, and 31.3°C in August.



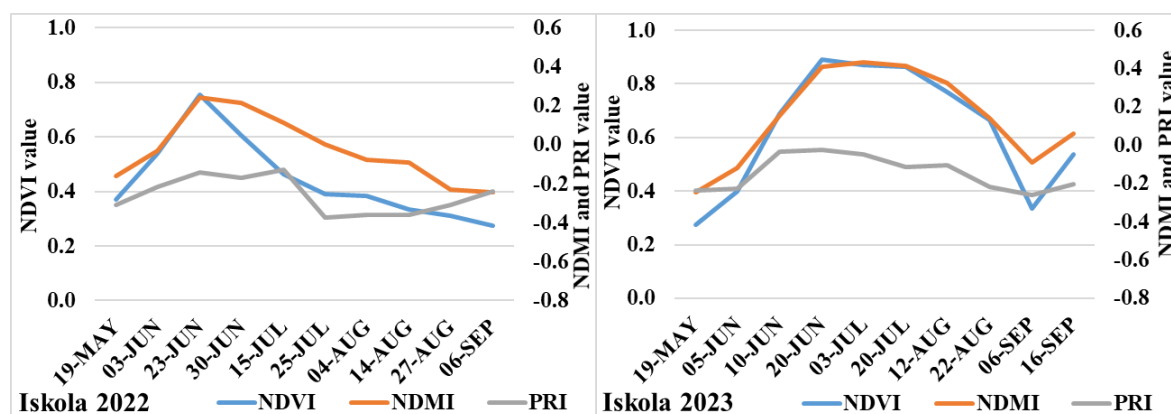
Graph 1. Monthly distribution of rainfall compared to the 30-year mean and study years.

During the experiment, plant physiological measurements such as NDVI (Normalized Difference Vegetation Index), PRI (Photochemical Reflectance Index), and NDMI (Normalized Difference Moisture Index) were applied. The hybrid used was DKC4897 (FAO 390–410), with a plant density of 74,000 plants per hectare. Fertilization included an autumn incorporation of 20 kg ha⁻¹ N, 52 kg ha⁻¹ P₂O₅, and 52 kg ha⁻¹ K₂O, followed by a spring application of 81 kg ha⁻¹ N, 21 kg ha⁻¹ CaO, and 15 kg ha⁻¹ MgO. Yield measurements at harvest were conducted across three fields: six measurements in the 5.55 ha "Iskola" field, two measurements in the 1.87 ha "Gencsi" field, and fourteen measurements in the 13.82 ha "Csajkos" field. Plant physiological data were collected using the OneSoil application and analyzed using IBM SPSS 26.0 and Microsoft Excel for statistical evaluation and yield analysis.

Results and discussion

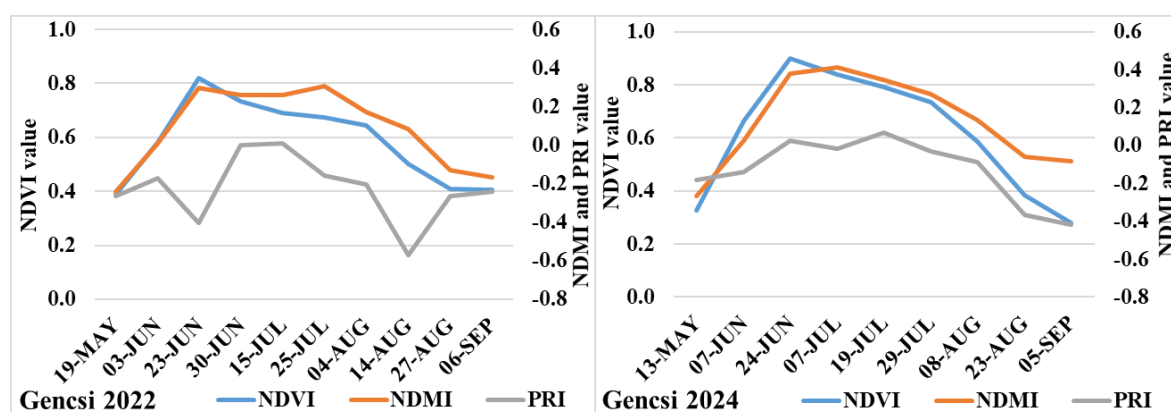
Assessing the development of maize in the same field in different years:

In 2022, the maize crop in the “Iskola” field (5.55 ha) reached peak physiological development in mid-June (NDVI: 0.76; NDMI: 0.25). Drought stress caused NDVI to decline from 0.76 (23 June) to 0.61 by late July, while NDMI decreased more gradually. PRI values remained consistently negative throughout the season, indicating persistent stress. In 2023, improved water supply resulted in better plant physiological values with NDVI reaching 0.89 and NDMI closely following, even after a disadvantageous (maize) forecrop (Graph 2). However, PRI values signalled early stress from 3 July onwards.



Graph 2. Physiological values of the “Iskola” field in 2022 and 2023

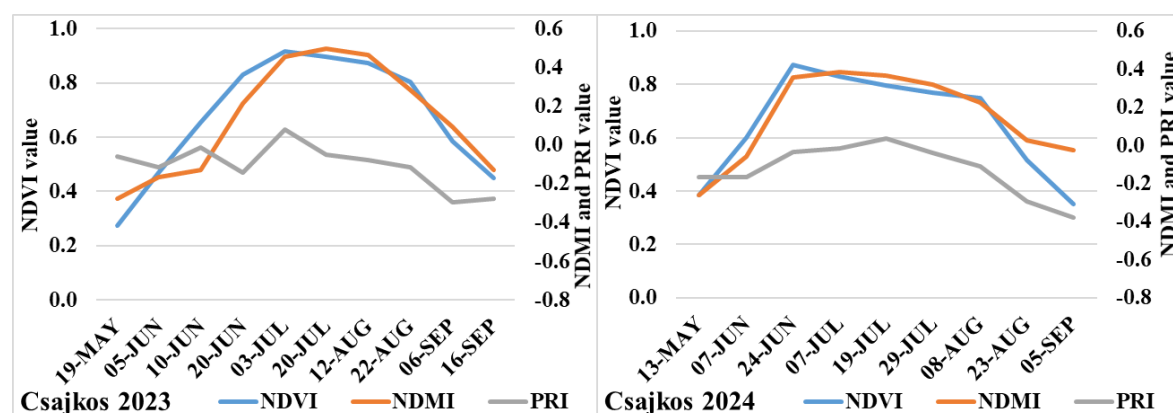
In the “Gencsi” field (1.87 ha), water availability was better in 2022, with higher NDVI (0.82) and NDMI (0.30) on 23 June compared to the regional average. Despite this, a clear downward trend emerged after 23 June, with PRI values declining earlier and reaching their lowest point by mid-August. In 2024, NDVI and NDMI again peaked in June (0.9 and 0.41, respectively) but declined steadily, turning sharply downward by late July. Although PRI briefly peaked on 19 July (0.07), field observations confirmed recurring drought stress (Graph 3).



Graph 3. Physiological values of the “Gencsi” field in 2022 and 2024

The “Csajkos” field (13.82 ha) showed delayed development in 2023. NDVI and NDMI moved synchronously, peaking at 0.92 on 3 July. The crop matured while retaining green stalks, supported by stable NDVI and NDMI trends. The PRI values were also favourable, peaking at 0.08 on 3 July. Our experimental results support Belhami et al. (2023)'s assertion that NDVI values above 0.7 in maize stands predict favourable yield performance. However, in 2024,

drought impacts were more severe in “Csajkos.” The NDVI (0.88) and NDMI (0.39) recorded in late June and early July gradually declined. Except for a slight positive PRI spike on 19 July (0.04), PRI remained negative throughout, indicating substantial crop stress (Graph 4).



Graph 4. Physiological values of the “Csajkos” field in 2023 and 2024

Assessing the development of maize in the same year in different growing areas

In 2022, even the short (1.2 km) distance between the “Iskola” and “Gencsi” fields resulted in notable differences. Despite the maize forecrop with higher water consumption being more unfavourable than triticale forecrop, higher yields (1.68 t ha^{-1}) and better plant physiological values were measured for “Gencsi” (NDVI: 0.82-0.65) compared to the “Iskola” (NDVI: 0.76-0.39) plot (Graphs 2–4). Based on NDVI and NDMI values, vegetative decline occurred earlier (2022.06.30.) in “Iskola”. This was attributed to differing environmental conditions: “Gencsi” was sheltered by tree rows on both western and eastern sides, mitigating desiccating winds, while “Iskola” was exposed to prevailing westerlies. According to PRI data, “Gencsi” exhibited more favourable values between late June and mid-August (0 to -0.16) compared to “Iskola” (-0.17 to -0.36). Ibrahim et al. (2023) also found a correlation between plant health and NDMI, NDVI values.

In 2023, NDMI values rose earlier in “Iskola” (June 10: 0.16; June 20: 0.41; July 3: 0.44) than in “Csajkos” (June 10: -0.13; June 20: 0.22; July 3: 0.46). NDVI values showed no significant differences between the fields from mid-May to late July. PRI values indicated that “Csajkos” achieved its most favourable condition on 3 July (0.08), although persistent stress was evident. In 2024, differences between “Gencsi” and “Csajkos” largely disappeared, with all three indices (NDVI, NDMI, PRI) following similar trends (Graphs 2–4).

Correlation between yield results and vegetation indices

In 2022, the severe drought caused substantial yield losses on the “Iskola” field, averaging only 1.02 t ha^{-1} . Lopes (2022) and Safwan et al. (2022) has already predicted a decline in harvests in Europe in 2022. Yield variability was significant, ranging from 0.2 t ha^{-1} on exposed sections to 2.1 t ha^{-1} in lower-lying zones. In contrast, the “Gencsi” field, benefiting from better microclimatic protection, achieved an average of 2.7 t ha^{-1} , ranging between 1.88 and 3.52 t ha^{-1} .

In 2023, yields markedly improved. The “Iskola” plot averaged 7.54 t ha^{-1} , while “Csajkos” outperformed it with an average of 8.18 t ha^{-1} . Nevertheless, substantial spatial variability persisted, especially near tree lines and wind-exposed eastern edges, where yields differed by up to 3 t ha^{-1} within the plot.

In 2024, drought again impacted yields but to a lesser extent than in 2022. Differences between “Gencsi” and “Csajkos” were minimal: the “Gencsi” field averaged 5.11 t ha^{-1} , while “Csajkos” yielded 4.38 t ha^{-1} .

Conclusions

The study aimed to detect drought effects via remote sensing and identify optimal intervention periods. Among the evaluated vegetation indices, NDVI and NDMI followed similar patterns, while PRI proved to be more sensitive in detecting early stress and forecasting critical periods. PRI values around or below -0.01 consistently indicated yield loss. Thus, continuous PRI monitoring could support timely interventions (e.g., irrigation) and help stabilize yields under increasingly variable and drought-prone conditions.

References

- Belmahi, M., Hanchane, M., Krakauer, N., Kessabi, R., Bouayad, H., Mahjoub, A., Zouhri, D., Zouhri, D. (2023). Analysis of relationship between grain yield and NDVI from MODIS in the Fez-Meknes region, Morocco. *Remote Sensing*, 15(11), 2707.
- Gitelson, A. A., Viña, A., Verma, S. B., Rundquist, D. C., Arkebauer, T. J., Keydan, G., Leavitt, B., Ciganda, V., Burba, G. G., Suyker, A. E. (2006). Relationship between gross primary production and chlorophyll content in crops: Implications for the synoptic monitoring of vegetation productivity. *Journal of Geophysical Research: Atmospheres*, 111(D8).
- Ibrahim, G., Rasul, A., Abdullah, H. (2023). Assessing how irrigation practices and soil moisture affect crop growth through monitoring Sentinel-1 and Sentinel-2 data. *Environmental Monitoring and Assessment*, 195(11).
- Laiskhanov, S. U., Smanov, Z. M., Kaimuldinova, K. D., Myrzaly, N. B., Ussenov, N. E., Poshanov, M. N., Azimkhanov, B. (2022). A study of the effects of soil salinity on the growth and development of maize (*Zea mays* L.) by using Sentinel-2 imagery. *Online Journal of Biological Sciences*, 22(3), 323-332.
- Lopes, M. (2022). Will temperature and rainfall changes prevent yield progress in Europe? *Food and Energy Security*, 11(2).
- Mohammed, S., Alsafadi, K., Enaruvbe, G., Bashir, B., Elbeltagi, A., Széles, A., Alsalman, A., Harsányi, E. (2022). Assessing the impacts of agricultural drought (SPI/SPEI) on maize and wheat yields across Hungary. *Scientific Reports*, 12(1).
- Nagy, A., Fehér, J., Tamás, J. (2018). Wheat and maize yield forecasting for the Tisza River catchment using MODIS NDVI time series and reported crop statistics. *Computers and Electronics in Agriculture*, 151, 41-49.
- Sárvári, M., Boros, B. (2009). The effect of crop production factors on yield quantity and quality of maize. *Cereal Research Communications*, 37, 53-56.
- Tamás, A., Kovács, E., Horváth, É., Juhász, C., Radócz, L., Rátónyi, T., Ragán, P. (2023). Assessment of NDVI dynamics of maize (*Zea mays* L.) and its relation to grain yield in a polyfactorial experiment based on remote sensing. *Agriculture*, 13(3), 689.

ORIGINAL SCIENTIFIC PAPER

Phytochemical profile of sweet potato (*Ipomoea batatas* L.) leaves

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Summary

The aim of this study was to determine the content of specialized metabolites in the leaves of three sweet potato varieties. A significant difference was observed among the bioactive leaf components analyzed across all studied varieties. The leaves of the purple sweet potato variety had the highest vitamin C content and a 34% higher polyphenol content compared to other varieties. On average, the purple variety also contained 23% more total flavonoids. Additionally, higher antioxidant capacity values were recorded in the leaves of the purple variety using the FRAP method. These findings suggest that sweet potato leaves, particularly those of the purple variety, are a valuable raw material rich in bioactive compounds.

Key words: organic waste residue, reuse, ascorbic acid, polyphenols, antioxidant capacity

Introduction

Sweet potatoes are primarily cultivated for its secondary root thickening. The above-ground part of the plant, specifically the leaves, is utilized in certain regions where their nutritional value is recognized. However, sweet potato leaves are more commonly used as animal feed or discarded as waste (Akinoso et al., 2022). Given that leaf yields are substantial and can be harvested 3-4 times a year (Bogović et al., 2013), and considering their rich nutritional profile sweet potato leaves have the potential to be consumed as a leafy vegetable and incorporated into daily meals (Ivane et al., 2024). A study by Hong et al. (2020) conducted on the leaves of 13 sweet potato varieties showed that vitamin C concentrations ranged from 10.78 to 152.95 mg 100 g⁻¹ of dry matter. Similarly, Tang et al. (2021) analyzed 11 sweet potato varieties and confirmed that sweet potato leaves are a good source of vitamin C, with levels varying between 99 and 511 mg 100 g⁻¹ of dry matter, depending on the variety. Padda and Picha (2007) investigated different parts of the sweet potato plant at various stages of maturity and found that the leaves contain significantly higher total phenolic content than the roots. Their findings also indicated that the phenolic content in leaves depends on plant age, with young leaves containing up to four times more total phenolics than older leaves. Similarly, research by Ooko Abong et al. (2020) reported a substantial difference in total phenolic content between sweet potato leaves and roots. The average phenolic content in leaves was 5800.26 mg GAE 100 g⁻¹ of dry matter, whereas the roots contained only 151.2 mg GAE 100 g⁻¹ of dry matter. Additionally, in their study on 9 varieties of orange, yellow, and white sweet potatoes, flavonoids were not detected in the roots of the white variety but were present in its leaves. The highest flavonoid concentration in leaves was observed in one of the orange-colored varieties. Hong et al. (2020) assessed the antioxidant capacity of 13 sweet potato varieties using the FRAP method, reporting values ranging from 3.94 ± 1.05 g TE g⁻¹ of dry matter to 16.44 ± 0.73 g TE g⁻¹ of dry matter. Based on the presented results and the reviewed literature, it should be emphasized that sweet potato leaves, in addition to the root, are a valuable source of various phytochemicals. Therefore, rather than being considered waste, they should primarily be regarded as a valuable by-product for the extraction of phytochemicals. Therefore, the aim of

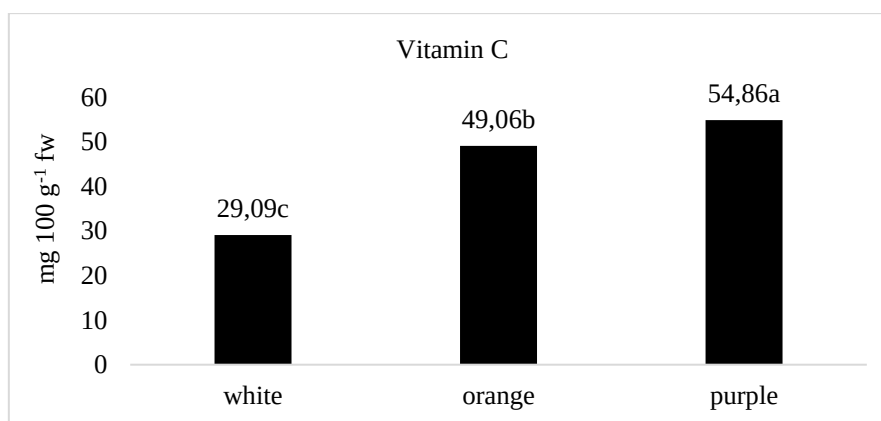
this study was to determine the content of specialized metabolites and evaluate the functional potential of the leaves of three sweet potato varieties.

Material and methods

The developed seedlings of the white, orange, and purple sweet potato varieties were planted on June 3rd, 2024, on polyethylene (PE) mulch with a planting distance of 120 × 40 cm at the experimental field (Maksimir research station, Department of Vegetable Crops University of Zagreb Faculty of Agriculture). A drip irrigation system was installed beneath the mulch. The leaf harvest was carried out one month after planting, on July 4th, during the morning hours, in the pre-flowering phase. After harvest, the leaves were transported to the laboratory of the Department of Sustainable Technologies and Renewable Energy Sources, University of Zagreb Faculty of Agriculture, where they were separated from the petioles and prepared for the required chemical analyses. The content of bioactive compounds was analyzed, including vitamin C, determined by titration with 2,6-dichloroindophenol, according to the standard method (AOAC, 2002), and expressed as mg 100 g⁻¹ of fresh weight (fw). Total phenolic compounds, including flavonoids and non-flavonoids, were quantified spectrophotometrically (UV-1900i, Shimadzu,) at 750 nm, with distilled water used as a blank using a method described by Ough and Amerine (1988). Gallic acid was used as an external standard for total phenolic compounds, and catechin for total non-flavonoids, while the content of total flavonoids was calculated as the difference between total phenolic compounds and non-flavonoids. The content of total polyphenolic compounds was expressed as mgGAE/CAT 100 g⁻¹ g fw. The antioxidant capacity was determined using the ABTS test according to Re et al. (1999). An ethanol extract prepared for isolating the total phenolic compounds was used for this determination. Antioxidant capacity was measured spectrophotometrically at 734 nm, with Trolox as an external standard. The final result of antioxidant capacity was expressed in µmol TE L⁻¹. Additionally, antioxidant capacity was assessed using the FRAP method (Benzie & Strain, 1996). All laboratory analyses were performed in triplicate. Significant differences between the properties of the analyzed varieties were determined using one-way ANOVA and the Duncan test in the SAS statistical package (ver. 9.3, 2010). The mean values were compared using the LSD test, with $p = 0.05$ considered the threshold for significance. In the graphs, different letters indicate significant statistical differences at $p \leq 0.0001$, along with the standard deviation.

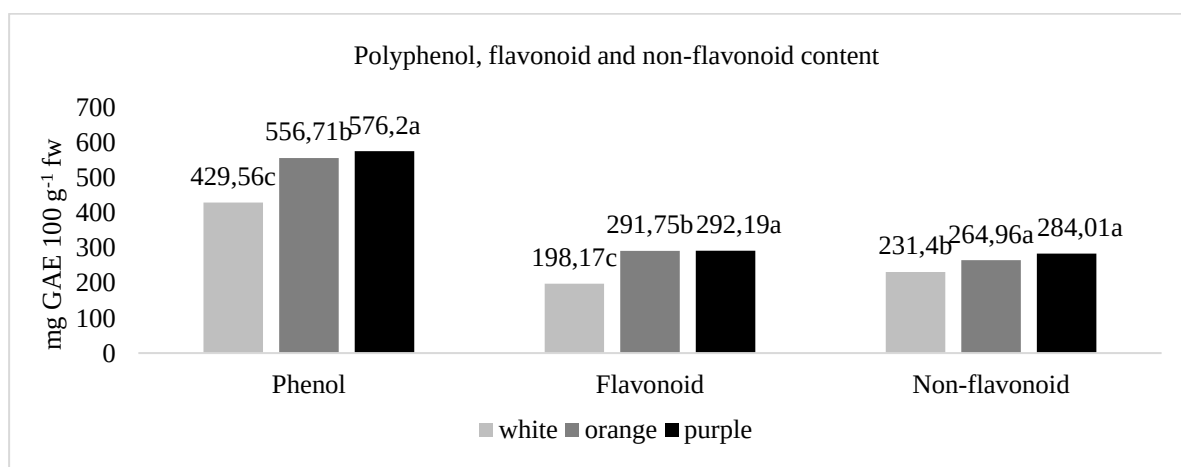
Results and discussion

The results of the analyzed bioactive compounds in the leaves of different sweet potato varieties are presented in Graphs 1, 2, and 3. A significant statistical difference ($p \leq 0.0001$) was observed between the bioactive compounds in the leaves of all the sweet potato varieties tested. The ascorbic acid content (Graph 1) ranged from 29.09 to 54.86 mg 100 g⁻¹ fw. The highest content was found in the leaves of the purple variety, while the lowest was found in the leaves of the white variety (29.09 mg 100 g⁻¹ fw). The ascorbic acid content in the leaves of the purple sweet potato variety was 89 % higher than in the white variety, and 12 % higher than in the orange variety. The ascorbic acid values reported in the study by Dinu et al. (2018) were significantly lower than those observed in this study (3.56 - 5.96 mg 100 g⁻¹ fw). As the authors noted, ascorbic acid levels are influenced by various factors and are directly related to chlorophyll content and photosynthetic rate, with higher ascorbic acid levels typically found in tissues with greater photosynthetic intensity. Islam (2014) reports an average ascorbic acid value of 7.2 mg 100 g⁻¹ fw in sweet potato leaves, while Suárez et al. (2020) report a asrobic acid value of 21.9 mg 100 g⁻¹ of dry matter, both of which are lower than the values found in this study.



Graph 1. Ascorbic acid content (mg 100 g⁻¹ fw) in the sweet potato leaf of white, orange and purple varieties

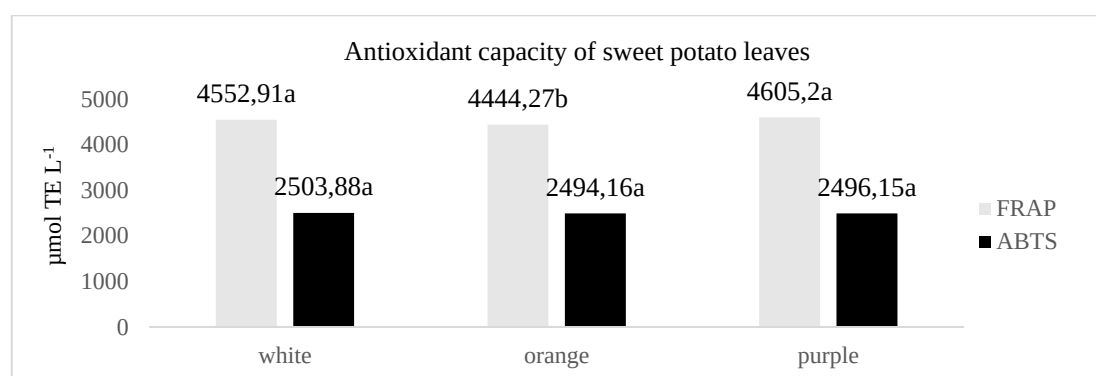
The content of total polyphenolic compounds content analyzed in sweet potato leaves is shown in Graph 2. The total polyphenol content in the leaves of the different sweet potato varieties ranged from 429.56 to 576.20 mgGAE 100 g⁻¹ fw. The highest content was found in the leaves of the purple sweet potato variety, while the lowest was found in the leaves of the white variety. The purple variety leaves had 34 % higher polyphenol content than the white variety and 3.5% higher than the orange variety. The highest content of total flavonoids was found in the leaves of the purple variety (284.01 mgGAE 100 g⁻¹ fw), while the lowest was found in the leaves of the white variety (231.40 mgGAE 100 g⁻¹ fw). On average, the purple sweet potato leaves had 23% higher total flavonoid content than the white variety and 7% higher than the orange variety. The non-flavonoid content values for the sweet potato leaf varieties analyzed ranged from 198.17 to 292.19 mgCAT 100 g⁻¹ fw. As in previous analyses, the white sweet potato leaves exhibited lower values than the orange and purple varieties. The results of the total polyphenol and total flavonoid content in the leaves of the different sweet potato varieties determined in this study are consistent with those found in other studies (Ghasemzadeh et al. 2012; Hue et al. 2012; Ojong et al. 2008).



Graph 2. The content of total polyphenols, flavonoids and non-flavonoids in sweet potato leaves of white, orange and purple varieties

The antioxidant capacity values of sweet potato leaves determined using the ABTS and FRAP methods are shown in Graph 3. According to the ABTS method, the antioxidant capacities of the leaves from different sweet potato varieties were not statistically different, with values ranging from 2494.16 to 2503.88 $\mu\text{mol TE L}^{-1}$. In contrast, when analyzing antioxidant capacity

using the FRAP method, significantly higher values were found in the leaves of the white ($4552.91 \mu\text{mol TE L}^{-1}$) and purple ($4605.20 \mu\text{mol TE L}^{-1}$) sweet potato varieties, with no significant difference between them. Lower values were found for the leaves of the orange sweet potato variety ($4444.27 \mu\text{mol TE L}^{-1}$). In the study by Suárez et al. (2020), which analyzed sweet potato leaves harvested at three different time points, antioxidant capacity determined by the ABTS method was higher in the later harvests (2nd and 3rd), though no significant difference was observed. A study by Hong et al. (2020), which analyzed antioxidant capacity using the FRAP method across 13 sweet potato varieties, reported higher values than those found in this study. Similarly, in the study by Dinu et al. (2021), which examined the antioxidant capacity of 6 sweet potato varieties using the ABTS method, the values were higher than those observed here, although the lowest values aligned with the results in this study. A study of 11 sweet potato leaf varieties by Jia et al. (2022) found antioxidant capacity values using the FRAP method to be similar to those in this study, but the values obtained using the ABTS method were significantly higher.



Graph 3. Antioxidant capacity (ABTS and FRAP method) of leaves of different sweet potato varieties

Conclusions

According to the obtained results, it can be concluded that sweet potato leaves are a highly valuable raw material. The bioactive compound content in the leaves of different sweet potato varieties varied significantly, with the purple variety showing the highest levels of ascorbic acid, total phenolic compounds, and flavonoids. Additionally, the antioxidant capacity, as determined by the FRAP method, was also higher in the purple and white varieties compared to the orange variety. These findings highlight the nutritional potential of sweet potato leaves, which can be considered a rich source of antioxidants and bioactive compounds. Future research should focus on identifying the optimal harvest time for maximizing bioactive compound content and exploring the potential of sweet potato leaves as a functional food ingredient.

References

- Akinoso R., Lawal A., Raji A., Osifuwa A. (2022). Quality characteristics of sweet potato leaves (*Ipomoea batatas* L.) as influenced by processing and storage conditions. *Croatian Journal of Food Science and Technology*, 14(2): 202-209.
- AOAC (2002). *Official methods of Analysis* (17th ed.). Washington, DC: Association of Official Analytical Chemists.
- Bogović, M., Belko, D., Božić, Đ., Fabek, S., Toth, N., Novak, B. i Radojčić Redovniković, I. (2013). Utjecaj folijarnog mineralnog gnojiva na sadržaj fenolnih spojeva u listu batata (*Ipomoea batatas* L.). *Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam*, 8(1-2): 29-35.

- Dinu M., Soare R., Băbeanu C., Hoza G. (2018). Analysis of nutritional composition and antioxidant activity of sweet potato (*Ipomoea batatas* L.) leaf and petiole. *Journal of Applied Botany and Food Quality*, 90: 120-125.
- Dinu M., Soare R., Hoza G., Becherescu A. D., Băbeanu C. (2021). Bioactive compounds content and antioxidant activity in the leaves of some sweet potato cultivars (*Ipomoea batatas* L.). *Scientific Papers. Series B, Horticulture*, 65(1): 415-423.
- Ghasemzadeh A., Omidvar V., Jaafar H.Z.E. (2012). Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas* L.). *Journal of Medicinal Plants Research*, 6(15): 2971-2976.
- Hong J., Mu T., Sun H., Richel A., Blecker C. (2020). Valorization of the green waste parts from sweet potato (*Ipomoea batatas* L.): Nutritional, phytochemical composition, and bioactivity evaluation. *Food Sci Nutr*, 8(8): 4086–4097.
- Hue S.M., Boyce A.N., Somasundram C. (2012). Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas* L.). *Australijan Journal of Crop Science*, 6(3): 375-380.
- Islam S. (2014.) Nutritional and Medicinal Qualities of Sweetpotato Tops and Leaves. University of Arkansas at Pine Bluff.
- Ivane N. M. A., Wang W., Ma Q., Wang J. (2024). Harnessing the health benefits of purple and yellow-fleshed sweet potatoes: Phytochemical composition, stabilization methods, and industrial utilization- A review. *Food Chemistry: X*, 23.
- Jia R., Tang C., Chen J., Zhang X., Wang Z. (2022). Total Phenolics and Anthocyanins Contents and Antioxidant Activity in Four Different Aerial Parts of Leafy Sweet Potato (*Ipomoea batatas* L.). *Molecules* 2022., 27, 3117.
- Ojong P.B., Njiti V., Guo Z., Gao M., Besong S., Barnes S.L. (2008). Variation of Flavonoid Content Among Sweetpotato Accessions. *J. Amer. Soc. Hort. Sci*, 133(6): 819–824.
- Ooko Abong G., Muzhingi T., Okoth M.W., Ng'ang'a F., Ochieng P.E., Mbogo D. M., Malavi D., Akhwale M., Ghimire S. (2020). Phytochemicals in Leaves and Roots of Selected Kenyan Orange Fleshed Sweet potato (OFSP) Varieties. *International Journal of Food Science*.
- Ough, C. S., Amerine, M. A. (1988.): *Methods for Analysis of Musts and Wines*. John Wiley and Sons. New York. USA
- Padda M.S., Picha D.H. (2007). Antioxidant Activity and Phenolic Composition in 'Beauregard' Sweetpotato Are Affected by Root Size and Leaf Age. *Journal of the American Society for Horticultural Science*, 132(4): 447–451.
- Re R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26 (9-10): 1231-1237.
- Suárez S., Mu T., Sun H., Añón M.C. (2020). Antioxidant activity, nutritional, and phenolic composition of sweet potato leaves as affected by harvesting period. *International Journal of Food Properties*, 23(1): 178-188.
- Tang C.C., Ameen A., Fang B.P., Liao M.H., Chen J.Y., Huang L.F., Zou H.D., Wang Z.Y. (2021). Nutritional composition and health benefits of leaf-vegetable sweet potato in South China. *Journal of Food Composition and Analysis*, 96.

Session 2

Animal Production and Food Quality

ORIGINAL SCIENTIFIC PAPER

Changes of goats' blood haemogram during early lactation

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Summary

Objective of this research was to determine changes of goats' blood haemogram during early lactation. The research was conducted on 22 Alpine goats in early lactation. Blood was sampled on the 15th, 30th and 60th day of lactation, and tested for parameters of erythrocytes and leukocytes count (RBC, WBC), content of haemoglobin (HGB) and haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), as well as the distribution of leukocytes. Blood tests confirmed significant decrease in the content of HGB, HCT and neutrophils, as well as a trend of decreasing RBC and WBC. Content of lymphocytes and monocytes in goats' blood was significantly increasing with the advance of lactation. There were no changes in the content of MCH, MCV and MCHC in the blood of goats in early lactation. Changes in the goats' blood haemogram confirmed by this research indicate the usefulness of animal blood testing for prevention of certain diseases and major losses in production. Early lactation shall be considered as an important factor in determination of a haemogram model.

Key words: haemogram, blood, goat, early lactation

Introduction

Lactation is a demanding and stressful period for goats, because it causes significant changes in the animal body, which reflect in haematological indicators (Antunović et al., 2013). Changes in haematological indicators confirm the fast and the best possible adaptation of goats to lactation. The goats' blood haemogram tests components of the blood, i.e. haemoglobin, blood cells count, and other blood indices. Haemogram is a good indicator of physiological health and welfare status of small ruminants (goat and sheep). Blood test is important in assessing the response of animal to various physiological stressful conditions, such as lactation, parturition and gravidity (Manat et al., 2016; Fazio et al., 2016). Roland et al. (2014) emphasised the haemogram provided valuable information in the diagnosis, surveillance, and formulation of a prognosis regarding the future progression of a disease. Mbassa and Poulsen (1991) pointed out that after kidding, goats showed significant changes in haematological indicators, especially in RBC, and in the content of HGB and HCT due to haemodilution. Erythrocyte constants (MCV, MCH, MCHC) provide information on the average cell size, haemoglobin content, and the ratio of haemoglobin concentration in erythrocytes (Roland et al., 2014). The transition period of goats lasts from 3 weeks before kidding to 3 weeks after kidding (Tharwat et al, 2015), which indicates that the early stage of goat lactation is very important, not only because of the increased milk production, but also because of reduced food consumption and gaining of the lost body weight (Drackley, 1999). As pointed out by many authors, haematological indicators in the goats' blood are significantly influenced not only by reproductive status, age, breed and diet (Livnjak and Hadžimusić, 2022; Antunović et al., 2020, and 2023; Arfuso et al., 2016; Piccione et al., 2014), but also by the range management and laboratory situation (McDougall et al., 1991). This paper investigates possible changes in the haemogram of goats in early lactation and compares them with available previous research.

Material and methods

The research into haematological indicators was conducted on 22 Alpine goats. All goats were in early lactation, and the research started on the 15th day of lactation and lasted until the 60th day of lactation. The goats were of good health and in satisfactory physical condition. The research was carried out on a small farm near Valpovo, which has been breeding goats for many years. It disposes of 40 adult goats. The criteria for selection of goats for this research were age, number and stage of lactation, and the number of kids in the litter. On average, selected goats were 5 years old, in the 4th lactation, with 1.5 kids in the litter. Goats were fed diets balanced at 16% crude protein and 11 MJ/kg DM ME, in the amount of 1.5 kg daily. Alfalfa hay, water and livestock salt were offered ad libitum. Sampling of goats' blood was carried out before morning feeding on the 15th, 30th and 60th day of lactation. Blood was taken from the jugular vein into sterile vacuum tubes with EDTA anticoagulant. Within two hours post sampling, goats' full blood was tested on an automatic three-part differential haematology analyser Sysmex pocH-100V. The test determined the erythrocytes and leukocytes count (RBC, WBC), content of haemoglobin (HGB) and haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Afterwards, blood smears were prepared and stained according to Pappenheim in order to determine the differential blood count. The relative ratio of individual leukocyte types was calculated as a percentage in relation to the total count. Research results were processed in TIBCO Statistica® 13.3.0., and differences between means were checked by the method of repeated measures at the significance level of 0.05.

Results and discussion

Analysis of haemogram of goats in early lactation, as of the results overviewed in the graphs 1 and 2, showed that there was significant lowering of HGB content in goats' blood on the 60th lactation day, as well as the content of HCT of the 30th lactation day, when comparing the results with the 15th lactation day. The content of HGB in goats' blood was also reduced on the 30th lactation day, and the content of HCT was reduced on the 60th lactation day, but without statistically significant difference. In the research of Antunović et al. (2013) carried out on lactating Alpine goats (from the 30th to the 60th lactation day), contents of HGB and HCT were significantly reducing ($P < 0.01$) as the lactation progressed. The decrease in HGB content can be connected with increased milk production (Hassan et al., 1986). Antunović et al. (2006) also confirmed a decrease in haemoglobin and haematocrit content in goats' blood in the first month of lactation. Tharwat et al. (2015) published similar results related to the reduced WBC and RBC, as well as the content of HGB and HCT in goats' blood in early lactation (from the 2nd to the 3rd week). Das and Singh (2000) also reported on changes in WBC. Compared to the results obtained in this research, in the research conducted in Italy, Zumbo et al. (2011) tested blood of goats in early lactation (on the 28th lactation day) to determine also the significant lowering of WBC and RBC, as well as significant lowering of HCT content. In this research, there was the trend of lowering RBC in the goats' blood as the lactation advanced, as well as lowering of WBC in goats' blood on the 60th lactation day (Figure 1). Similar, yet significant changes in blood of goats being in lactation from the 30th to the 60th day were reported by Antunović et al. (2013), as they established significant lowering ($P < 0.01$) of RBC and WBC as the lactation advanced. Due to increased transport from blood to milk, WBC and neutrophils in blood decreased, allowing for more efficient phagocytosis and defence of the mammary gland against various pathogens (Paape et al., 1992). Reduced RBC, HGB and HCT in blood of goats from the period of gravidity to the middle lactation were determined also by Mbassa and Poulsen (1991). When comparing results obtained in this research with reference values, it is confirmed that they were within the limits determined by Feldman et al. (2002) as follows: WBC ($\times 10^3/\mu\text{L}$) 3-13, RBC ($\times 10^6/\mu\text{L}$)

8-18, HGB 8-12 g/dL, HCT 22-38%. Slightly lower content of HGB in blood of goats in early lactation can be associated with the decrease in the blood's oxygen-carrying capacity, leading to symptoms of anaemia (Aaron et al., 2003). However, as pointed out by Cole et al. (1997), anaemia is a common abnormality seen in the blood profiles of ruminants defined as haematocrits less than 24%, and RBC less than 5×10^{12} cells/L, or HGB concentration less than 8 g/dL. In this research, no such changes were recorded for the first two indicators that indicate anaemia. This needs to be investigated in future research, since the health status of the researched goats did not indicate the existence of anaemia. Similar content of HGB in blood of indigenous Croatian goats (Croatian white goat and Istrian goat) of different age were reported by Antunović et al. (2023 and 2020), and those blood tests were performed on the same device as used in this research. The mentioned changes may indicate the influence of chosen methodology and laboratory processing of blood samples.

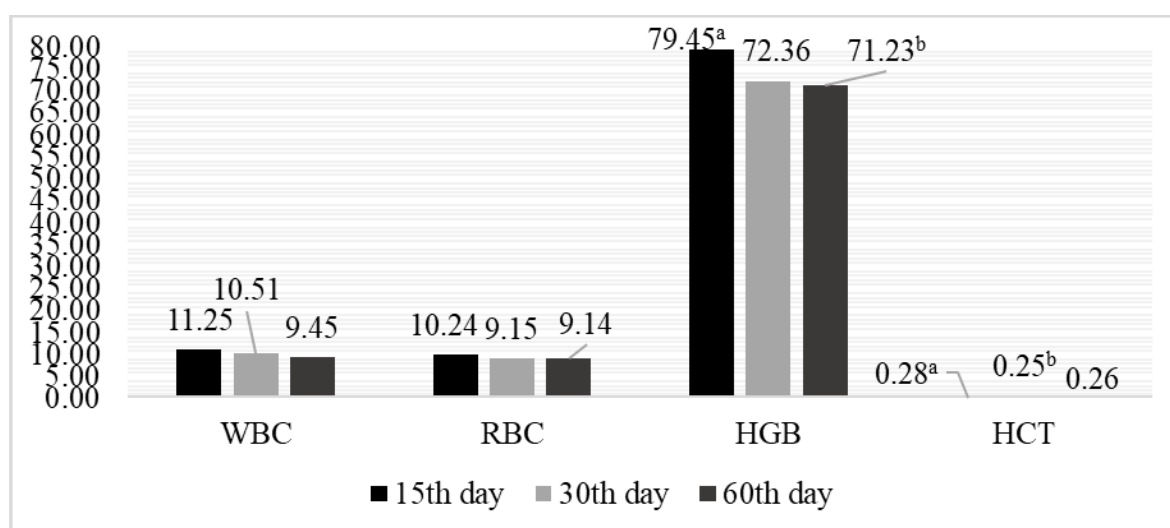


Figure 1. Number of white blood cell: WBC ($\times 10^9$ L) and erythrocytes: RBC ($\times 10^{12}$ L) as well as content of haemoglobin (HGB, g/L) and haematocrit (HCT, %) in the blood of goats during early lactation (a, b - $P < 0.05$)

There were no significant changes in the content of MCH, MCV and MCHC in goats' blood in early lactation (Figure 2). In the research of Antunović et al. (2013) carried out on lactating Alpine goats (from the 30th to the 60th day of lactation), there were also no changes determined in the content of MCH and MCHC, while there was significant lowering of the content of MCV as the lactation advanced. Similar results related to MCH and MCHC in blood of goats in early lactation (from the 2nd to the 3rd week) were reported by Tharwat et al. (2015). According to Feldman et al. (2002), and the following reference values: MCV 16-25 fL, MCH 2,2-8 pg, MCHC 300-360 g/L, the research proved slightly lower content of MCHC and higher content of MCV. In this respect, a slightly higher MCV compared to reference values may indicate a cell regeneration response (Antunović et al., 2014), which is expected because of the stress to which goats are exposed in early lactation. Lower MCHC may occur because of reticulocytosis or iron deficiency (Polizopoulou, 2010), however, no such changes were confirmed in this research.

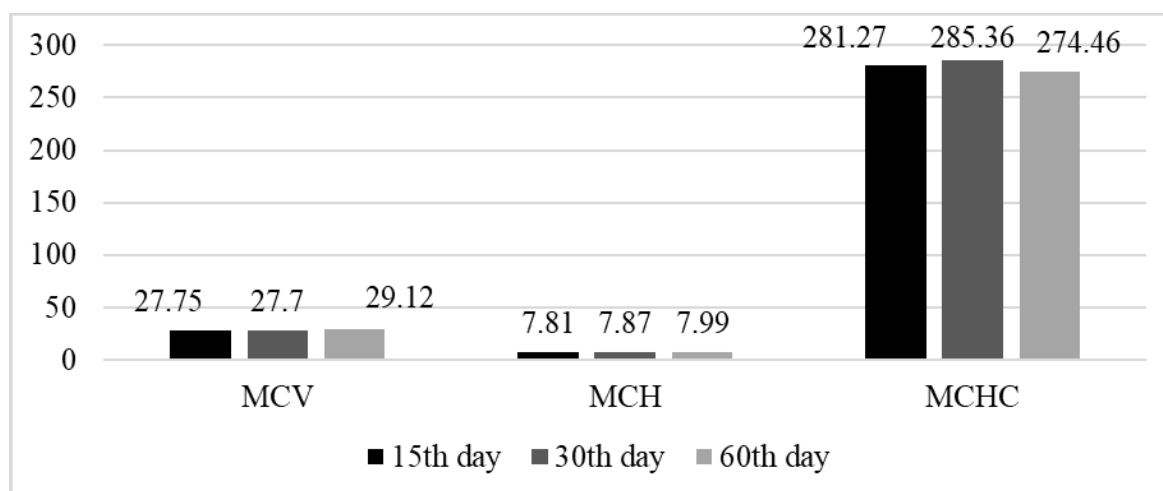


Figure 2. Content of average haemoglobin content in erythrocytes (MCH, fL), mean volume of red blood cells (MCV, pg) and mean haemoglobin concentration (MCHC, g/L) in goats during early lactation

The differential blood count of goats in early lactation is shown in the Table 1.

Table 1. Differential blood count of goats during early lactation

Indicator	Days of early lactation, % (mean $\pm$ sd)			Reference values* (%)
	15.	30.	60.	
Lymphocytes	44.68 ^a $\pm$ 10.82	52.14 ^b $\pm$ 12.45	52.86 ^b $\pm$ 13.13	50-78
Neutrophils	49.41 ^a $\pm$ 11.54	43.05 ^{ab} $\pm$ 11.85	39.96 ^b $\pm$ 13.17	30-48
Eusinophils	3.86 $\pm$ 2.90	2.68 $\pm$ 2.63	4.55 $\pm$ 3.76	1-8
Basophils	1.00 $\pm$ 1.38	0.86 $\pm$ 0.89	0.95 $\pm$ 0.90	0-1
Monocytes	1.05 ^a $\pm$ 1.56	1.73 ^{ab} $\pm$ 1.96	2.45 ^b $\pm$ 2.30	0-4

sd - standard deviation; * Feldman et al. (2002); a, b - ($P < 0.05$)

Analysis of the differential blood count showed that goats in early lactation exhibited significant increase in the content of lymphocytes and monocytes, and the content of neutrophils decreased as lactation progressed. There were no significant changes in the content of eosinophils and basophils in the blood of goats in early lactation. When testing the blood of lactating goats, Antunović et al. (2013) reported on the significant increase of eosinophils. Tharwat et al. (2015) confirmed significant increase in monocyte content and a slight decrease in neutrophils in blood of goats in early lactation (from the 2nd to the 3rd week of lactation). Das and Singh (2000) also determined that lymphocytes and neutrophils were low during early lactation. Similar result for leukocyte distribution in Istrian goats was reported by Antunović et al. (2020). By comparing the results of leukocyte distribution in the blood of goats obtained in this research with the reference values (Feldman et al., 2002), it is concluded that all obtained values were within physiological limits.

Conclusions

Determined changes in goats' blood haemogram (significant decrease of HGB, HCT and neutrophils, the trend of lowering of RBC and WBC, and significant increase in lymphocytes and monocytes) confirm the need of goats' blood testing during early lactation. Blood tests shall be properly performed and blood shall be analysed by applying appropriate laboratory methods. Changes in blood test results for goats in early lactation shall be considered in assessment of health condition of animals in order to undertake timely action to avoid the occurrence of certain diseases and to prevent major losses. Results of blood tests obtained in this research indicate

that early lactation of goats should be taken as an important factor in determining the model of goats haemogram.

Acknowledgement

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References

- Aaron, S.D., Vandemheen K.L., Naftel S.A., Lewis M.J., Rodger M.A. (2003). Tropical tetracaine prior to arterial puncture: a randomized, placebo-controlled clinical trial. *Respiratory Medicine*. 97: 1195-1199.
- Antunović Z., Šperanda M., Senčić Đ., Šerić V., Šperanda T., Gutzmirtl D. (2006). Metabolički profil mliječnih koza u ekološkoj proizvodnji. *Krmiva*. 48 (5): 231-236.
- Antunović Z., Novoselec J., Klir Ž., Đidara M. (2013). Hematological parameters in the Alpine goats during lactation. *Poljoprivreda*. 19 (2): 40-43.
- Antunović Z., Marković B., Novoselec J., Marković M., Klir Ž., Radonjić D. (2014). Hematološki pokazatelji ugroženih pasmina ovaca - dubrovačke rude i zetske žuje. *Zbornik radova 49. hrvatskog i 9. međunarodnog Simpozij agronoma*. Dubrovnik, Hrvatska, 16.-21.02.2014, 291-295.
- Antunović Z., Novaković K., Klir Ž., Šerić V., Mioč B., Šperanda M., Ronta M., Novoselec J. (2020). Blood metabolic profile and acid-base status of Istrian goats- a critically endangered Croatian goat – in relation to age. *Veterinarski arhiv*. 90 (1): 27-38.
- Antunović Z., Erceg O., Klir Šalavardić Ž., Mioč B., Đidara M., Novoselec J. (2023). Blood metabolic profile of Croatian white goat. *Veterinarski arhiv*. 92 (6): 713 – 722.
- Arfuso F., Fazio F., Rizzo M., Marafioti S., Zanghì E., Piccione, G. (2016). Factors affecting the hematological parameters in different goat breeds from Italy. *Annals of Animal Science*. 16 (3): 743-757.
- Cole D.J., Roussel A.J., Whitney M.S. (1997). Interpreting a bovine CBC: collecting and sample and evaluating the erythron. *Veterinary Medicine*. 92(5): 460-468.
- Das M., Singh M. (2000). Variation in blood leucocytes, somatic cell count, yield and composition of milk crossbred goats. *Small Ruminant Research*. 35 (2): 169-174.
- Drackley J.R. (1999). Biology of dairy cows during the transition period: the final frontier? *Journal of Dairy Science*. 82(11): 2259-2273.
- Fazio F., Giangrosso G., Marafioti S., Zanghì E., Arfuso F., Piccione G. (2016). Blood haemogram in *Ovis aries* and *Capra hircus*: effect of storage time. *Canadian Journal of Animal Science*. 96 (1): 32-36.
- Feldman B., Zink J., Jain N. (2002). *Schalm's Veterinary Hematology*. Philadelphia, PA, Baltimore, New York, London, Buenos Aires, Hong Kong, Sidney, Tokyo: Lippincott Williams and Wilkins, pp 1376.
- Hassan G.A., El-Nouty F.D., Samak M.A., Salam H.M. (1986). Relationship between milk production and some blood constituents in Egyptian Baladi goats. *Beitr Trop Landwirtschaft Veterinarmed*. 24 (2): 213-219.
- Livnjak A., Hadžimusić N. (2022). Hematological parameters of Alpine goats in Bosnia and Herzegovina. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine*. 79 (2): 42-46.
- Manat T.D., Chaudhary S.S., Singh V.K., Patel S.B., Puri G. (2016). Hematobiochemical profile in Surti goats during post-partum period. *Veterinary World*. 9(1): 19-24.
- Mbassa G.K., Poulsen J.S.D. (1991). Influence of pregnancy, lactation and environment on haematological profiles in Danish landrace dairy goats (*Capra hircus*) of different parity. *Comparative Biochemical Physiology*. 100 (2): 403-412.

- McDougall S., Lephherd E.E., Smith S. (1991). Haematological and biochemical reference values for grazing Saanen goats, *Australian Veterinary Journal*. 68(11): 370–372.
- Paape M.J., Capuco A.V., Lefcourt A., Burvenich C., Miller R.H. (1992). Physiological response of dairy cows to milking. In: Ipema A.H., Lippus A., Metz J., Rossing W. (Eds.), *Proceedinmgs of International Symposium Prosp. Autom. Milking*. Pudco. Sci. Publ. Wageningen, The Netherlands. EAAP Publ. 65: 93-105.
- Piccione G., Monteverde V., Rizzo M., Vazzana I., Assenza A., Zumbo A., Niutta P.P. (2014). Reference intervals of some electrophoretic and haematological parameters in Italian goats: comparison between Girgentana and Aspromontana breeds. *Journal of Applied Animal Research*. 42 (4): 434–439.
- Polizopoulou Z.S. (2010). Haematological test in sheep health management. *Small Ruminant Research*. 92 (1-3): 88-91.
- Roland L., Drillich M., Iwersen M. (2014). Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation*. 26 (5): 592-598
- Tharwat M., Ali A., Al-Sobayil F. (2015). Hematological and biochemical profiles in goats during the transition period. *Comparative Clinical Pathology*. 24: 1-7.
- Zumbo A., Sciano S., Messina V., Casella S., di Rosa A.R., Piccione, G. (2011). Haematological profile of Messinese goat kids and their dams during first month post-partum, *Animal Sciences Papers and Reports*. 29 (3): 223–230.

REVIEW ARTICLE

The Issue of Ethics and Equine Welfare – Eventing and Flat Racing

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Summary

Equestrian sport struggle to maintain social licence to operate since the abundance of scientific research gained important data about behaviour and different needs of equine species while on the other hand social media is full of videos showing negative examples of neglect and abuse of horses for different purposes. The concept of welfare has gained significant prominence in recent years, serving as a starting point for the establishment of sound principles and maintenance standards for the welfare of horses in the broader equestrian industry. This objective is pursued through the integration of different sectors (agriculture, veterinary science, kinesiology, economics, etc.) as they collectively contribute to the organization and management of different components of the equestrian sector. Globally renowned and well attended, horse racing and eventing are considered the most dangerous equestrian sports with the highest rate of fatal injuries, which has a direct impact on the welfare of horses and riders. The aim of this paper is to examine the challenges associated with flat racing and eventing, including the real risks to the welfare, health, and fatalities of horses. The study refers to existing regulations and ethical frameworks that apply to all equestrian sports to enhance welfare standards in these two particular sports.

Key words: horse welfare, flat racing, eventing

Introduction

Equestrian sports and cultural events featuring horses have long captured people's interest. With the modernization of society, the farm working animal became a status symbol, while at the same time the importance of global horse breeding increased. Within the global equine industry, there are several different sectors in which experts from agriculture, veterinary medicine, kinesiology, business and other professions work together. It is of great importance as it attracts entrepreneurs and investors from various industries, that further contribute to the overall economic value of the global equestrian industry (Connors and Feldman, 2009). According to Kibler and Thompson (2020), the current nature of horse trading through online advertising and auctions is drastically changing the market dynamics as the geographic area for buying and selling sport horses expands. However, it is interesting that according to Bryant and Stowe (2020), less than 50% of transactions at auction sales are profitable, and the median profit is almost always negative. Despite this, the top horses in the sport fetch high purchase prices. The total value of the global equestrian industry is expressed in billions of dollars, and Connors and Feldman (2009) confirm that the import and export of horses around the world is the main reason for this. At a European level, the annual economic and value impact of equestrian sport is €52.1 billion, with flat racing accounting for as much as €20.3 billion (FEI, 2020). Zhang (2017) adds that sports betting as a whole and a separate industry within sport horse breeding

in the UK and Ireland is worth up to £7.1 billion per year. Sports betting has a dual character, so for Peirson and Smith (2010) responsible behaviour is important because it is a known situation to share confidential information about competing horses. The desire for profit points to the competitive nature of humans, which sometimes supersedes the most important component of equestrian sport - the welfare of the horse. Respecting the principles of welfare is the foundation of all equestrian sports, even if each of them has its own ethical and animal welfare characteristics. For example, terms such as doping, maximum utilization of the horse capacity and illegal betting are associated with the flat racing, which attracts various interest groups and generates a great deal of media attention. Eventing, on the other hand, is the most demanding equestrian sport for both horse and rider, resulting in numerous injuries and deaths in horses. It therefore requires maximum regulation and an improvement in competition standards. These two equestrian sports are considered one of the most dangerous sports and it is important to note that injuries to riding pairs can be prevented: i) through the interaction of experience and fitness status of horse and rider, ii) and by improving the standards regarding the requirements of each equestrian sport (Crawford et al. 2020; FEI, 2022).

Flat races

The history of flat racing dates back several thousand years to Ancient Greece and Rome, and it is not surprising that over time, it gained the most popularity compared to all other equestrian sports (Willekes, 2023). Some of the most famous flat races in the world are the Kentucky Derby, the Prix de l'Arc de Triomphe, the Melbourne Cup and others. Flat races have always been watched by a large number of spectators, and media coverage additionally contributed to their attractiveness (Bell et al., 2020). The popularity of flat racing and horse breeding led to the establishment of an official studbook for the English thoroughbred breed in 1791. Today, it is the second most popular sport in the UK and Ireland after football (Zhang, 2017). One of the problems of racing is betting, which is widespread worldwide and offers opportunities for illegal profits (e.g. race fixing). Race fixing is possible through the use of banned substances, the illegal influencing on people involved in the functioning of flat racing and the sharing of confidential information about competing horses. It was Peirson and Smith (2010) who concluded that two-year-olds who have not yet raced are 16% more likely to win than two-year-olds who have raced. Research by the same authors points to the dangers of releasing confidential information that can strongly influence betting privileges. Considering the high amount of legal betting that is as much as \$200 million in one day during the Kentucky Derby or Kentucky, the use of banned substances that affect horse performance is not unknown (Silfer, 2018 cited in Tuttle, 2018). According to Wong and Wan (2014), the biggest problem is the relative freedom of the competent organisations in the general regulation of doping controls as well as the existing exceptions for permitted substances despite the proposed guidelines of the IFHAe (International Federation of Horseracing Authorities). The technological development of various illegal substances that affect horse performance is progressing faster than the development of tests to determine them, so it is necessary to test horses at races and during training as well as to keep samples for possible analysis years after the competition. Wilkin et al. (2017) point out the problem of the manipulation of horse performance. In their opinion, advances in genetic engineering and the detection of specific genes responsible for horse performance could lead to another level of doping in flat racing known as "genetic doping" which is very difficult to detect and would severely compromise the integrity of the sport. Unethical treatment of horses can also be observed in the racing such as tongue tying, excessive use of whips, excessive strain on horses or the use of electric shockers. Tongue tying of horses is prohibited, and Marsh et al. (2019) found that tongue-tying results in behaviour that betrays discomfort (laying the ears back, pronounced head movement and mouth opening). Whips are allowed in races, but their excessive use is punishable as it can cause pain, discomfort and injury

to the horse. A study by Evans and McGreevy (2011) found that increased use of the whip, particularly in the last 200 m before the finish line, was not associated with a significant change in speed (the highest speeds had already been reached) but was associated with horse fatigue. Accordingly, the use of whips on tired horses during the race can hardly be justified. One of the characteristics of the flat racing is the early start and end of the sporting career of young horses, and some of the factors that influence the length of a horse's career in flat racing are the age at entry into the sport, the number of starts and the owner's financial profit (Özen et al., 2021). The same authors note that in Turkey, the average time spent in flat racing is only 17.79 months and that low financial profit can shorten a performance career by 2.28 times. Entering the flat racing too young increases the likelihood of musculoskeletal injuries, which are most common in English Thoroughbreds (Physick-Sheard et al., 2019). Although there is no consolidated data on mortality at a global level, according to the latest data from the Jockey Club (2023), there has been a significant decrease in deaths among racehorses. In 2009, there was an average of 2 deaths per 1,000 starts, and in 2022 the number of deaths fell to 1.25, a significant decrease of 37.5%. Lyle et al (2010) conducted a study of 268 English Thoroughbreds that died suddenly in races and concluded that some of the most common causes were heart failure, lung failure, pulmonary haemorrhage, haemorrhage associated with pelvic fractures or idiopathic rupture of blood vessels, and spinal cord injury. When analysing the descriptive epidemiology of fractures on race tracks of the Japan Racing Association (JRA) from 1980 to 2000, Maeda et al. (2016) found an incidence of fractures during races of 1-2% and 0.1% during training. The frequency of fractures depends on various factors such as the type of track, the surface and the weather conditions. The most common fractures of the front legs occur when changing the lead leg and in the third and fourth corners of oval tracks. Specifically, in the period from 1983 to 1986, there were a total of 259 fractures, although the number of fractures decreased due to the geometric redesign of the tracks (increasing the diameter of the turns). In terms of surface, the injury rate is higher on clay surfaces than on grass or synthetic surfaces (Maeda et al., 2016; Peterson et al., 2021). It is important to emphasize that with the new regulations for the proper handling of horses and the improvement of the terrain for races in flat racing, the rate of injury and death of horses is decreasing (Maeda et al., 2016; The Jockey Club, 2023).

Eventing - a test of the horses' versatility

Eventing is a test of the versatility of horses. Once used to test horses for military and official purposes, today it is a challenge to overcome the most demanding obstacles for the most experienced horses and riders (Foreman, 2014). The name eventing comes from the three-stage, i.e. three-day competition including dressage, show jumping and cross-country (Cameron-Whytock et al., 2023). Eventing is an Olympic sport and is governed by the Federation Equestre Internationale (FEI) and all horses used in this sport are protected by the FEI International Regulations, which govern the above areas through the FEI General Regulations, FEI Veterinary Regulations, FEI Controlled Substances Regulations (OEDCM) and others, in addition to EU and national legislation relating to equine welfare and biosecurity. Although the above regulations apply to all equestrian disciplines under the jurisdiction of the FEI, they are particularly important for this sport as injuries to horse and rider, sometimes fatal, are by far the most common. Fixed obstacles can cause rotational falls, where the horse runs at full speed into an obstacle that he cannot overcome, resulting in a sudden change of trajectory, a turn and a fall of horse and rider (Flogard, 2009). In a rotational fall, horses most commonly suffer a skull fracture, particularly at the base of the skull, with consequent injuries to the vital structures of the head and neck and bleeding of the horse through the carotid artery, which usually ruptures (Foreman, 2014). According to Cameron-Whytock et al. (2023), almost half of all deaths in this sport are caused by falls in the cross country over fixed obstacles. Their study in the UK identified 16 risk factors for horse falls in the cross phase of competitions. The authors believe

that important recommendations can be derived from their work for the introduction of new rules for eventing that would reduce the injury rate among horses and riders. For example, limiting the number of penalty points in dressage or changing the qualification requirements for advancement at different levels of competition could be useful to reduce the risk of falls. The time between starts in competitions also needs to be researched further, as the mandatory hold time in endurance could reduce the risk of falls and, therefore, the risk of injury or death. Of the total 217,066 cross-country starts between 2010 and 2021, according to official FEI data, 398 ended in a fall of the horse, representing 5.45%, with 117 horses fatally injured, representing 34% overall. According to the FEI (2022), 2.5% of the total number of falls between 2010 and 2021 were those with serious consequences, and 90% of falls occur during the cross-country phase of the competition. In addition to fatal accidents, there are numerous other injuries, particularly in horses that have previously competed in flat races, as their racing careers are relatively short (Ecker, 1995). Although English Thoroughbreds are faster and more enduring than horses of some other breeds, they suffer stress fractures of the patella, patellar desmitis, rhabdomyolysis and soft tissue injuries much more frequently than horses of other breeds. They are ridden too early due to flat racing, and even if they survive their racing careers unscathed, they usually suffer injuries when used in eventing. Furthermore, hemorrhage in lungs due to extreme work-related stress (EIPH) usually occurs during the cross-country phase of the competition and is accompanied by spontaneous nosebleeds (epistaxis). In addition, stress-related pneumonia and pleuropneumonia are common in horses in the USA, especially those traveling more than 500 km from one competition to the next (Casey, 2007). Also, according to Cameron-Whytock et al (2023), the risk of falling is greater in horses that compete too often and expose themselves to great exertion. Fortunately, the so-called over-riding of horses and the forcing of exhausted horses is illegal and subject to the regulations of the competent authorities (FEI, 2023). Cardiac problems also occur as a result of the strain in the cross-country phase, such as potentially fatal atrial fibrillation (AF) with cardiac arrest, as seen at the 1996 Atlanta Olympics, where two of the 99 horses that qualified for the cross-country phase developed AF, although they were saved by the rapid intervention of the veterinarian on duty (Foreman, 2014). Rupture of the aorta caused by exertion and stress is possible in horses during the cross-country phase, where horses practically bleed to death very quickly and no help is available. It is clear from all of the above that the use of horses in eventing is a high-risk event for both the horses and the riders, despite all the safety measures, trained and well-equipped veterinary services at the competition venue and strict international and FEI regulations regarding the welfare of horses. Given the fact that riders voluntarily participate in this high-risk activity and horses are introduced to it based on our needs and assessments, the current condition of horses in eventing requires further analysis and research, as well as the introduction of new methods and changes to improve the standard of horse welfare.

Conclusions

Horse welfare is an important factor in all equestrian sports, including flat racing and eventing, which are among the most challenging sports for both horse and rider. It is important to note that the number of injuries and fatalities is decreasing year on year thanks to better regulations and better conditions for flat racing. In eventing number of injuries and deaths of horse and rider is decreasing, and the increasing number of falls is characterized as harmless. Although the horse industry itself and potential financial gains influence equestrian sport on a global level, and in certain situations they call into question the welfare of horses, the support of responsible bodies and institutions (at international and national level) and the revision and adoption of regulations in terms of ethics and defined rules in competitions put balance in it. The above measures must be applied responsibly in order to reduce injuries and fatalities of both horses and riders.

References

- Aerts, J., Piwczyński, D., Ghiasi, H., Sitkowska, B., Kolenda, M., & Önder, H. (2021). Genetic parameters estimation of milking traits in Polish Holstein-Friesians based on automatic milking system data. *Animals*, 11(7): 1943.
- Bar, D., & Solomon, R. (2010). Rumination collars: What can they tell us? In *Proceedings of the First North American Conference on Precision Dairy Management* (pp. 214–216). Toronto, Canada.
- Behren, L. E., König, S., & May, K. (2023). Genomic selection for dairy cattle behavior considering novel traits in a changing technical production environment. *Genes*, 14(10): 1933.
- Brito, L. F., Oliveira, H. R., McConn, B. R., & Schinckel, A. P. (2020). Large-scale phenotyping of livestock welfare in commercial production systems: A new frontier in animal breeding. *Frontiers in Genetics*, 11: 793.
- Cavani, L., Brown, W. E., Parker Gaddis, K. L., Tempelman, R. J., VandeHaar, M. J., White, H. M., Peñagaricano, F., & Weigel, K. A. (2022). Estimates of genetic parameters for feeding behavior traits and their associations with feed efficiency in Holstein cows. *Journal of Dairy Science*, 105(9): 7564–7574.
- Eckelkamp, E. A. (2019). Invited review: Current state of wearable precision dairy technologies in disease detection. *Applied Animal Science*, 35(3): 209–220.
- Farris, E. J. (1954). Activity of dairy cows during estrus. *Journal of the American Veterinary Medical Association*, 125: 117–120.
- Fraser, E. D. G., & Campbell, M. (2019). Agriculture 5.0: Reconciling production with planetary health. *One Earth*, 1(3): 276–279.
- Gur, P. (n.d.). Cow Welfare – the Afimilk way. Afimilk Ltd. (Industry article noting Afikim's introduction of the first electronic milk meter and heat-detection pedometer)
- Hoffmann, G., Strutzke, S., Fiske, D., Heinicke, J., & Mylostyvyi, R. (2024). A new approach to recording rumination behavior in dairy cows. *Sensors*, 24(17): 5521.
- Kaur, A., Cavani, L., Parker Gaddis, K. L., Tempelman, R. J., VandeHaar, M. J., White, H. M., Peñagaricano, F., & Weigel, K. A. (2023). Genetic relationships between behavioral traits and feed efficiency traits in lactating Holstein cows. *Journal of Dairy Science*, 106(10): 8141–8149.
- Kiddy, C. A. (1977). Variation in physical activity as an indication of estrus in dairy cows. *Journal of Dairy Science*, 60(2): 235–243.
- Kleen, J. L., Guatteo, R. (2023). Precision Livestock Farming: What does it contain and what are the perspectives? *Animals*, 13(5): 779.
- Koltes, J. E., Cole, J. B., Clemmens, R., Dilger, R. N., Kramer, L. M., Lunney, J. K., McCue, M. E., McKay, S. D., Mateescu, R. G., Murdoch, B. M., Reuter, R., Rexroad, C. E., Rosa, G. J. M., Serão, N. V. L., White, S. N., Woodward-Greene, M. J., Worku, M., Zhang, H., & Reecy, J. M. (2019). A vision for development and utilization of high-throughput phenotyping and big data analytics in livestock. *Frontiers in Genetics*, 10: 1197.
- Kramer, M., Erbe, M., Bapst, B., Bieber, A., & Simianer, H. (2013). Estimation of genetic parameters for novel functional traits in Brown Swiss cattle. *Journal of Dairy Science*, 96(9): 5954–5964.
- Lamanna, M., Bovo, M., & Cavallini, D. (2025). Wearable collar technologies for dairy cows: A systematized review of current applications and future innovations in precision livestock farming. *Animals*, 15(3): 458. <https://doi.org/10.3390/ani15030458>
- Lemal, P., Tran, M.-N., Atashi, H., Schroyen, M., & Gengler, N. (2024). Adding behavior traits to select for heat tolerance in dairy cattle. *JDS Communications*, 5: 368–373. DOI: 10.3168/jdsc.2023-0421
- Lewis, G. S., & Newman, S. K. (1984). Changes throughout estrous cycles of variables that might indicate estrus in dairy cows. *Journal of Dairy Science*, 67(1): 146–152.
- Luginbuhl, J. M., Pond, K. R., Russ, J. C., & Burns, J. C. (1987). A simple electronic device and computer interface system for monitoring chewing behavior of stall-fed ruminant animals. *Journal of Dairy Science*, 70(6): 1307–1312.
- Luo, H., Hu, L., Brito, L. F., Dou, J., Sammad, A., Chang, Y., Ma, L., Guo, G., Liu, L., Zhai, L., Xu, Q., & Wang, Y. (2022). Weighted single-step GWAS and RNA sequencing reveals key candidate genes associated with physiological indicators of heat stress in Holstein cattle. *Journal of Animal Science and Biotechnology*, 13: 88.

- Mayberry, D. E., Ash, A., Prestwidge, D., Godde, C., Harrison, M. T., & Eckard, R. J. (2023). Opportunities and challenges for integrating precision livestock technologies into dairy cattle breeding programmes. *Animal Production Science*, 63(6): 578–586.
- Mazier, H., Tal, S., Aizinbud, E., & Bargai, U. (2006). A field investigation of the use of the pedometer for the early detection of lameness in cattle. *The Canadian Veterinary Journal*, 47(9): 883–886.
- Menezes, G. L., Pontes Ferreira, R. E., Chinelato, L. S., et al. (2024). Artificial intelligence for livestock: a narrative review of the applications of computer vision systems and large language models for animal farming. *Animal Frontiers*, 14(6): 36–47.
- Moretti, R., de Rezende, M. P. G., Biffani, S., & Bozzi, R. (2018). Heritability and genetic correlations between rumination time and production traits in Holstein dairy cows during different lactation phases. *Journal of Animal Breeding and Genetics*, 135(4): 293–299.
- Nascimento, B. M., Cavani, L., Caputo, M. J., Marinho, M. N., Borchers, M. R., Wallace, R. L., Santos, J. E. P., White, H. M., Peñagaricano, F., & Weigel, K. A. (2024). Genetic relationships between behavioral traits and feed efficiency traits in lactating Holstein cows. *Journal of Dairy Science*, 107(10): 8141–8149.
- Neethirajan, S. (2023). Digital phenotyping: A game changer for the broiler industry. *Animals*, 13(16): 2585.
- Neethirajan, S., & Kemp, B. (2021). Digital phenotyping in livestock farming. *Animals*, 11(7): 2009.
- Pedrosa, V. B., Boerman, J. P., Gloria, L. S., Chen, S.-Y., Montes, M. E., Doucette, J. S., & Brito, L. F. (2023). Genomic-based genetic parameters for milkability traits derived from automatic milking systems in North American Holstein cattle. *Journal of Dairy Science*, 106(4): 2613–2629.
- Pedrosa, V. B., Chen, S. Y., Gloria, L. S., Doucette, J. S., & Silva, M. A. (2024). Machine learning methods for genomic prediction of cow behavioral traits measured by automatic milking systems in North American Holstein cattle. *Journal of Dairy Science*.
- Piwczyński, D., Kolenda, M., Sitkowska, B., & Aerts, J. (2021). Genetic parameters of workability traits in the population of Polish Holstein-Friesian cows based on conventional and genomic data. *Animals*, 11(8), 2443.
- Prasad, S. (2023). RFID technology and its application in milk production management. *AgriGate – An International Multidisciplinary e-Magazine*, 2(8): 56–62.
- Santos, L. V., Brügemann, K., Ebinghaus, A., & König, S. (2018). Genetic parameters for longitudinal behavior and health indicator traits generated in automatic milking systems. *Archives Animal Breeding*, 61: 161–171.
- Seidel, A., Krattenmacher, N., & Thaller, G. (2020). Dealing with complexity of new phenotypes in modern dairy cattle breeding. *Animal Frontiers*, 10(2): 23–29.
- Senger, P. L. (1994). The estrus detection problem: New concepts, technologies, and possibilities. *Journal of Dairy Science*, 77(9): 2745–2753.
- Shamah, D. (2014, December 12). Agritech firm SCR acquired for \$250 million. *The Times of Israel*. (Reports SCR's first collar tags in 1998 and Heatime collar system launch in 2006)
- UN: World Population Prospects: The 2017 Revision.
- Upadhyay, V. R., Ramesh, V., Kumar, H., & Singh, S. K. (2024). Phenomics in livestock research: Bottlenecks and promises of digital phenotyping and other quantification techniques on a global scale. *OMICS: A Journal of Integrative Biology*.
- Wethal, K. B., Ødegård, J., Svendsen, M., & Heringstad, B. (2020). Genetic analysis of mastitis-related traits in Norwegian Red cows. *Journal of Dairy Science*, 103(8): 7188–7198.
- Wiggans, G. R., & Carrillo, J. A. (2022). Genomic selection in United States dairy cattle. *Frontiers in Genetics*, 13, 994466.

REVIEW ARTICLE

The automatic lameness detection in dairy cows

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Summary

The prevalence of lameness on farms is high, posing a major challenge to both farm welfare and profitability. One approach to prevent lameness is through the use of advanced technologies that monitor cow activity or detect certain abnormalities in movement, body mass distribution, or body temperature. Therefore, the aim of this paper was to present various methods of automated lameness detection. The most commonly used method used to identify lame cows is the manual locomotion scoring system, but it is labour-intensive and physically demanding, especially on large dairy farms. To overcome the limitations of visual evaluation and to provide an objective and efficient early lameness detection monitoring tool, automated detection methods have been developed in recent decades. The market offers several automated solutions for lameness detection in dairy cows, each utilizing different sensors for tracking the various parameters, such as parameters during cows locomotion, feeding and grooming behaviour, as well as production and health parameters. Despite the challenges, automatic detection of lame cows offers great potential and should be further developed. The solution to improve its reliability may lie in integrating different observational parameters and sensors to increase the accuracy and effectiveness.

Key words: lameness, detection, automatic, dairy cows

Introduction

Besides mastitis and reproduction disorders, lameness is the most important cause of economic losses on dairy farms. It affects both the health and welfare of dairy cows and is usually caused by painful limb disorders (Dippel et al., 2009). Lameness is characterised as an abnormality in a cow's gait. Because of the pain, animals don't want to lean on a sore leg, so they try to transfer the weight of the body to other extremities, which results in an abnormal walking behaviour. Those abnormalities can be evident by deviations from a normal gait, including slower walking speed (Chapinal et al., 2010; Flower et al., 2005), shorter steps (Flower et al., 2005), an irregular stride, a curved back, and a lowered head (Maertens et al., 2011). Based on these features, lame cows can be identified and then selectively examined and treated if necessary. In the UK, prevalence of lameness ranges between 20 and 37% (Barker et al., 2010; Clarkson et al., 1996; Weaver et al., 2005), while in the USA it ranges between 13 and 55% (Cook et al., 2016; Shearer, 2010; Von Keyserlingk et al., 2012). Lameness has a negative effect on dairy herd performance and farm profitability as it has been associated with a negative effect on milk yield (Archer et al., 2010; Green et al., 2002), reproductive performance (Walker et al., 2010), as well as increasing the risk of culling (Booth et al., 2004). On average, a lame cow with *dermatitis digitalis* costs between €307.50 and €391.80 per year or €12.10 per week per lame cow, respectively (Robcis et al., 2023). The most common method for identifying lame cows is the manual locomotion scoring system, which uses a numerical rating system. For example, some authors used a 2-point scale (Groehn et al., 1992), a 4-point scale (Cook, 2003; Vokey et al., 2001), or a 13-point scale (Offinger et al., 2013). Although very useful, manual locomotion

scoring is labour-intensive and physically demanding, especially on large dairy farms (Leach et al., 2010). From a welfare and farm profitability perspective, lameness is undesirable. One way to prevent lameness is through the use of advanced technologies that monitor cow activity or detect certain abnormalities in movement, body mass distribution, or body temperature. Therefore, the aim of this paper was to present various methods of automated lameness detection.

Automated lameness detection methods

In order to overcome the limitations of visual evaluation and to provide an objective and efficient monitoring tool for the early detection of lameness, automated lameness detection methods have been developed in recent decades. Mainly these methods can be divided into two groups: direct and indirect. Direct methods include the kinematic and kinetic gait analysis, while indirect methods include thermographic imaging, feeding and grooming behaviour, as well as production and health parameters.

Direct methods

Kinematic and Kinetic

Kinematic methods include automatic recognition and analysis of temporal and spatial movement parameters such as cow gait analysis, which tracks changes in the position of specific body segments over time (Alsaad et al., 2019). This type of automatic lameness detection includes monitoring parameters during cows locomotion, such as stride length, trekking distance, the back line, the range of movement for different joints, head position, etc. For this purpose, it can be used with image-processing techniques and 3D cameras (Myint et al., 2024; Viazzi et al., 2013) or accelerometers (Chapinal et al., 2011; Thorup et al., 2015). Kinetic methods, on the other hand, analyze forces applied to the body during movement. For example, using 1- or 3-dimensional ground reaction force plates or weight distribution platforms (Maertens et al., 2011; Pastell et al., 2010).

Indirect methods

Indirect methods do not rely on gait characteristics but on some other parameters, such as the temperature of the body part using infrared thermography (Alsaad et al., 2015; Wood et al., 2015; Zheng et al., 2022), and devices that collect information from sensors for movement (Mandel et al., 2018). Besides that, indirect methods can utilize data for eating habits (Nechanitzky et al., 2016; Thorup et al., 2016), milk production (Borderas et al., 2008; Miguel-Pacheco et al., 2014), or lying time (Siachos et al., 2024).

The limitations of automated lameness detection methods

Monitoring animal behaviour on farms is challenging, not only due to the increasing number of animals but also because of the cost of equipment and the large environmental impact. Additionally, the challenge lies in proper data interpretation and finding proper algorithms with high sensitivity and specificity. Another challenge is to identify lame cows and to examine/treat them immediately. While there are many prototypes on the market, there are still in the research/development phase and have not yet been commercialised.

Conclusions

For both financial and animal welfare reasons, it is important to decrease the prevalence of lameness on dairy farms. Despite the challenges, automated detection of lame cows offers great potential and should be further developed. The solution to improve its reliability may lie in integrating different observational parameters and sensors to increase the accuracy and effectiveness.

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References

- Alsaad, M., Schaefer, A., Büscher, W., & Steiner, A. (2015). The Role of Infrared Thermography as a Non-Invasive Tool for the Detection of Lameness in Cattle. *Sensors*, 15(6): 14513–14525.
- Alsaad, M., Fadul, M., & Steiner, A. (2019). Automatic lameness detection in cattle. *The Veterinary Journal*, 246, 35–44.
- Archer, S. C., Green, M. J., & Huxley, J. N. (2010). Association between milk yield and serial locomotion score assessments in UK dairy cows. *Journal of Dairy Science*, 93(9): 4045–4053.
- Barker, Z. E., Leach, K. A., Whay, H. R., Bell, N. J., & Main, D. C. J. (2010). Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. *Journal of Dairy Science*, 93(3): 932–941.
- Booth, C. J., Warnick, L. D., Gröhn, Y. T., Maizon, D. O., Guard, C. L., & Janssen, D. (2004). Effect of Lameness on Culling in Dairy Cows. *Journal of Dairy Science*, 87(12): 4115–4122.
- Borderas, T. F., Fournier, A., Rushen, J., & De Passillé, A. M. B. (2008). Effect of lameness on dairy cows' visits to automatic milking systems. *Canadian Journal of Animal Science*, 88(1): 1–8.
- Chapinal, N., De Passillé, A. M., & Rushen, J. (2010). Correlated changes in behavioral indicators of lameness in dairy cows following hoof trimming. *Journal of Dairy Science*, 93(12): 5758–5763.
- Chapinal, N., De Passillé, A. M., Pastell, M., Hänninen, L., Munksgaard, L., & Rushen, J. (2011). Measurement of acceleration while walking as an automated method for gait assessment in dairy cattle. *Journal of Dairy Science*, 94(6): 2895–2901.
- Clarkson, M. J., Downham, D. Y., Faull, W. B., Hughes, J. W., Manson, F. J., Merritt, J. B., Murray, R. D., Russell, W. B., Sutherst, J. E., & Ward, W. R. (1996). Incidence and prevalence of lameness in dairy cattle. *The Veterinary Record*, 138(23): 563–567.
- Cook, N. B. (2003). Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface. *Journal of the American Veterinary Medical Association*, 223(9): 1324–1328.
- Cook, N. B., Hess, J. P., Foy, M. R., Bennett, T. B., & Brotzman, R. L. (2016). Management characteristics, lameness, and body injuries of dairy cattle housed in high-performance dairy herds in Wisconsin. *Journal of Dairy Science*, 99(7): 5879–5891.
- Dippel, S., Dolezal, M., Brenninkmeyer, C., Brinkmann, J., March, S., Knierim, U., & Winckler, C. (2009). Risk factors for lameness in freestall-housed dairy cows across two breeds, farming systems, and countries. *Journal of Dairy Science*, 92(11): 5476–5486.
- Flower, F. C., Sanderson, D. J., & Weary, D. M. (2005). Hoof Pathologies Influence Kinematic Measures of Dairy Cow Gait. *Journal of Dairy Science*, 88(9): 3166–3173.
- Green, L. E., Hedges, V. J., Schukken, Y. H., Blowey, R. W., & Packington, A. J. (2002). The Impact of Clinical Lameness on the Milk Yield of Dairy Cows. *Journal of Dairy Science*, 85(9): 2250–2256.
- Groehn, J. A., Kaneene, J. B., & Foster, D. (1992). Risk factors associated with lameness in lactating dairy cattle in Michigan. *Preventive Veterinary Medicine*, 14(1–2): 77–85.
- Leach, K. A., Whay, H. R., Maggs, C. M., Barker, Z. E., Paul, E. S., Bell, A. K., & Main, D. C. J. (2010). Working towards a reduction in cattle lameness: 1. Understanding barriers to lameness control on dairy farms. *Research in Veterinary Science*, 89(2): 311–317.
- Maertens, W., Vangeyte, J., Baert, J., Jantuan, A., Mertens, K. C., De Campeneere, S., Pluk, A., Opsomer, G., Van Weyenberg, S., & Van Nuffel, A. (2011). Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. *Biosystems Engineering*, 110(1): 29–39.
- Mandel, R., Harazy, H., Gygax, L., Nicol, C. J., Ben-David, A., Whay, H. R., & Klement, E. (2018). Short communication: Detection of lameness in dairy cows using a grooming device. *Journal of Dairy Science*, 101(2): 1511–1517.
- Miguel-Pacheco, G. G., Kaler, J., Remnant, J., Cheyne, L., Abbott, C., French, A. P., Pridmore, T. P., & Huxley, J. N. (2014). Behavioural changes in dairy cows with lameness in an automatic milking system. *Applied Animal Behaviour Science*, 150: 1–8.

- Myint, B. B., Onizuka, T., Tin, P., Aikawa, M., Kobayashi, I., & Zin, T. T. (2024). Development of a real-time cattle lameness detection system using a single side-view camera. *Scientific Reports*, 14(1): 13734.
- Nechanitzky, K., Starke, A., Vidondo, B., Müller, H., Reckardt, M., Friedli, K., & Steiner, A. (2016). Analysis of behavioral changes in dairy cows associated with claw horn lesions. *Journal of Dairy Science*, 99(4): 2904–2914.
- Offinger, J., Herdtweck, S., Rizk, A., Starke, A., Heppelmann, M., Meyer, H., Janßen, S., Beyerbach, M., & Rehage, J. (2013). Postoperative analgesic efficacy of meloxicam in lame dairy cows undergoing resection of the distal interphalangeal joint. *Journal of Dairy Science*, 96(2): 866–876.
- Pastell, M., Hänninen, L., De Passillé, A. M., & Rushen, J. (2010). Measures of weight distribution of dairy cows to detect lameness and the presence of hoof lesions. *Journal of Dairy Science*, 93(3): 954–960.
- Robcis, R., Ferchiou, A., Berrada, M., Ndiaye, Y., Herman, N., Lhermie, G., & Raboisson, D. (2023). Cost of lameness in dairy herds: An integrated bioeconomic modeling approach. *Journal of Dairy Science*, 106(4): 2519–2534.
- Shearer, J. K. (2010). *Lameness and Welfare of Dairy Cattle* (0 ed.). Iowa State University.
- Thorup, V. M., Munksgaard, L., Robert, P.-E., Erhard, H. W., Thomsen, P. T., & Friggens, N. C. (2015). Lameness detection via leg-mounted accelerometers on dairy cows on four commercial farms. *Animal*, 9(10): 1704–1712. <https://doi.org/10.1017/S1751731115000890>
- Thorup, V. M., Nielsen, B. L., Robert, P.-E., Giger-Reverdin, S., Konka, J., Michie, C., & Friggens, N. C. (2016). Lameness Affects Cow Feeding But Not Rumination Behavior as Characterized from Sensor Data. *Frontiers in Veterinary Science*, 3.
- Viazzi, S., Bahr, C., Schlageter-Tello, A., Van Hertem, T., Romanini, C. E. B., Pluk, A., Halachmi, I., Lokhorst, C., & Berckmans, D. (2013). Analysis of individual classification of lameness using automatic measurement of back posture in dairy cattle. *Journal of Dairy Science*, 96(1): 257–266.
- Vokey, F. J., Guard, C. L., Erb, H. N., & Galton, D. M. (2001). Effects of Alley and Stall Surfaces on Indices of Claw and Leg Health in Dairy Cattle Housed in a Free-Stall Barn. *Journal of Dairy Science*, 84(12): 2686–2699.
- Von Keyserlingk, M. A. G., Barrientos, A., Ito, K., Galo, E., & Weary, D. M. (2012). Benchmarking cow comfort on North American freestall dairies: Lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. *Journal of Dairy Science*, 95(12): 7399–7408.
- Walker, S., Smith, R., Jones, D., Routly, J., Morris, M., & Dobson, H. (2010). The Effect of a Chronic Stressor, Lameness, on Detailed Sexual Behaviour and Hormonal Profiles in Milk and Plasma of Dairy Cattle. *Reproduction in Domestic Animals*, 45(1): 109–117.
- Weaver, A. D., St. Jean, G., & Steiner, A. (2005). *Bovine surgery and lameness* (2nd ed). Blackwell publ.
- Wood, S., Lin, Y., Knowles, T. G., & Main, D. C. J. (2015). Infrared thermometry for lesion monitoring in cattle lameness. *Veterinary Record*, 176(12): 308–308.
- Zheng, S., Zhou, C., Jiang, X., Huang, J., & Xu, D. (2022). Progress on Infrared Imaging Technology in Animal Production: A Review. *Sensors*, 22(3): 705.

EXPERT ARTICLE

Raw milk quality - example of a dairy farm ZZ Livač

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Summary

The aim of this paper is to present the results of testing the chemical and microbiological quality of raw milk on the "Livač" farm, as raw material for cheese production.

During 2022, milk production on the cow farm was observed. The results obtained showed that the average daily milk production on the farm was 1011,29 kilograms, and the total annual production was 369,469 kilograms. The milk was in the standard quality system throughout the year, with an average of 248,677 somatic cells and 42,562 microorganisms per milliliter of milk. The milk fat content was 4,17% and the protein content was 3,32%.

The milk fat content was 4,17% and the protein content was 3,32%.

Considering that there were an average of 75 cows milked, these results are quite poor and unsatisfactory. These results were also greatly influenced by the unfavorable weather conditions that preceded the production of animal feed on the farm.

Key words: milk production, somatic cell content, microorganisms, milk quality

Introduction

Cattle breeding is the most valuable branch of livestock production, if the total profit is observed. Within cattle breeding, milk production is of great importance, and it is the main product for which dairy animals are kept (Jurković, 2015).

In the world, the production of cow's milk dominates with over 90% of the total production. At the global level, over 740 million tons of cow's milk are produced, and in addition, there is a constant increase in production, which indicates that the demand for milk is also growing (Faostat, 2021).

Milk production plays one of the vital roles in the agricultural economy, but it is also one of the most important branches of agriculture in our country. However, it is important to emphasize that although our area has excellent conditions for cattle breeding, milk production lags behind Western European countries (Roljić, 2019). The biggest problem is the inadequate way of keeping cattle, which inevitably affects the reduction of average milk production.

Milk contains proteins, fats (milk fat), carbohydrates (sugar - lactose), minerals, vitamins and other substances (Đorđević, 1987). On average, cow's milk contains about 12.6% dry matter, 87.4% water, 3.9% milk fat, 3.3% protein, 4.7% sugar and about 0.7% ash (Havranek and Rupić, 1996).

The way the cows are kept has a great influence on the quality, composition and production of milk. The way of keeping must ensure adequate accommodation, nutrition and care, all with the aim of achieving the highest possible productivity. In order to achieve profitable milk production, care must be taken to choose the appropriate way of keeping, as it significantly affects the productivity and health of the cows, the milking efficiency, the amount of food, etc. (Uremović, 2004).

It is also important to point out that ambient (microclimatic) conditions have a great influence on the health and well-being of animals and on the amount of milk produced. These include: temperature, air humidity and air circulation (ventilation). It is known that as a result of temperatures higher than 30 °C, milk production drops significantly, as does food consumption. The optimal air temperature in buildings where dairy cows stay should be from 10 to 15 °C, and the relative humidity should vary from 50 to 75% (Jurković, 2015). The goal of good hygiene and production practice is to produce milk with low somatic cells and microorganisms per ml of milk. On top farms, these parameters range up to 30,000 microorganisms per ml of milk and up to 300,000 somatic cells per ml of milk (Raić, 2017).

According to the Rulebook on the quality of fresh raw milk and conditions for the work of an authorized laboratory (Official Gazette of RS, 81/15), it is considered that raw milk that will be thermally processed during further processing is of standard quality in terms of somatic cells and the number of microorganisms only if it meets the following criteria: that the total number of microorganisms is up to 100,000 per ml of milk and that the total number of somatic cells is up to 400,000 per ml milk.

The aim of this paper is to show the results of testing the chemical and microbiological quality of raw milk at the "Livač" farm.

Material and methods

The composition and quality of raw milk was monitored on a cow farm that belongs to the agricultural cooperative "Livač" and is located in Aleksandrovac, municipality of Laktasi. The cooperative was founded in 2006 and deals with agricultural and livestock production. The farm is organized in such a way that dairy cows, breeding broodstock, cattle fattening and Trappist cheese production take place on it.

The "Livač" farm has a stationary milking parlor, the so-called "fish bone", with a capacity of 2x6. Hygiene in this type of milking parlor is at an excellent level, which is reflected in the microbiological quality of the milk itself. The milk does not come into contact with the environment, but is stored in a lactofreezer through pipes and cooled to a temperature of +4°C to +8°C. In the milking parlor, there are means for disinfection and washing of udders, which is carried out after entering the cows. After each milking, the milking parlor, milking machines and access corridors are cleaned.

The average number of cows in milking during 2022 was 75 heads.

Data for the quality of raw milk at the farm "Livač" were obtained on the basis of the control carried out by the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska, which is carried out by authorized control assistants. Control is performed twice a month, when milk samples are taken from lactofreezers. The analysis of somatic cells is performed once, on the first sample, and the control of microorganisms is performed twice. Sample analysis is performed at the "Dr Vaso Butozan" Institute and the results are delivered via SMS. Based on them, the monthly average is calculated on the basis of the Ordinance on the quality of fresh raw milk and conditions for the work of an authorized laboratory (Official Gazette of RS 81/15). The collected data is systematized and presented by month, tabularly and graphically.

Results and discussion

Milk production on the farm varied from month to month, with a total of 369,469 liters of milk produced, while the average monthly amount of milk produced was 30,789 liters. The daily average in 2022 was 1,011 kilograms of milk.

Table 1. Number of somatic cells and microorganisms per ml of milk in raw milk

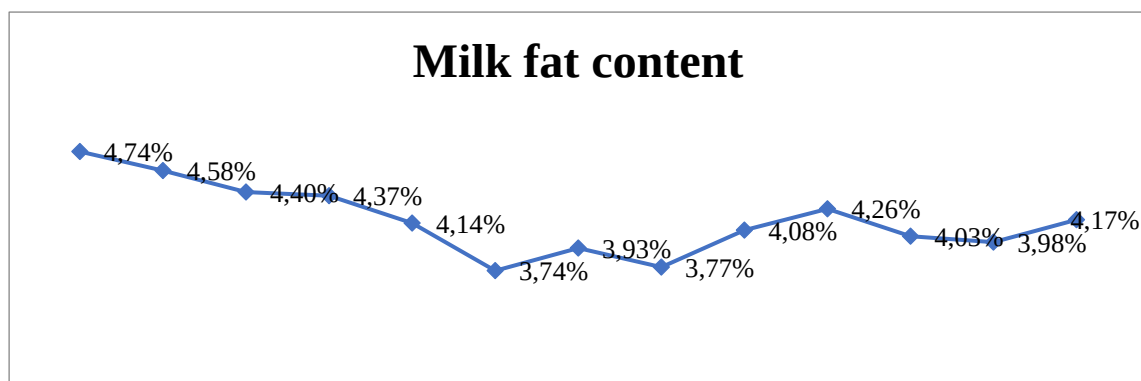
Year of 2022	Somatic cells ml/milk	Microorganisms ml/milk
January	260,400	47,688
February	246,617	57,719
March	298,412	65,118
April	250,890	46,528
May	238,324	23,020
June	223,270	26,820
July	262,019	25,917
August	261,625	28,031
September	293,803	54,682
October	249,427	80,491
November	215,233	31,116
December	184,115	23,625
Average	248,677	42,562

Table 1 shows the microbiological quality of milk at the "Livač" farm by calendar month. On average, raw milk contained 248,677 somatic cells per ml of milk, as well as 42,562 microorganisms per ml of milk. Observing the average values of the analyzed microbiological parameters, it can be concluded that they meet the conditions given by the Ordinance on the quality of fresh raw milk and the conditions for the work of the authorized laboratory (Official Gazette of RS 81/15). In addition, table 2 shows that the lowest number of somatic cells per ml of milk was recorded in the month of December (184,115), while in March the number of somatic cells per ml of milk was the highest in the observed period (298,412). On the other hand, the lowest number of microorganisms per ml of milk was recorded in May (23,020), and the highest in October (80,491).

From the microbiological analysis results presented above, it can be seen that the "Livač" farm largely adheres to good hygiene practices, and that the health of the dairy cows is good. In the study by Kopecki (2018), which examined the production and quality of milk on the Kopecki-Lipovljani farm, slightly different microbiological results were obtained in terms of the number of somatic cells and microorganisms per ml of milk. According to the author, milk produced in 2016 had an average of 120,000 somatic cells and 6,000 microorganisms per ml of milk. In our study, the number of somatic cells was twice as high, and the number of microorganisms was many times higher.

Graph. 1 shows the milk fat content in raw milk produced on the "Livač" farm by month for 2022. Raw milk from the farm contained an average of 4.17% milk fat.

The highest value of protein content in milk was recorded in January (3.72%), while the lowest value was recorded in August (3.13%). Similar to milk fat content, protein content varies and shows certain trends. Thus, the protein content decreases from January to May, then there is a slight increase in June and July, as well as in the period from August to October.



Graph 1. Fat content (%) in milk

It is common for the protein content in milk to vary, but it is subject to a number of factors. The protein content also varies seasonally in such a way that cows consume less food during the hot summer months (Rajčević et al., 1997). In our research, a similar trend was recorded, where a drop in protein content was evident from January to May, while in the period from August to October, when a seasonal drop in temperature is also recorded, an increase in protein content was observed. The influence of nutrition on the protein content of cow's milk has not yet been sufficiently examined. It is known that diets containing smaller amounts of protein than prescribed by the norms have a greater effect on the reduction of protein content in milk (Grubić and Adamović, 2003). The raw milk that was produced on this farm contained an average of 8.83% of dry fat-free matter.

According to Milković (2014), the content of dry fat-free matter in raw milk can decrease by even more than 1% when the external temperatures are high. The reason for this phenomenon is a decrease in the content of sugar and protein in the blood, i.e. blood plasma (Mitić et al., 1987). Similar conclusions were reached in our research, where the lowest content of dry fat-free matter was recorded during the summer month - August, and in addition, a drop in the content of this parameter was also noticed during the warmer months.

Conclusions

Based on the results of research into the quality of raw milk at the "Livač" farm, the following conclusions can be drawn:

- A total of 369,469 liters of milk were produced at the "Livač" farm in 2022, while the average monthly amount of milk produced was 30,789 liters. Daily milk production was 1,011 liters of milk.
- The milk produced at the "Livač" farm belongs to standard quality milk in terms of chemical and microbiological quality.
- Satisfactory microbiological parameters indicate good hygiene practices at the "Livač" farm, as well as good throat health.
- When it comes to the content of milk fat, protein and dry fat-free matter, there are certain seasonal variations, where the content of these parameters decreases during the summer months. This is a normal phenomenon, as during the hot summer months the consumption of food by cows decreases, and this is also reflected in the analyzed parameters.
- The freezing point of the milk produced at the "Livač" farm is not subject to large variations..

References

- Đorđević, J. (1987). Milk - chemistry and physics of milk, Scientific book, Belgrade
- Grubić, G., Adamović, M. (2003). Nutrition of high producing cows. PKB Agro-economics, Belgrade.
- Havranek, J., Rupić, V. (2003). Milk from farm to dairy; Croatian Dairy Association, Zagreb.
- Jurković, M. (2015). The quality of milk in the system of the purchase organization of the Dairy "Mlijekoprodukt" D.O.O. Kozarska Dubica, Final thesis, University of Banja Luka, Faculty of Agriculture.
- Kopeći I. (2018). Milk production and quality on the Kopečki-Lipovljani farm, College of Agriculture in Križevci, final thesis.
- Milković, T. (2014). Seasonal changes in milk production on the Grube d.o.o. farm – Potnjeni, Croatia
- Mitić, N.A., Ferčej, J., Zeremski, D. Lazarević, Lj. (1987). Cattle Breeding, Institute for Textbooks and Teaching Aids. Belgrade.
- Rulebook on the quality of fresh raw milk and conditions for the work of authorized laboratories, Official Gazette of the Republic of Srpska, no. 81/2015
- Rulebook on conditions and methods of obtaining financial incentives for the development of agriculture and villages, Official Gazette of the Republic of Srpska, no.11/2023
- Railić, N. (2017). Purchase of milk in the area of the municipality of Prnjavor, Final thesis, University of Banja Luka, Faculty of Agriculture
- Rajčević, M., Zadnik, T., Levstek, J., Vidio, A. (1997). Reflection of summer feeding of cows on milk parameters and blood, *Krmiva* 39: 6, 287-297
- Roljić, S. (2019). The state of cattle breeding in the Republic of Srpska, Final thesis, University of Banja Luka, Faculty of Agriculture
- Uremović, Z., (2004). Cattle breeding, Croatian Dairy Association, Zagreb
- Websites:
- Faostat - <https://www.fao.org/faostat/en/#data/QCL> , accessed 01/09/2023.

ORIGINAL SCIENTIFIC PAPER

Exploring the Potential of Autochthonous Probiotic Starter Cultures Strains in White-Brined Cheese Production

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Summary

This work aimed to investigate the possibility of using autochthonous probiotic strains *Lactobacillus plantarum* and *Lactococcus lactis* subsp. *lactis* in the production of white-brined cheese. Probiotic white-brined cheese (PWBC) was compared with standard white-brined cheese (SWBC), produced with a commercial starter culture, by physicochemical, sensory properties and lactic acid bacterial count. PWBC was rated higher in terms of sensory properties than SWBC. In both cheeses the number of lactococci and lactobacilli were higher than 10⁸ per gram. The results of this research indicate that starter cultures containing selected autochthonous lactic acid bacteria strains can be successfully used in commercial production of white-brined cheese.

Key words: white-brined cheese, autochthonous probiotic strains

Introduction

The white-brined cheeses (WBC) are the most popular cheeses with a long tradition of production and consumption in Serbia and the region. These cheeses are traditionally produced from raw milk without starter cultures, and these are full-fat cheeses of intense flavour, dry-salted, ripened and stored in brine. Their microbiota varies depending on the ripening period (Golić et al., 2013; Levkov et al., 2017), and affects the specific texture and flavour of the final product (Farahani et al., 2017; Pino et al., 2019). Consumer interest in a healthy lifestyle and demand for additional benefits from food besides a nutritional effect have promoted a functional food market worldwide (Baker et al., 2022). Milk products, including cheeses, represent a good base for developing new products with functional properties, especially those with probiotic bacteria. SWBC are produced in dairy plants, using commercial starter cultures, which include mesophilic and/or thermophilic bacteria, the most important are lactococci. *Lactococcus lactis* is the main lactic acid bacteria (LAB) used in cheese production as an industrial starter culture worldwide. In addition, this species (which consists of the subspecies *lactis* and *cremoris*) is abundant with a large number of known strains that play a significant role in milk fermentation both in traditional processes and in industrial applications (Malesevic et al., 2021; Pytka et al., 2022). During fermentation, LAB can produce significant amounts of bioactive substances, such as peptides, bacteriocins, lactic acid, exopolysaccharides, enzymes, and others. *Lactococcus lactis* as one of the best-known and well-characterized species of LAB serves as a model organism for studying LAB (Fernandez et al., 2011; Kondrotiene et al., 2024). Saleena et al. (2023), reported that *L. lactis* is generally considered safe for human usage, but its influence on the gastrointestinal microbiota and its synergistic action with other LAB strains on immune modulation require further research. *Lactobacillus plantarum* shows possible

benefits in the treatment of digestive tract diseases, particularly irritable bowel syndrome, in people of all ages (Pavlović et al., 2017).

This work aimed to investigate the possibility of using autochthonous probiotic strains in the production of white-brined cheese.

Material and methods

Cheese making - In the experimental dairy of the Agricultural school in Futog, WBC was produced from milk from the school farm. The whole milk was pasteurized in a duplicator (100 l) at 73 °C for 20 sec. Then, 100 l was cooled to 32 °C, and the whole quantity was split into two parts (2x50 l). In one part was added starter culture with commercial strains *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, and *Leuconostoc* (CHN 11, Christian Hansen) and in another probiotic culture with autochthonous strains *Lactococcus lactis* subsp. *lactis* and *Lactobacillus plantarum* (Labbank collection, Institute of Molecular Genetics and Genetic engineering). These lactic acid bacterial strains were isolated from traditionally produced cheeses and previously identified as well as characterized as functional starter cultures.

Chemical and sensory analyses - Milk chemical composition was determined by the infrared test method (ISO 9622:2020) by Milcoscan FT in the accredited Laboratory for Milk Quality Control, Faculty of Agriculture in Novi Sad. The analysis of cheeses was carried out in the Laboratory for quality control of animal feed and animal products at the Department of Animal Husbandry, Faculty of Agriculture in Novi Sad, using accredited methods.

The sensory evaluation of the cheeses was done 28 days after production, and was performed by 10 participants using the scoring method 1 to 5. The following sensory characteristics of the cheese were evaluated: external appearance, consistency, odour, and taste. The maximum number of points was 20. Sensory evaluation of the cheese was carried out based on the Rulebook on evaluation of the quality of milk and milk products at the International Agricultural Fair in Novi Sad (2016).

Microbiological analysis - of cheese was carried out in the Laboratory Molecular Microbiology (Probiotic and Microbiota-Host Interaction Group) of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade. The viability of total LAB was monitored after the manufacturing process and after 10, 20 and 30 days of production. *Leuconostoc* and *Lactobacillus* were enumerated following pour plating on MRS agar and incubated at 30 °C for 48h, and *Lactococcus* ssp. on medium GM17 incubated at 30 °C for 48h. Microbiological analyses were carried out in triplicate.

Results and discussion

Raw milk for cheese production didn't contain antibiotics or other inhibitors. Table 1. shows the chemical composition of the used raw milk.

Table 1. Chemical composition of raw milk

Parameter	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	TS (%)	SCC (*1000/ml)	pH
Value	3.68	3.28	4.78	8.71	12.36	142	6,6

TS- total solids; SNF - solids non-fat, SCC- somatic cells count.

The average chemical composition of the analyzed milk meets the criteria prescribed by the Rulebook on the quality of raw milk (2017), which states that cow's raw milk must have at least 3.2% milk fat and at least 3.0% protein, and solids non-fat 8.50%. Based on the results, in Table 2, it can be seen that the yield for PWBC is a bit lower than the yield for SWBC. Research Leboš-Pavnuc (2012) and Ilić et al. (2016) showed that starter culture influenced the cheese yield.

Table 2. Processing parameters

Parameter	Milk volume (l)	Cheese mass (kg)	Coagulation time (min)
SWBC	50	7.8	60
PWBC	50	7.6	50

PWBC- Probiotic white brined cheese; SWBC- standard white-brined cheese

The chemical composition of probiotics and commercial white-brined cheeses, after 28 days of ripening, is presented in Table 3.

Table 3. Chemical composition of the probiotic and commercial white-brined cheese after 28 days of ripening

Trait	DM (%)	Fat (%)	Protein (%)	FDM (%)	MFFB (%)	Na (%)	pH
PWBC	52.04	25.28	19.88	48.58	64.19	1.10	4.09
SWBC	52.48	26.52	18.74	50.53	64.67	1.88	3.86

PWBC- Probiotic white brined cheese; SWBC- standard white-brined cheese, DM – dry matter, FDM – fat in dry matter; MFFB-Moisture in the free-fat basis

The use of different starter cultures did not affect the chemical composition of the cheeses, and the values of chemical parameters of investigated cheeses were similar to the literature data for white-brined cheeses (Radulović et al., 2011; Miočinović et al., 2012). Lower pH values and higher content of salt were determined in SWBC, compared to PWBC, which affected the sensory evaluation of the cheese. The data for sensory evaluation of PWBC and SWBC after 28 days of ripening is given in Table 4. The PWBC received a higher total score than the SWBC. Both cheeses were scored as acceptable products with good sensory characteristics.

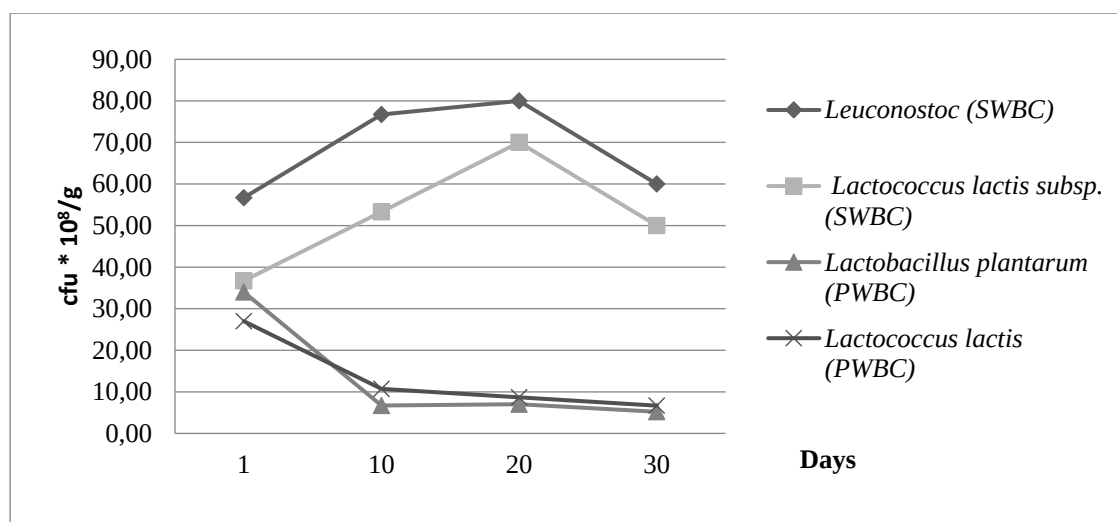
Table 4. The sensory evaluation of cheeses after 28 days of ripening

Trait	Appearance exterior	Texture	Odor	Taste	Total
PWBC	4.40	4.70	4.80	4.10	18.00
SWBC	4.80	4.70	4.80	3.20	17.50

PWBC- Probiotic white brined cheese; SWBC- standard white-brined cheese, DM – dry matter,

Besides the results, it can be seen that the PWBC had a little higher total score (18 points) than the SWBC cheese (17.5 points). The main difference between cheese samples was in the taste because SWBC has higher acidity and a higher content of salt. Buriti et al. (2007) confirm that there were not any changes in taste with improvement in nutritional value (product enriched with probiotics). The effect of probiotic bacteria on flavour characteristics depends mainly on the type and strains added. Also, it depends on the metabolic activity of the strains during cheese production and storage (Karimi et al., 2012).

Miočinović et al. (2012) found that cheeses made with autochthonous bacteria had higher flavour scores, and were more acceptable than cheese made with commercial probiotic bacteria. The viability of probiotic strains during cheese ripening was also determined, in Graph 1, the movement of the number of individual LAB species during the 30-day ripening period is visible. In both cheeses, the number of lactococci and lactobacilli were higher than 10^8 per gram during the whole observed period.



Graph 1. Changes in the lactic acid bacteria count during the ripening of standard (SWBC) and probiotic white-brined cheeses (PWBC)

In SWBC, the number of lactococci and leuconostoc increased, reaching a maximum number on the 20th day, and then a slight decrease. The number of lactococci and lactobacilli, in PWBC, had a maximum number after processing, decreased after 10 days of ripening and remained at the same number until 30 days. Radulović et al. (2011) found that the number of lactobacilli increased during ripening, and reached maximum at the end of ripening.

Conclusions

The probiotic white brined cheese had good sensory ratings, and the chemical composition of the cheese is appropriate for that type of cheese, and the number of lactococci and lactobacilli were higher than 10^8 per gram during the whole observed period. The results of this research indicate that starter cultures containing selected autochthonous lactic acid bacteria strains can be successfully used in commercial production of white brined cheese.

Acknowledgement

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References

- Buriti, F. C., Okazaki, T. Y., Alegro, J. H., Saad, S. M. (2007). Effect of a probiotic mixed culture on texture profile and sensory performance of Minas fresh cheese in comparison with the traditional products. *Archivos latinoamericanos de nutricion*. 5(2): 179-185.
- Baker, M.T., Lu, P., Parrella, J.A., Leggette, H.R. (2022). Consumer Acceptance toward Functional Foods: A Scoping Review. *International Journal of Environmental Research and Public Health*, 19, 1217.
- Farahani, Z., Rasooli, I., Owlia, P. (2017). Isolation, identification and characterization of indigenous lactic acid bacteria for flavour improvement. *International Food Research Journal*, 24: 428–436.
- Fernandez, E., Alegria, A., Delgado, S., Martin, M. C., Mayo, B. (2011). Comparative phenotypic and molecular genetic profiling of wild *Lactococcus lactis* subsp. *lactis* strains of the *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* genotypes, isolated from starter-free cheeses made of raw milk. *Applied and Environmental Microbiology*. 77: 5324-5335
- Golić, N., Čadež, N., Terzić-Vidojević, A., Šuranská, H., Beganović, J., Lozo, J., Topisirović, L. (2013). Evaluation of lactic acid bacteria and yeast diversity in traditional white pickled and fresh soft

- cheeses from the mountain regions of Serbia and lowland regions of Croatia. *International Journal of Food Microbiology*, 166 (2): 294-300.
- Ilić, M., Milanović, S., Carić, M., Lončar, E., Malbaša, R., Kanurić, K., Hromiš, N. (2016). Packaging materials and cheese production. *Mljekarstvo* 66(2): 91-98
- ISO. (2020). Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry. [ISO 9622: 2020/IDF 141]. Geneva, Switzerland: International Organization for Standardization.
- Karimi, R., Sohrabvandi, S., Mortazavian, A. M. (2012). Sensory characteristics of probiotic cheese. *Comprehensive Reviews in Food Science and Food Safety*, 11(5): 437-452.
- Kondrotiene, K., Zavistanaviciute, P., Aksomaitiene, J., Novoslavskij, A., Malakauskas, M. (2024). *Lactococcus lactis* in Dairy Fermentation—Health-Promoting and Probiotic Properties. *Fermentation*, 10, 16.
- Leboš Pavunc, A., Beganović, J., Kos, B., Uroić, K., Blažić, M., Šušković, J. (2012). Characterization and application of autochthonous starter cultures for fresh cheese production. *Food Technol Biotechnol*. 50(2): 141-51.
- Levkov, V., Mojsova, S., Nastova, R., Srbinska, S., Gjorgovska, N. (2017). Identification and phenotypic characteristics of lactic acid bacteria isolated from some traditional cheeses produced in the Republic of Macedonia. *Macedonian Journal of Animal Science*, 7: 79–87.
- Malešević, M., Stanisavljević, N., Miljković, M., Jovčić, B., Filipi, B., Studholme, D. J., Kojic, M. (2021). The large plasmidome of *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* S50 confers its biotechnological properties. *International Journal of Food Microbiology*. 16: 337:108935
- Miočinović, J. B., Radulović, Z. T., Radovanović, M. M., Nedeljković, A. D., Trpković, G. D., Paunović, D. D., Pudja, P. D. (2012). The ripening of white brined cheeses made with commercial and potential autochthonous probiotic strains. *CEFood 2012 - Proceedings of 6th Central European Congress on Food*, 1298-1302.
- Pavlović, A., Agić, A., Đorđević, V., Dugalić, P. (2017). The efficiency of use of probiotic monoculture *Lactobacillus plantarum* 299v/lv299v in maintaining the health of digestive system. V Professional and scientific works, Health care 3/2017
- Pino, A., Russo, N., Van Hoorde, K., De Angelis, M., Sferrazzo, G., Randazzo, C. L., Caggia, C. (2019). Piacentinu Ennese PDO cheese as reservoir of promising probiotic bacteria. *Microorganisms*, 7, 254.
- Pytko, M., Kordowska-Wiater, M., Wajs, J., Glibowski, P., Sajnaga, E. (2022). Usefulness of Potentially Probiotic *L. lactis* Isolates from Polish Fermented Cow Milk for the Production of Cottage Cheese. *Applied Sciences*. 12(23): 12088
- Radulović, Z., Miočinović, J., Puđa, P., Barać, M., Miloradović, Z., Paunović, D., Obradović, D. (2011). Lactic acid bacteria in white brined cheese production, *Mljekarstvo* 61(1): 15-25.
- Rulebook on evaluation of the quality of milk and milk products at the International Agricultural Fair in Novi Sad (2016).
- Rulebook on the quality of raw milk, Official Gazette of SR, 106/2017
- Saleena, L. A. K., Teo, M. Y. M. How, Y. H., In, L. L. A., Pui, L. P. (2023). Immunomodulatory action of *Lactococcus lactis*. *Journal of Bioscience and Bioengineering*. 135(1): 1-9.

REVIEW ARTICLE

Water in animal nutrition: importance and specificity

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Summary

Water is often overlooked as an important nutrient in animal nutrition. However, sufficient quantities of high-quality and potable water are necessary for watering and maintaining good health and animal production. This paper elaborates the importance and function of water in animal organisms. Furthermore, the paper brings detailed information regarding the daily water requirements of different domestic animals together with the explanation of respective legal acts of the European Union that regulate access to fresh and clean water for animals. At the end of this paper, in depth analysis of the significance of the water quality for sustainable animal production has been reviewed. The paper concludes how free access to clean and fresh water is essential for animal production of today and tomorrow, emphasizing that given the necessary growth trend in the production of almost all animal products, especially meat, it is possible to expect additional pressure on current freshwater supplies for the needs of animal production in the future.

Key words: water, animal nutrition, livestock production

Introduction

Water, as the most essential, irreplaceable, and limited resource, holds priceless importance due to its fundamental role in most physiological processes of living organisms (Habuda Stanić et al., 2023). Water is often overlooked as an important factor in animal nutrition, although sufficient quantities of high-quality and healthy water are necessary for watering and maintaining good health and animal production. In a physiological sense, water is the basic ingredient of all body fluids, including saliva, lymph, and blood. Also, it plays an important role in all other processes in the animal's body. It dissolves minerals, vitamins, and other substances, thereby improving their transport through the body, protects tissues and organs, regulates body temperature, facilitates digestion and the proper flow of food through the digestive tract, ensures good absorption of nutrients and helps eliminate harmful substances from the animal's body (Singh et al., 2020; Socha et al., 2003). The hygienic quality of water for animal drinking is essential, because some diseases and reduced production can be caused by poor water quality. Insufficient water supply negatively affects animal growth performance and welfare (Singh et al., 2020). Water for animal drinking occupies the most important place in terms of livestock production. It is a very important factor in animal welfare and affects the physiological needs of animals, their behavior, health, productivity, reproduction, and emotions. Protecting domestic animals from hunger and thirst is crucial for good, positive animal welfare. Food and water are essential for life and therefore play a critical role in animal welfare (Marjanović and Tofant, 2008; Parkes, 2005). The development of increasingly large-

scale industry, agriculture, and globalization has led to greater needs for water, but on the other hand, it has increasingly led to pollution. Increasingly frequent climate changes have a significant impact on modern trends in agricultural production. For these reasons, producers must adapt to new challenges and constantly find new skills and solutions to try to ensure sustainable agricultural production (Habuda Stanić et al., 2023; Faour-Klingbeil and Todd, 2018). This paper aims to demonstrate the importance of water and its specificity in animal nutrition as one of the essential inorganic components of living organisms.

The importance and function of water in the organism

Water is one of the most important components of the animal organism, therefore, for the normal functioning and productive capacity of animals, a balanced intake and release of water from the organism is of primary importance. Water is the most important inorganic component of living organisms, which it is represented in a very high percentage, but it is important to note that of the total amount of water in the organism, only 10% is free water, while the remaining 90% is bound to water (Ward and McKague, 2007). Certainly, when talking about water, we must not forget the fact that the water content in an animal's body is closely related to the animal's age, with the water content being highest in newborns and lowest in older animals, but it is also specifically related to the animal's nutrition. Younger and leaner animals contain more water in their bodies, so in lean cow's water makes up about 70% of their body mass, while in very fat cows' water may only make up 40% of their body mass. Due to differences in the ratio between muscle and fat tissue, female and castrated animals have a lower percentage of water in their bodies compared to uncastrated male animals (Sjaastad et al., 2017). Its main function is to dissolve organic and inorganic substances and is essential for metabolic processes in cells. If we talk about water outside the cells, its function is to transport nutrients and metabolites, regulate osmotic pressure, and we certainly cannot forget its important role as a temperature regulator because it absorbs the generated heat and distributes it throughout the animal's body and uses it during evaporation or sweating, and it removes harmful substances formed as by-products of intermediary metabolism in the cell from the body (Wagner and Engle, 2021; Wakchaure et al., 2015; Schlink et al., 2010). If only 10 to 15% of body water is lost, problems such as decreased appetite, weakness, and fatigue of the animal appear, and blood circulation becomes difficult, which leads to an increase in the body temperature of the animal's organism. When water is not consumed for a long time, all body processes stop, which can ultimately cause the death of the animal (Wagner and Engle, 2021). The dominant routes of water excretion from the animal's body are through urine and feces, but we must not forget that there are significant differences depending on the type of animal, but water excretion through the skin and lungs can be as high as 35-45%, as well as water loss through the final products (milk, eggs) of the animals we breed (Wakchaure et al., 2015; Senčić et al., 2021). Under severe stress, cattle and other animal species can also lose significant amounts of water through increased salivation (Schlink et al., 2010).

Water requirements

The daily water requirements of animals depend on several factors, the most important of which are the type of food consumed by the animal, the temperature of the environment in which the animal lives, age, physiological state, type of animal and production method, availability of water, its quality and the temperature of the water consumed by the animal (Carson, 2000; Wagner and Engle, 2021; Petersen et al., 2016; Schlink et al., 2010). Due to the previously mentioned factors, it is much more realistic to express water requirements based on the amount of dry matter in the feed. For 1 kg of dry matter, it is necessary to provide cattle with 4-8 liters of water, pigs require 2.5-3 liters, while horses and sheep need 2-4 liters of water (Ward and McKague, 2007; Schlink et al., 2010; Senčić et al., 2021). Alongside the previously mentioned

ratios of water consumption per kg of dry matter of feed, Table 1 presents the average daily water requirements for different species and categories of animals.

Table 1. Average daily water requirements (L/day)

Species and categories of animals		L/day
Dairy cow		40-100
Beef cattle		22-66
Sheep, goat		4-5
Piglets	suckling (< 9 kg body mass)	0.7-1
	weaned (< 30 kg body mass)	1-3
Fattening pigs	30-50	3-6
	50-80	5-8
	80-120	8.5-11
Breeding pigs	sows	8-15
	boars	12-15
Laying hen; broiler chickens		0.2-0.4

Source: Domaćinović et al., 2015

When it comes to water requirements, animals can consume water in two ways: through drinking water and food, i.e. individual components found in the diet, but also through metabolic water (Carson 2000; Sjaastad et al., 2017). The water content in food is highly variable. It can vary from 10-12% in some dried grains (corn, wheat, barley), mixtures and concentrates, to around 90% in some voluminous and succulent feeds (Schlink et al., 2010; Wakchaure et al., 2015; Wagner and Engle, 2021). Furthermore, the type of feeding also greatly affects the amount of water that an animal consumes each day. For example, cattle grazing on lush grass that can contain up to 75% water need much less additional water for drinking compared to animals fed dry food, concentrates, or hay that contains only 10% water (Wakchaure et al., 2015). On the other hand, the metabolic water is created by oxidation in cells and usually represents 8-13% of the total water in the body. However, the metabolic water in some species of animals that live in deserts and that hibernate can make up to 100% of the water supply (Sjaastad et al., 2017; Senčić et al., 2021). According to the European Union Directive 2001/93/EC, all animals older than 2 weeks must have access to fresh and clean water. This constant supply of water to animals has a positive impact on their production results. Any reduction in free water intake is undesirable as it will limit animal production and impair the health status of the animals, which will lead to reduced profitability for the producer (Pavičić et al., 2008; Wakchaure et al., 2015; Domaćinović et al., 2019).

Water quality

As water is one of the most important nutrients for animals, the availability of drinking water in sufficient quantity and acceptable quality is a very important, and sometimes decisive parameter for livestock production (Carson, 2000). Water symbolizes a key role in animal welfare. It has a significant impact on the physiological needs of animals, as well as on their behavior, health, productivity, reproduction, and emotions. In the Republic of Croatia, the regulation states that the quality of water for animal drinking must be the same as that for human drinking, and therefore it is assessed according to the Regulation on the health safety of drinking water (Official Gazette 64/2023.) (Marjanović and Tofant, 2008). The hygienic quality of drinking water is of great importance; as poor quality can lead to the occurrence of various animal diseases as well as reduced production. The water for animals should be transparent, tasteless, and odorless, and should also not contain harmful chemicals, pathogenic microorganisms, or other negative substances that could endanger the health of the animals

(Carson, 2000; Matković, 2007; Domaćinović et al., 2019; Wagner and Engle, 2021). The animal should be provided with easily accessible water of constant quality, the recommended temperature of which should be between 5 and 15 °C, the pH should be in the range of 6.5 to 8.5, and it should not contain heavy metals at all (Matković, 2007; Domaćinović et al., 2019). A small sanitary analysis is used to examine the water used for drinking, and more detailed, complex water analyses are carried out if necessary.

Methods such as physical-chemical, organoleptic, and microbiological are used to determine various water properties, including color, turbidity, taste, smell, pH, temperature, electrical conductivity, amount of organic matter, chlorides, and concentrations of ammonia, nitrates, nitrites, bacteria, as well as water hardness (Matković, 2007; Wakchaure et al., 2015; Habuda Stanić et al., 2023). It is also important to pay attention to the health and safety of water for animal drinking. According to the Regulation on Health Safety (NN 125/2017), the total number of live bacteria in one milliliter of water must not exceed 20 colonies that develop on a nutrient medium after 72 hours of incubation at a temperature of 37°C. In 100 ml of water, there must be no total coliform bacteria or sporogenic sulfite-reducing anaerobic bacteria, because the coliform group of bacteria is traditionally an indicator used to assess the degree of water pollution, and thus the sanitary quality of a water sample (Matković, 2007; Carson, 2000). Salinity is an expression of the number of soluble salts in a particular water sample and is one of the most important parameters used to assess water quality. In an attempt to enable the widespread use of salt water for all species and categories of animals, guidelines are offered that should be followed to avoid health problems for animals consuming such water. Water containing less than 1000 mg of soluble salts per liter does not pose a danger to any animal. Water containing between 1000 and 5000 mg of soluble salts per liter may cause mild, temporary diarrhea, or animals may initially refuse to consume such water. Water containing 5000 to 7000 mg of soluble salts per liter is a health risk for pregnant animals, lactating animals, and animals under severe stress. Water containing more than 7000 mg of soluble salts per liter should be considered unsafe for animal consumption, especially for poultry, pigs, and horses, which are most sensitive to elevated salt concentrations in water (Carson, 2000; Ward and McKague, 2007). Any identified poorer quality of water usually results in less consumption of such water by the animal, which is ultimately reflected in worse feed conversion ratios, reduced animal growth, poorer animal production, as well as in some non-specific disease states of such animals, which consequently in severe cases can lead to the death of animals and large economic losses for the producer (Ward and McKague, 2007; Wakchaure et al., 2015).

Conclusions

Free access to clean and fresh water is essential for animal production today and tomorrow. In this context, it is highly important to ensure monitoring of water quality along with the feed analysis. Given the necessary growth trend in the production of almost all animal products, especially meat, it is possible to expect additional pressure on current freshwater supplies for future animal production needs.

References

- Carson, T. L. (2000). Current knowledge of water quality and safety for livestock. *Veterinary Clinics of North America: Food Animal Practice*. 16(3): 455-464.
- Domaćinović, M., Antunović, Z., Džomba, E., Opačak, A., Baban, M., Mužić, S. (2015). *Specijalna hranidba domaćih životinja*. Poljoprivredni fakultet u Osijeku, Sveučilište Josipa Jurja Strossmayera u Osijeku. Osijek.
- Domaćinović, M., Đidara, M., Solić, D., Šperanda, M. (2019). *Dobra proizvođačka praksa u animalnoj proizvodnji*. Fakultet agrobiotehničkih znanosti Osijek, Sveučilište Josipa Jurja Strossmayera u Osijeku.

European Communities: Commission Directive 2001/93/EC

Faour-Klingbeil, D., Todd, E. C. (2018). The impact of climate change on raw and untreated wastewater use for agriculture, especially in arid regions: a review. *Foodborne pathogens and disease*. 15(2): 61-72.

Habuda-Stanić, M., Sučić, H., Santo, V., Šiljeg, M., Romić, Ž., Pavlović, O., Begić, S., Cvrk, R., Stjepanović, M., Pala, A., Ergović Ravančić, M., Bogdanović, T. (2023). Voda i zdravlje. Primarna i sekundarna prevencija bolesti suvremenog čovjeka. Medicinska naklada, Zagreb, 39-55.

Marjanović, S., Tofant, A. (2008). Kvaliteta vode za napajanje goveda-čimbenik dobrobiti. *Meso*. 10(2): 127-131.

Matković, K. (2007): Kvaliteta vode za piće muznih životinja. *Mljekarski list*. 43(12): 20-22.

NN 64/2023; Pravilnik o parametrima sukladnosti, metodama analiza i monitorinzima vode namijenjene za ljudsku potrošnju.

NN 125/2017; Pravilnik o parametrima sukladnosti, metodama analize, monitoringu i planovima sigurnosti vode za ljudsku potrošnju te načinu vođenja registra pravnih osoba koje obavljaju djelatnost javne vodoopskrbe.

Pavičić, Ž., Ostović, M., Tofant, A., Balenović, T., Ekert Kabalin, A. (2008). Značenje higijene pojilica u suvremenom svinjogojstvu. *Stočarstvo: Časopis za unapređenje stočarstva*. 62(4): 317-322.

Parkes, K. (2005). Farm Animal Welfare Council annual review 2003/2004. *Animal Welfare*. 14(1): 87-88.

Petersen, M. K., Muscha, J. M., Mulliniks, J. T., Roberts, A. J. (2016). Water temperature impacts water consumption by range cattle in winter. *Journal of animal science*. 94(10): 4297-4306.

Schlink, A. C., Nguyen, M. L., Viljoen, G. J. (2010). Water requirements for livestock production: a global perspective. *Rev. Sci. Tech*. 29(3): 603-619.

Senčić, Đ., Antunović, Z., Novoselec, J., Samac, D., Prakatur, I., Bobić, T., Klir, Ž. (2021). Tehnologija animalne proizvodnje. Fakultet agrobiotehničkih znanosti Osijek.

Singh, A. K., Bhakat, C., Yadav, D. K., Kansal, G., Rajput, M. S. (2020). Importance of measuring water intake in dairy animals: A review. *International Journal of Advances in Agricultural Science and Technology*. 7(2): 23-30.

Sjaastad, Ø. V., Sand, O., Hove, K. (2017): Fiziologija domaćih životinja. Naklada Slap, Jastrebarsko.

Socha, M. T., Ensley, S. M., Tomlinson, D. J., Johnson, A. B. (2003). Variability of water composition and potential impact on animal performance. In *Proc. from the Intermountain Nutrition Conference*, Salt Lake City, UT, 85-96.

Wagner, J. J., Engle, T. E. (2021). Invited review: water consumption, and drinking behavior of beef cattle, and effects of water quality. *Applied Animal Science*. 37 (4): 418-435.

Wakchaure, R., Ganguly, S., Praveen, P. K. (2015). Role of water in livestock. *The Recent Advances in Academic Science Journal*, 1: 56-60.

Ward, D., McKague, K. (2007). Water requirements of livestock. *Factsheet, Ministry of Agriculture, Food and Rural Affairs*. 22(54): 38.

REVIEW ARTICLE

From farm to slaughterhouse: cortisol as a stress indicator in sows and its effects on welfareIvona Djurkin Kušec¹, Marko Bagarić², Kristina Gvozdanović¹, Goran Kušec¹¹*Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University in Osijek, Vladimira Preloga 1, Osijek, Croatia (idurkin@fazos.hr)*²*Bayer d.d., Radnička cesta 80, Zagreb, Croatia***Summary**

Cortisol, an important biomarker of stress, is often used to assess animal welfare in livestock, particularly in pigs at different stages of production. The transition from farm to slaughter is a critical phase where stress can significantly affect animal welfare, meat quality and overall productivity. This review examines the role of cortisol as a physiological indicator of stress in sows, explores the factors that contribute to cortisol fluctuations and discusses the impact on animal welfare, methods of cortisol measurement and possible strategies to minimise stress during transport and slaughter are also discussed. Overall, elevated cortisol levels confirm significant stress during transport and slaughter, but implementing proper handling and transport practices can mitigate these effects, improving sow welfare.

Key words: pigs, sows, stress, biomarker, cortisol**Introduction**

Farm animal welfare is a growing concern because of its ethical, economic and quality implications (Hemsworth et al., 2015). In modern pig production sows are exposed to various stress factors throughout their life cycle, from gestation and farrowing to transport and slaughter. Stress is not only a welfare concern, but also a productivity concern, as increased stress responses negatively affect reproduction, immunity and meat quality (Zhang et al., 2021). Stress in pigs is usually assessed using different physiological and behavioural markers, with cortisol being one of the most widely studied because of its direct link with the hypothalamic-pituitary-adrenal (HPA) axis response to stressors (Heimbürge et al., 2019). The transition from farm to slaughterhouse is one of the most stressful phases for sows, as they experience handling, transport, mixing with unfamiliar animals, feed and water deprivation and slaughterhouse procedures, all of which can trigger acute and chronic stress (Dalla Costa et al., 2020). Studies show that culled sows have significantly higher cortisol levels compared to normal fattening pigs, which indicates that this category is particularly susceptible to stress during handling and transport (Terlouw et al., 2020). In addition to cortisol, other stress biomarkers such as catecholamines, acute phase proteins, lactate levels and heart rate variability have also been investigated (Heimbürge et al., 2019). However, the measurement of blood cortisol levels, and especially using non-invasive methods, such as hair cortisol concentration (HCC) and faecal cortisol metabolites (FCM) are still gold standard for assessing stress levels in pigs (Zhang et al., 2021).

The aim of this review is to describe cortisol as a stress biomarker, identify the main stress factors to which sows are exposed from farm to slaughter, discuss their impact on animal welfare and explore strategies to reduce stress through improved handling, transport and slaughter practices.

Cortisol as a biomarker of stress in sows

Cortisol, a primary glucocorticoid hormone secreted by the adrenal cortex in response to stress, plays a central role in energy metabolism, immune modulation and physiological adaptation to environmental stressors (Otten et al., 2019; Sapolsky et al., 2000). It is a key component of the hypothalamic-pituitary-adrenal (HPA) axis which is activated in response to acute and chronic stressors (Moberg, 2000). Cortisol fluctuations influence numerous physiological processes, including glucose homeostasis, cardiovascular function and reproductive performance, thus making it a most significant biomarker for the assessment of stress and welfare in animals, including sows (Möstl and Palme, 2002). Cortisol levels in sows are influenced by various intrinsic and extrinsic factors, including genetic predisposition, social hierarchy, environmental conditions, nutritional status, and human-animal interactions (Reale et al., 2025; Goymann, 2012; Ruis et al., 2001; Heimbürge et al. 2019). For example, housing conditions, such as group or individual housing, with or without enrichment, have been identified as significant factors influencing cortisol levels in sows (Hemsworth et al., 2015; Zhang et al., 2021; Terlouw, 2020). Social stress, such as regrouping or competition for resources, can also significantly increase cortisol levels which can affect reproductive performance of sows (Jarvis et al., 2006).

Cortisol can be measured through different biological substrates, each offering unique insights into the stress response of an animal. Plasma and serum cortisol are widely used indicators of acute stress (Zhang et al., 2021). However, these methods are invasive and may themselves trigger a stress response, which could confound the results obtained (Palme, 2019). Salivary cortisol, on the other hand, is a non-invasive alternative that reflects the level of free cortisol in the blood and is therefore suitable for the assessment of acute stress without the need for blood sampling (Cook et al., 2000). Hair cortisol concentration (HCC) has been proven as a biomarker for measuring cortisol secretion over period of weeks to months and is therefore used for the assessment of chronic stress (Davenport et al., 2006; Russell et al., 2012). Faecal cortisol metabolites (FCM) provide a non-invasive means of monitoring cumulative stress responses, reflecting HPA axis activity over a period of hours to days (Möstl et al., 2005). As the method is non-invasive and the sampling procedure avoids the acute stress associated with handling, it is frequently used in animal welfare research (Palme et al., 2013). The best method for measuring cortisol in sows depends on the specific research objective, whether it is studying short-term stress, long-term stress, or monitoring welfare in different housing systems. Using multiple cortisol measurement tools can give us a deeper understanding of how pigs respond to stress, ultimately leading to better welfare measures and management strategies (Terlouw et al., 2020; Palme, 2019).

Stressors affecting sows from farm to slaughter

From farm to slaughter, sows are exposed to many stress factors that significantly affect their welfare and productivity. Culling is the removal of sows from production due to health problems, poor reproductive performance or age-related factors. This is an important animal welfare concern because the majority of culled sows are transported and slaughtered immediately after culling, which can increase stress (Dalla Costa et al., 2020). Studies show that cull sows have higher cortisol levels compared to market pigs due to their compromised body condition, longer transport time and greater susceptibility to injury and fatigue (Zhang et al., 2021). In addition, inadequate handling or inadequate housing conditions can lead to an additional increase in cortisol levels with detrimental effect on carcass and meat quality (Terlouw et al., 2020). Transport is one of the biggest stress factors for sows because it exposes them to numerous physical and psychological challenges. These include loading and unloading, encounters with unfamiliar animals, temperature fluctuations and the vibrations caused by vehicle movement (Faucitano et al., 2024). Extended transport increases cortisol, creatine kinase (CK) and lactate, which are all indicators of physiological and muscular stress (Chin et

al., 2015). Transport stress can be further compounded by factors such as overcrowding, poor ventilation, rough handling and duration of transport (Schwartzkopf-Genswein et al., 2012).

Social stress is another important stress factor for sows, where hierarchical competition often leads to aggressive interactions resulting in injuries, increased cortisol release and reduced feed intake, which ultimately affects animal welfare and productivity (Arey and Edwards, 1998; Rault et al., 2019).

Heat stress impairs thermoregulation, increases respiration rate, heart rate and reduces feed intake and reproductive performance (Lucy and Safranski, 2017). However, cold stress can increase the metabolic demands and lead to excessive energy loss and muscle fatigue (Sanz-Fernandez et al., 2017).

Handling practises have a significant impact on stress responses in sows. Rough or aversive handling techniques lead to increased cortisol levels, increased heart rate and poorer meat quality (Hemsworth et al., 2011). In abattoirs, pre-slaughter stress, including tethering, electric prodding and stunning methods, can cause acute stress that further affects carcass welfare and characteristics (Grandin, 2014).

Feed and water deprivation prior to transport and slaughter can also cause metabolic stress, leading to hypoglycaemia, dehydration and increased muscle catabolism (Jongman et al., 2020). Prolonged periods of fasting exacerbate stress responses, with increased cortisol and creatine kinase levels observed in feed-deprived sows (Lambooy, 2007).

Strategies to improve sow welfare from farm to slaughter

Ensuring high welfare standards for sows from farm to slaughter is essential for ethical, economic and production reasons. Chronic stress impairs immune function, increases susceptibility to disease and reduces reproductive efficiency (Otten et al., 2019). Transport and slaughter stress have a negative impact on meat quality and result in detrimental meat quality traits such as pale, soft, exudative (PSE) and dark, firm, dry (DFD) meat (Terlouw et al., 2020). Therefore, appropriate management practises must be implemented to reduce stress and maximise animal welfare. Appropriate handling techniques, such as calm movements without excessive force, reduce anxiety reactions (Hemsworth et al., 2015). Well-designed loading ramps, appropriate stocking density during transport and limiting mixing with unfamiliar animals reduce stress and risk of injury (Mota-Rojas et al., 2021). Timely culling decisions, veterinary assessments and appropriate housing conditions prevent both acute and chronic stress in sows (Zhang et al., 2021; Gvozdanović et al., 2023). Slaughterhouses should minimise acute stress by shortening stabling times, using efficient stunning methods and implementing noise reduction strategies (Grandin, 2014). Social stress from encounters with unfamiliar animals should be reduced by keeping familiar groups together, while environmental factors such as extreme temperatures, noise and poor ventilation should be controlled to minimise stress and reduce cortisol levels (Mota-Rojas et al., 2021). Excessive feed deprivation before slaughter increases stress and muscle fatigue, and therefore optimised fasting periods need to be implemented, while continuous water access is crucial to prevent dehydration (Otten et al., 2019). Future research should focus on non-invasive monitoring technologies such as infrared thermography and wearable sensors for real-time stress assessment, along with genetic and nutritional strategies to increase stress resistance and improve meat quality (Zhang et al., 2021).

Conclusions

Cortisol is an important biomarker for the assessment of stress in sows. Its measurement can provide valuable insights into the effects of handling, transport, culling and slaughter on sow welfare. Reducing stress at every stage of the production process is important not only because of its effect on pig productivity and meat quality, but also for ethical reasons. The use of low-stress handling techniques, well-managed transport systems and humane slaughter practises can

effectively mitigate stress responses, leading to improved welfare and efficiency in pork production. Further research into non-invasive stress monitoring methods will be essential for improving sow welfare and for creating a more sustainable future in livestock production.

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References

- Arey, D. S. and Edwards, S. A. (1998). Factors influencing aggression between sows after mixing and the consequences for welfare and production. *Livestock Production Science*. 56(1): 61-70.
- Chin, T. Y., Barker, T., Marchant-Forde, J. N. (2015). Assessment of creatine kinase as an indicator of muscle fatigue and stress in pigs during transport. *Livestock Science*. 178: 63-69.
- Dalla Costa, O. A., Devillers, N., Paranhos da Costa, M. J. R., Faucitano, L. (2020). Physiological indicators of stress in pigs transported to slaughter. *Animal Production Science*. 60(10): 1206-1216.
- Faucitano, L., Ribas, J., Lambooij, E. B. (2024). Transport of pigs. In T. Grandin (Ed.), *Livestock handling and transport* (6th ed., pp. 357-389). CABI Publishing.
- Gvozdanović, K., Djurkin Kušec, I., Đidara, M., Blažetić, S., Komlenić, M., Kuterovac, K., Radišić, Ž., Kušec, G. (2023). Changes in Carcass Composition, Meat Quality Traits, and Stress Levels in Culled Sows Exposed to Different On-Farm Rest Periods. *Processes*. 11(7):1961.
- Grandin, T. (2014). *Livestock handling and transport* (4th ed.). CABI Publishing.
- Heimbürge, S., Kanitz, E., Otten, W. (2019). The use of hair cortisol for the assessment of stress in animals. *General and Comparative Endocrinology*. 270: 10–17
- Hemsworth, P. H., Mellor, D. J., Cronin, G. M., Tilbrook, A. J. (2015). Scientific assessment of animal welfare. *New Zealand Veterinary Journal*. 63(1): 24–30.
- Lambooij, E. (2007). Transport of pigs. In *Animal Welfare at Slaughter* (pp. 11-26). Wageningen Academic Publishers.
- Lucy, M. C. and Safranski, T. J. (2017). Heat stress in pregnant sows: Thermal responses and subsequent performance of sows and their offspring. *Molecular Reproduction and Development*. 84(9): 946-956.
- Mota-Rojas, D., Martínez-Burnes, J., Villanueva-García, D., Mora-Medina, P., González-Lozano, M., Gómez-Prado, J. (2021). Stress and pain in swine: Physiological mechanisms and animal welfare implications. *Animals*. 11(7): 1984.
- Rault, J. L., Lay, D. C., Marchant-Forde, J. N. (2019). Mixing socially unfamiliar pigs elevates plasma markers of stress and behavior through duration of 24 hours. *Journal of Animal Science*. 97(5): 1771-1780.
- Réale, D., Martin, J. G. A., Coltman, D. W., Poissant, J., & Festa-Bianchet, M. (2025). Genetic determinism of cortisol levels: A selection experiment on hypothalamic-pituitary-adrenal axis activity and robustness in pigs. *Frontiers in Genetics*. 16: 1461385
- Sanz-Fernandez, M. V., Pearce, S. C., Gabler, N. K., Ross, J. W. (2017). Effects of heat stress on animal performance and carcass composition. *Annual Review of Animal Biosciences*. 5:171-180.
- Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A., & Crowe, T. G. (2012). Road transport of cattle, swine, and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. *Meat Science*. 92(3): 227-243

- Cook, N. J., Schaefer, A. L., Gonyou, H. W. (2000). Salivary vs. serum cortisol for the assessment of stress in swine. *Canadian Journal of Animal Science*. 80(4): 687-693.
- Davenport, M. D., Tiefenbacher, S., Lutz, C. K., Novak, M. A., Meyer, J. S. (2006). Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology*. 147(3): 255-261.
- Goymann, W. (2012). On the use of non-invasive hormone research in uncontrolled, natural environments: The problem with sex, diet, metabolic rate, and the individual. *Methods in Ecology and Evolution*. 3(4): 757-765.
- Jarvis, S., Moinard, C., Robson, S. K., Baxter, E. M., Ormandy, E., Douglas, A. J., Lawrence, A. B. (2006). The effect of parity and environmental enrichment on the behaviour and physiology of pre-parturient pigs. *Applied Animal Behaviour Science*. 100(1-2): 100-116.
- Möstl, E. and Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, 23(1-2), 67-74.
- Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U., Palme, R. (2005). Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications*. 29(2): 275-285.
- Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg and J. A. Mench (Eds.), *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare* (pp. 1-21). CAB International.
- Otten, W., Kanitz, E., Tuchscherer, M. (2019). The impact of stress on immune cell functions in farm animals. *Veterinary Immunology and Immunopathology*. 208: 80-87.
- Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior*. 199: 229-243.
- Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., Möstl, E. (2013). Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and non-invasive measurement in fecal samples. *Annals of the New York Academy of Sciences*. 1040(1): 162-171.
- Ruis, M. A., Te Brake, J. H., Engel, B., Buist, W. G., Blokhuis, H. J., Koolhaas, J. M. (2001). Adaptation to social isolation: Acute and long-term stress responses of growing gilts with different coping characteristics. *Physiology & Behavior*. 73(4): 541-551.
- Russell, E., Koren, G., Rieder, M., & Van Uum, S. (2012). The detection of cortisol in human hair: Clinical applications and research implications. *Journal of Clinical Endocrinology & Metabolism*. 97(2): 220-229.
- Sapolsky, R. M., Romero, L. M., Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*. 21(1): 55-89.
- Terlouw, C., Bourguet, C., Deiss, V. (2020). Stress and meat quality. In F. Toldrá (Ed.), *Advances in Food and Nutrition Research*, Vol. 91 (pp. 81-134). Academic Press
- Zhang, X., Sun, Y., Lei, L. (2021). Salivary cortisol as a non-invasive biomarker for stress assessment in pigs. *Animal Welfare*. 30(1): 45-58.

EXPERT ARTICLE

What and how high are the internal capacities of domestic farmers in milk production?

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Summary

Numerous objective external, and subjective internal factors that show a long-term negative trend lead to adverse results in domestic milk production and affect the overall state of production state with varying intensity. Considering the significant influence of these external factors on the financial performance of dairy farms, this article aims to provide a more detailed overview of the internal factors that also have a significant impact on the basic production indicators of domestic dairy farms and which can be influenced by farmers.

Based on the current production conditions on domestic dairy farms, it is clear that many important internal factors are lacking. Improving these factors could increase production and the financial success of the farm. Even if ambitions to become a dairy-exporting country are not realistic at this stage, it is reasonable to expect that we can achieve self-sufficiency in this vital animal feed.

Key words: dairy cow, dairy farm, food, production facilities, breed

Introduction

According to Central Bureau of Statistics (CBS), there has been a continuous decline in all production indicators is evident: decline in the number of farmers, the number of dairy animals and total milk production. While the number of farmers fell from 12,600 in 2013 to 2,630 in 2024, the number of dairy cows fell from the initial 180,555 cows to 71,000 in 2023 over the same period (HAPiH, 2023). Total milk production fell from 786 million liters in 2012 to 377 million liters in 2023 (CBS, 2024). According to these data, the level of self-sufficiency in domestic milk production of this important food item in the Republic of Croatia is less than 40%, which is unacceptable if we take into account the real natural resources and the production and professional capacities of domestic farmers. In addition to the above-mentioned extremely negative indicators for domestic milk production over the last ten years, there are also some positive indicators relating to the increase in milk production per cow, the increase in the quantity of milk per supplier, and the improvement in milk quality (today over 97% of milk is in quality class E). The positive indicators mentioned are the result of the cessation of production by a large number of small producers with lower technological standards in production and provide little consolation for the overall unfavorable picture of the situation in milk production.

These unfavorable results of domestic milk production with a long-term negative trend are the result of numerous objective external factors and internal subjective factors, each of which affects the overall state of production to varying degrees. Without diminishing the very strong

influence of the aforementioned external factors on the financial results of dairy farms, this professional article aims to provide a more precise overview of internal factors that also have a significant impact on the basic production indicators of domestic dairy farms and which the farmer can influence to a large extent. Internal production factors under the farmer's control include breed structure, feed, facilities, production techniques and technologies, and the farmer's expertise.

Breed structure on dairy farms

From the previously significant presence of the Simmental breed (combined production traits) among smaller dairy producers, there has recently been a clear trend towards the choice of the Holstein dairy breed. Although the genetically determined maximum production potential of both breeds can only be achieved through knowledge and application of the best technical and technological solutions in production, this is particularly true for the Holstein dairy breed (Ivanković and Mijić, 2020). Due to its extremely high milk yield, the Holstein breed is defined by the profession as a "small milk factory", and for its high production results, it rightly demands the best production conditions from farmers.

In domestic dairy farms where production is based on the Holstein breed, the technology is often not suitable for this high-milk breed. Inadequate housing capacities with questionable hygienic conditions in the biozone and very unfavorable effects of climatic conditions (especially in the summer months) on health and production status are observed. Insufficient quantities of dominant feedstuffs have also been observed, which are also of weaker to bad nutritional and hygienic value, and an insufficient number of daily feedings have been recorded (three times per day is recommended for this breed). Many farmers prepare one or two meal recipes for all livestock, which is professionally unacceptable given the significantly different individual needs of the animals. The previous statements are confirmed by the observation of a large number of animals in inadequate conditions. In addition to the lack of daily animal monitoring, diseases are not detected and treated in time, as well as the more frequent "silent" calvings characteristic of this breed, which reduce the fertilization success, resulting in "long" lactations (over 400 days), a lack of quality female calves and the inability to service the herd from their breeding. All the described shortcomings in the practical production of domestic farmers are the reason for the reduced utilization of the genetic production capabilities of the Holstein breed by up to 50%. Based on the above, the choice of the highly productive Holstein breed in domestic industrial milk production is professionally justified by farmers with a high technological level of production development. Therefore, the advice of the profession would be that when introducing highly productive dairy animals into the production of newer facilities equipped with modern technology, farmers should change old work and business habits so that the production results match the capabilities of the animals (Domaćinović et al., 2015).

Food in milk production

In practice, domestic dairy farms often do not have enough arable land to produce basic feed (hay, haylage, silage) and they make up for the lack of these feed by buying it on the open market. Often, the quantity and quality of feed offered on the market are questionable, with nutritional efficiency less than 1.5 ST food/kg of milk. As a result, feed consumption is reduced, and the proper satisfaction of the total nutritional needs of production animals becomes questionable. In these circumstances, farmers resort to a professionally and cost-unacceptable solution, replacing the shortage of roughage by increasing the proportion of concentrated feed (Haluška, 2004). Fresh green roughage, although nutritionally, physiologically and financially competitive, is still unjustifiably rarely represented in meals. Analysis of the chemical composition of bulky feedstuffs, where greater variations in nutritional value are expected (at the level of the year and mowing), has not yet become a regular technological measure and is

rarely carried out by many farmers (HAPiH data, 2023). The results of these periodic analyses often indicate poorer and variable nutritional quality of the dominant components of the meal. Based on the experience of experts in the field, the most common errors in production and preparation are harvest time outside the optimal phenophase of plant growth, improper management of the preservation process, and inappropriate storage conditions. Ignorance of the nutritional value of the components seriously questions the correct composition of the entire meal recipe and meeting the overall needs of dairy animals, especially high-milk animals, which are most sensitive to improvisation in feeding. The quality of the meal can be determined based on daily monitoring of the dynamics and quality of its consumption by analyzing the structure and consistency of feces and by analyzing the quality of milk, which is not carried out regularly. Failure to carry out a microbiological analysis of feed in these climatically extreme growing years creates a real possibility that feed contaminated with mycotoxins threatens the health of animals, as well as the hygienic value of milk, which therefore loses its market value and problems arise with purchasers.

Dairy farm facilities

In addition to a certain number of farms where the facilities are of top quality in every respect, a significant number of dairy farms still base their production in facilities that do not meet today's trends in modern milk production techniques and technology. Sometimes, these are very old facilities, poorly dimensioned, built of materials that do not provide the expected comfort to the animals during production (feeding, milking, resting). The microclimate in these facilities is particularly questionable during the extremely hot summer months, which causes the animals to change their standard behavior patterns, and then the welfare of the animals is also called into question. In these facilities, due to the values of the microclimate (temperature and humidity) being above those desirable for the animals, the oxidative stress of the organism has reduced food consumption, which directly affects the reduction in production by more than 30%. Furthermore, in some farmers with medium and larger production capacity, the construction of auxiliary facilities for storing voluminous feed and litter (sheds, silos) is insufficient, which directly affects the deterioration of the nutritional and health quality of fermented and dry feed during storage. Unprotected feed stored outdoors can lose up to 40% of its initial DM over a year due to weather conditions (Leto, 2021). At the same time, with the stronger development of putrefactive processes with increased feed moisture (presence of mold and mycotoxins), animals react by reducing or completely rejecting such feed and the entire TMR meal. Losses of nutritional and hygienic value of feed under inappropriate storage conditions are negatively reflected in increased feed prices and poorer production results. To apply modern production technology and the latest automatic robotic techniques, all facilities (production and auxiliary) must be close and well-connected in terms of communication; otherwise, inappropriate facilities can be a serious obstacle to innovation with new technical systems (Mijić and Bobić, 2019).

Production technique and technology

It is not uncommon for domestic farmers to believe that investing in automatic technology can reduce the time spent in contact with an animal, which is not the right approach. Automatic technology in dairy farms (PLF systems, Precision Livestock Farming) offers a large amount of useful information daily for individual monitoring of production animals, which is a valuable tool for every farmer in monitoring the entire production. Highly efficient and widely applicable technology can be of particular help to an experienced and professional farmer in the early recognition of errors, initiating immediate solutions to minimize damage to the technology, facility, animals, and product. Therefore, technology helps the farmer but does not replace him in managing production (Domaćinović et al., 2023). It is not possible to talk about modern milk

production technology if the production animal is not in a good living environment, if the health care is insufficient (medium and large farms without permanent veterinary services), if there are poor microclimate conditions, increased animal population, frequent stressful situations, etc. (Šperanda, 2019). How can we expect a continuously high production output of animals if their different individual production capabilities and needs are not considered, organizing feeding only on one or two production groups, and a maximum of two nutritionally different meals. Justifying this with easier work organization, many farmers apply a shortened version of the introduction to dairying or do not implement it at all. The above omissions should be added to the insufficient number of daily feedings (one feeding per day), which is especially professionally unacceptable in the summer period when sensory properties change rapidly, the hygienic value of food is impaired, and animals reduce consumption. In today's conditions of technically modernized milk production (up to robotization), the practice of omitting mineral and vitamin components from the meal has been observed, as well as feeding dairy cows a multi-component meal without a known recipe for the quantitative proportions of individual feed ingredients in the meal, the so-called "feeding by eye".

Farmer expertise

Although the expertise of domestic farmers has improved in the last twenty years compared to the previous period, even today, it cannot be said that the level of expertise of farm owners and the workforce of a large number of farmers is satisfactory. A significant number of farmers have a primary school education, a certain number have secondary education, and a very small percentage of them have a university degree. To all of the above, it should be added that not all farmers with a high school or college degree have the appropriate profile, a secondary or higher education diploma, majoring in zootechnics or veterinary medicine. If previously in extensive conditions of animal husbandry and milk production, work and management were based on basic qualifications, today the unanimous opinion of the profession is that for the proper and successful management of new modern dairy farms, a higher education is required, especially in the circumstances of further intensification of farm milk production in all segments of production (Domaćinović et al., 2019). Considering how important a high level of expertise and experience of farmers is for successful business results in this technologically demanding direction of cattle production, the authors are inclined to claim that this was one of the reasons for the closure of many dairy farms in the last ten years.

Conclusions

In the need to change the previous negative trends in the domestic dairy sector, while considering the competitiveness and trends of the global European milk market, farmers are expected to contribute to increased productivity and profitability in milk production through more serious systematic solutions to improve internal production conditions. So, it is unquestionable that there are unused significant production capacities among domestic farmers whose improvements in the direction of higher quality technological solutions can bring greater financial benefits in business without additional investments. Improving the internal conditions of production, raising it to a higher level, will result in a greater production effect and will have a beneficial effect on the external conditions of production. If the ambitions to be a milk-exporting country are not realistic, then the expectations to be self-sufficient in this important animal food are very realistic.

References

- Domaćinović, M., Z. Antunović, E. Džomba, A. Opačak, M. Baban, S. Mužić (2015). Specijalna hranidba domaćih životinja, Poljoprivredni fakultet u Osijeku.
- Domaćinović, M., M. Đidara, D. Solić, Marcela Šperanda (2019). Dobra proizvođačka praksa u animalnoj proizvodnji. Fakultet agrobiotehničkih znanosti u Osijeku.
- Domaćinović, M., Mijić, P., Novoselec, J., Domaćinović, A., Solić, D., Prakatur, I. (2023). Prednosti i prijetnje tehnologije preciznoga praćenja i upravljanja na mliječnim farmama. *Poljoprivreda*. 29(2): 70-78.
- Egner H., Riehm H., Domingo W. R. (1960). Investigations on the chemical soil analysis as a basis for assessing the soil nutrient status II. Chemical extraction methods for phosphorus and potassium determination. *Kungliga Lantbrukshögskolans Annaler* 26: 199-215.
- Haluška, J. (2004). Osnovna pravila hranidbe visokomliječnih krava. *Stočarstvo*. 58(5): 395-400.
- Ivanković, A., P. Mijić (2020). *Govedarstvo*, Sveučilište u Zagrebu Agronomski fakultet, Zagreb.
- Leto J. (2021): Čimbenici koji utječu na kvalitetu krme, *Mljekarski list*, 58,(2), 20-23.
- Mijić, P., Bobić, T. (2019). Automatizirani muzni sustavi ili robotizirana mužnja krava: prednosti i nedostaci. In *14. savjetovanje uzgajivača goveda u Republici Hrvatskoj*, Plitvička jezera, 68-77.
- Šperanda Marcela (2019). Kako sačuvati zdravlje životinja u proizvodnji? In *14. savjetovanje uzgajivača goveda u Republici Hrvatskoj*, Plitvička jezera, 48-54.
- <https://www.agroklub.com/stocarstvo/za-pravilnu-hranidbu-krava-pratiti-faze-laktacije/24466/>
- <https://podaci.dzs.hr/2024/hr/77204>
- Hrvatska agencija za poljoprivredu i hranu (2023). Kontrola kvalitete stočarskih proizvoda. Godišnje izvješće za 2023. godinu.

ORIGINAL SCIENTIFIC PAPER

A survey of managed honey bee colony losses in Croatia from 2020 to 2024Janja Filipi¹, Barbara Predovan¹, Maja Marica Dražić²¹*Department of ecology, agronomy and aquaculture, University of Zadar, Trg kneza Višeslava 9, Zadar, Croatia (jfilipi@unizd.hr)*²*Ministry of Agriculture, Forestry and Fisheries, Ilica 101, Zagreb***Summary**

Honey bees as most important pollinators are suffering severe losses due to a complex interaction of multiple stressors. We conducted an international standardized survey on honey bee colony losses in Croatia for winters 2020/2021 till 2023/2024. Altogether we collected 844 valid responses from beekeepers wintering 54.284 honey bee colonies. Overall winter loss rates were highest in winter 2020/2021 (29.50%; CI: 26.87 – 32.28%) and lowest in winter 2023/2024 (13.37%; CI: 11.00 – 16.16%). Winter colony loss rates were variable between years and operation size. There are many differences influencing honey bee survival such as weather, pest and pathogens, management and many others. To address specific risk factors, a higher response rate is needed, along with more detailed research and case studies.

Key words: Honey bees, winter losses, operation size

Introduction

Over the past two decades, honey bees, as crucial pollinators, have been experiencing significant losses on a global scale (Cox-Foster et al., 2007; van Engelsdorp et al., 2009, 2010; Neumann and Carreck, 2010; van der Zee et al., 2012; Brodschneider et al., 2019). These declines pose a severe threat to biodiversity, agriculture, and food security. Within the COLOSS (nonprofit scientific organization) core project on “Monitoring Honey Bee Colony Losses” collects and analyze colony losses worldwide (Brodschneider and Gray, 2021) reporting on decline of honey bee colonies and risk factors (Brodschneider et al., 2016, 2018, Gray et al., 2019, 2020). A multitude of biotic and abiotic factors contribute to honey bee survival and overall colony health (Steinhauer et al., 2018). Honey bee colony losses are the result of a complex interaction of multiple stressors. The parasitic mite *Varroa destructor* Anderson and Trueman remain one of the most significant threats to honey bee colonies worldwide, acting as both a direct parasite and a vector for numerous viral infections (Le Conte et al., 2010; McMenamin and Genersch, 2015). Other harmful pathogens such as *Vairimorpha* sp. (formerly *Nosema* sp.), also contribute to colony health decline (Steinhauer et al., 2018). The rise of novel pests and pathogens presents a growing concern for beekeeping. Changes in disease dynamics, including virus prevalence and virulence, exacerbate colony mortality rates (McMenamin and Genersch, 2015). The loss of natural forage areas due to urbanization, monoculture farming, and habitat destruction limits the diversity and abundance of nectar and pollen sources. Poor nutrition weakens honey bees' immune responses, making them more susceptible to diseases and environmental stressors (Brodschneider and Crailsheim, 2009). Widespread use of agrochemicals poses a substantial risk to honey bee health. Pesticides can impair foraging behavior, weaken immune function, and contribute to colony collapse when combined with other stress factors (Tosi et al., 2018). Hive management, including disease control measures, can negatively impact colony resilience (Jacques et al., 2017). Rising global temperatures, unpredictable weather patterns, and extreme climatic events disrupt floral resources, alter

seasonal bee activity, and increase stress levels, further endangering colony survival (Overturf et al., 2022).

The aim of this study was to present winter honey bee colony losses in Croatia and to identify loss rates among years and by the operations size.

Materials and methods

Data on honey bee colony losses were collected using the standardized COLOSS questionnaire each spring between 2021 and 2024 (van der Zee et al., 2013). Questionnaire is anonymous and available online using Lime Survey also in paper form. Incomplete or multiple entries were removed from data set before analyses. The statistical analyses were calculated according to van der Zee et al. (2013) using R statistical program. Beekeepers were asked the number of colonies before winter and in following spring. The overall proportion of colonies lost is calculated as the number of lost colonies and divided by the number of colonies before winter. Beekeepers were categorized by operation size in five classes according to the number of colonies before winter (A 1 – 15, B 16 – 30, C 31 – 50, D 51 – 150, E >150).

Results and discussion

Participation in the survey vary among years. Altogether we collected 844 valid responses from beekeepers who wintered 54.284 honey bee colonies over four-year period. The number of beekeeper's responses, wintered colonies and loss rates are presented in the table 1. Winter losses rates fluctuated from year to year. The highest loss rates were occurred in winter 2020/2021 (29.50%; CI: 26,87 – 32.28%) and lowest in winter 2023/2024 (13.37%; CI: 11.00 – 16.16%). Similar results were reported in Brodschneider et al. (2019), Mazur et al. (2022), Gray et al. (2022).

Table 1. Number of beekeeper's responses, colonies before winter and number of lost colonies with loss rates (CI) per year

Winter	2020-21	2021-22	2022-23	2023-24
No of beekeeper's responses	298	172	234	140
No of colonies before winter	19.495	10.475	16.820	7.494
No of lost colonies	5.751	1.712	3.431	1.002
Loss rate (%)	29,50	16,34	20,40	13,37
Confidence interval	26.87– 32.28	13.39– 19.81	17.59– 23.53	11.00– 16.16

Figure 1 illustrates the distribution of winter losses per beekeeper (% of colonies lost), segmented by year. The distribution highlights substantial variability at the individual beekeeper level. The histograms reveal that in most years, a majority of beekeepers experienced moderate losses (0–30%). However, a consistent minority reported severe losses including complete colony loss (100%), especially in years with higher overall mortality, which is similar to the results presented in Brodschneider et al. (2019).

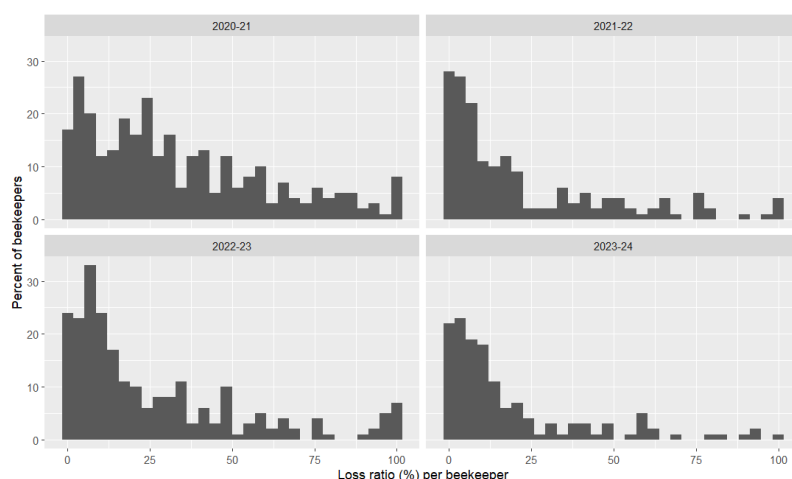


Figure 1. The distribution of winter colony losses per beekeeper (% of colonies lost) over four winter periods (2020–21 to 2023–24). Each subplot represents a histogram for one winter season, where the x-axis shows the percentage of colony loss per beekeeper, and the y-axis indicates the number of beekeepers (count) within each loss percentage interval.

The number of colonies per beekeeper is important information from economy point of view, in that regard we divided loss rates per operation size (fig. 3). The majority of beekeepers (51,7 – 62,2%) are fitting the first three categories up to 50 colonies in our data set, having similar distribution as the other European countries (Brodschneider et al., 2016, Gray et al., 2022). Small operation beekeepers up to 50 colonies have higher losses in all reported years comparing to the beekeepers with more than 150 colonies as showed in the figure 2. Same pattern of losses is also found in the research of Gray et al. (2022).

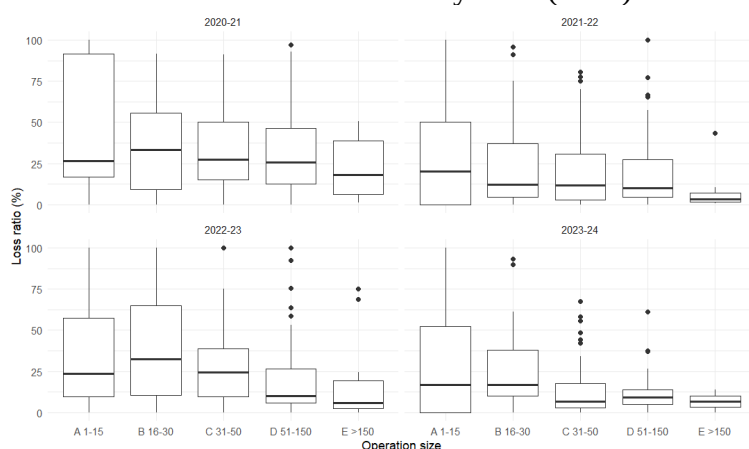


Figure 2. Boxplots of colony loss rates (%) across different operation size categories for each winter season from 2020–21 to 2023–24. The boxplot illustrates the median, interquartile range (Q1–Q3), minimum and maximum values and individual data points outside this range, which are plotted as outliers. Operation sizes are categorized into five groups: A (1–15 colonies), B (16–30), C (31–50), D (51–150), and E (>150 colonies).

Conclusions

Honey bee colony losses are worldwide problem influenced by complex interaction of multiple stressors. Honey bee colony losses were greatly variable between years and operation size. Beekeepers with less than 50 colonies had more severe losses than beekeepers with more than 150 colonies, emphasizing the vulnerability of small-scale beekeeping. These findings underscore the importance of targeted support and recommendations for smaller operations, including improved access to knowledge, resources, and technical guidance. Many factors

influence honey bee survival including weather, pest and pathogens, management and others. To address specific risk factors, a higher response rate is needed, along with more detailed research and case studies.

References

- Brodschneider, R., and Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, 41(3): 278-294.
- Brodschneider R., Gray A., van der Zee R., Adjlane N., Brusbardis V., Charriere J.-D., Chlebo R., Coffey M.F., Crailsheim K., Dahle B., Danihlik J., Danneels E., de Graaf D.C., Dražić M.M., Fedoriak M., Forsythe I., Golubovski M., Gregorc A., Grzęda U., Hubbuck I., İvgin Tunca R., Kauko L., Kilpinen O., Kretavicius J., Kristiansen P., Martikkala M., Martin-Hernandez R., Mutinelli F., Peterson M., Otten C., Ozkirim A., Raudmets A., Simon-Delso N., Soroker V., Topolska G., Vallon J., Vejsnas F., Woehl S. (2016). Preliminary analysis of loss rates of honey bee colonies during winter 2015/16 from the COLOSS survey. *J. Apic. Res.* 55, 375-378. <https://doi.org/10.1080/00218839.2016.1260240>
- Brodschneider R., Gray A., Adjlane N., Ballis A., Brusbardis V., Charriere J.-D., Chlebo R., Coffey M. F., Dahle B., Graaf D. C., Dražić M. M., Evans G., Fedoriak M., Forsythe I., Gregorc A., Grzęda U., Hetzroni A., Kauko L., Kristiansen P., Martikkala M., Martin-Hernandez R., Medina-Flores C. A., Mutinelli F., Raudmets A., Ryzhikov V. A., Simon-Delso N., Stevanovic J., Uzunov A., Vejsnas F., Wohl S., Zammit-Mangion M., Danihlik J. (2018). Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. *J. Apic. Res.* 57: 452-457.
- Brodschneider R., Brus J., Danihlik, J. (2019). Comparison of apiculture and winter mortality of honey bee colonies (*Apis mellifera*) in Austria and Czechia. *Agric. Ecosyst. Environ.* 274, 24-32.
- Brodschneider R., Gray A. (2021). How COLOSS Monitoring and Research on Lost Honey Bee Colonies Can Support Colony Survival. *Bee World*, 99(1): 8-10.
- Cox-Foster D. L., Conlan S., Holmes E. C., Palacios G., Evans J. D., Moran N. A., Quan P. L., Brieseman T., Hornig M., Geiser D. M., Martinson V., vanEngelsdorp D., Kalkstein A. L., Drysdale A., Hui J., Zhai J., Cui L., Hutchison S. K., Simons J. F., Egholm M., Pettis J. S., Lipkin W. I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318: 283-287. DOI: 10.1126/science.1146498
- Gray A., Brodschneider R., Adjlane N., Ballis A., Brusbardis V., Charrière J. D., Chlebo R., Coffey M. F., Cornelissen B., Amaro da Costa C., Csaki T., Dahle B., Danihlik J., Dražić M. M., Evans G., Fedoria M., Forsythe I., de Graaf D. C., Gregorc A., Johannesen J., Kaukov L., Kristiansen P., Matrikkala M., Martin – Hernandez R., Medina – Flores C. A., Mutinelli F., Patalano S., Petrov P., Raudmets A., Ryzhikov V. A., Simon-Delso N., Stevanovic J., Topolska G., Uzunov A., Vejsnaes F., Williams A., Zammit-Mangion M., Soroker V. (2019). Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources. *Journal of Apicultural Research*, 58(4): 479-485.
- Gray A., Adjlane N., Arab A., Ballis A., Brusbardis V., Charrière J. D., Chlebo R., Coffey M. F., Cornelissen B., Amaro da Costa C., Dahle B., Danihlik J., Dražić M. M., Evans G., Fedoria M., Forsythe I., Gajda A., de Graaf D. C., Gregorc A., Ilievat I., Johannesen J., Kaukov L., Kristiansen P., Matrikkala M., Martin – Hernandez R., Medina – Flores C. A., Mutinelli F., Patalano S., Raudmets A., San Martin G., Soroker V., Stevanovic J., Uzunov A., Vejsnaes F., Williams A., Zammit-Mangion M., Brodschneider R. (2020). Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018-2019, and the effects of a new queen on the risk of colony winter loss. *Journal of Apicultural Research*, 59(5): 744-751.
- Gray A., Adjlane N., Arab A., Ballis A., Brusbardis V., Bugeja Douglas A., Cadahia L., Charriere J. D., Chlebo R., Coffey M. F., Cornelissen B., Amaro da Costal C., Danneels E., Danihlik J., Dobrescou C., Evans G., Fedoriak M., Forsythe I., Gregorc A., Ilieva Arakelyan I., Johannesen J., Kaukov L., Kristiansen P., Martikkala M., Martin - Hernandez R., Mazur E., Medina-Flores C. A., Mutinelli F., Omar E. M., Patalano S., Raudmets A., San Martin G., Soroker V., Stahlmann-Brownah P., Stevanovic J., Uzunov A., Vejsnaes F., Williams A., Brodschneider, R. (2022). Honey bee colony loss rates in 37 countries using the COLOSS survey for winter 2019-2020: the combined effects of operation size, migration and queen replacement. *Journal of Apicultural Research*, 62(2): 204-210.

- Jacques A., Laurent M., Epilobee Consortium, Ribière-Chabert M., Saussac M., Bougeard S., Budge G. E., Hendrikx P., Chauzat M. P. (2017). A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. *PLoS ONE*, 12.
- Le Conte Y., Ellis M., Ritter W. (2010). Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie*. 41(3):353–63. DOI: 10.1051/apido/2010017
- Mazur E. D., Czopowicz M., Gajda A. M. (2022). Two Faces of the Screened Bottom Boards—An Ambiguous Influence on the Honey Bee Winter Colony Loss Rate *Insects*. 2022; 13(12):1128.
- McMenamin A., Genersch E. (2015). Honey bee colony losses and associated viruses. *Current Opinion in Insect Science* 2015, 8:121–129.
- Neumann P., Carreck N. L. (2010). Honey bee colony losses. *J. Apic. Res.* 49: 1–6.
- Overturf K. A., Steinhauer N., Molinari R., Wilson M. E., Watt A. C., Cross R. M., vanEngelsdorp D., Williams G. R., Rogers S. R. (2022). Winter weather predicts honey bee colony loss at the national scale. *Ecological Indicators*, Vol 145, 109709.
- Steinhauer N., Kulhanek K., Antúnez K., Human H., Chantawannakul P., Chauzat M. P., vanEngelsdorp D. (2018). Drivers of colony losses, *Current Opinion in Insect Science*. 26: 142–148.
- Tosi S., Costa C., Vesco U., Quaglia G., Guido G. (2018). A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. *Science of The Total Environment*. 615: 208–218.
- van der Zee, R., Pisa L., Andonov S., Brodschneider R., Charriere J. - D., Chlebo R., Coffey M. F., Crailsheim K., Dahle B., Gajda A., Gray A., Drazic M. M., Higes M., Kauko L., Kence A., Kence M., Kezic N., Kiprijanovska H., Kralj J., Kristiansen P., Hernandez R.M., Mutinelli F., Nguyen B. K., Otten C., Ozkirim A., Pernal S. F., Peterson M., Ramsay G., Santrac V., Soroker V., Topolska G., Uzunov A., Vejsnas F., Wei S., Wilkins S. (2012). Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. *J. Apic. Res.* 51: 100–114.
- van der Zee R., Gray A., Holzmann C., Pisa L., Brodschneider R., Chlebo R., Coffey M. F., Kence A., Kristiansen P., Mutinelli F., Nguyen B. K., Noureddine A., Peterson M., Soroker V., Topolska G., Vejsnas F., Wilkins S. (2013). Standard survey methods for estimating colony losses and explanatory risk factors in *Apis mellifera*. *J. Apic. Res.* 52: 1–36.
- VanEngelsdorp D; Evans J. D., Saegerman C., Mullin C., Haubruge E., Nguyen B. K., Fraizer M., Fraizer J., Cox - Foster D., Chen Y., Underwood R., Tarpay D. R., Pettis J. S. (2009). Colony Collapse Disorder: A descriptive study. *PloS ONE* 4:e6481.
- VanEngelsdorp D., Hayes J. Jr., Underwood R. M., Pettis J. S. (2010). A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research* 49(1): 7–14.

REVIEW ARTICLE

From sensors to data: Application of digital technologies in intensive pig production

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Summary

The aim of this review is to highlight the impact of digital technologies, in particular sensor systems, on intensive pig production. The use of sensor technologies has a positive impact on animal welfare, productivity and the sustainability of production cycles. Sensors such as environmental sensors, animal behaviour sensors, identification and tracking sensors and biosensors collect data through real-time monitoring to help farmers make management decisions. Although these technologies offer many advantages, there are still challenges such as high costs, data accuracy and infrastructure requirements. Future trends in sensors in pig production indicate that artificial intelligence, robotics and IoT will take their place in pig production.

Key words: pigs, farm, sensors, data, productivity

Introduction

Intensive pig production is defined as a system in which pigs are kept at high stocking densities, with specific management practises and in a controlled environment to maximise production efficiency. The demand for pork and pork products increases due to global population growth and a shift in dietary preferences towards animal-based proteins (Fang, 2022). Due to the continuous increase in the population and increased needs for animal products, it is necessary to apply new technologies and practices such as precision livestock farming (PLF) to make pig production more sustainable.

Precision livestock production combines the use of sensors, tracking and communication systems and software solutions that follow the product from the producer to the consumer. It refers to a range of digital technologies that aim to improve the quality and quantity of production while optimising labour requirements (Gómez et al., 2021). For instance, sensor systems can track environmental conditions and health parameters, allowing farmers to respond in real time on potential welfare issues (Schillings et al., 2021). Integration of advanced digital technologies can help in early detection of health issues, significantly reducing economic losses and improving overall production efficiency. The use of digital technologies such as the Internet of Things (IoT), automated systems and artificial intelligence allows advanced monitoring and optimisation of production processes (Andronie et al., 2021; Rath et al., 2024). Digital tools lead to a faster response to changes in environmental conditions on production farms and in the health and production conditions of animals. Digitalisation plays a key role in modern pig production, allowing better management practices and animal welfare as well as optimised productivity. Additionally, implementing these digital innovations allows that pig production changes according to the consumer demands for ethical animal care and sustainability. The aim of this review paper is to analyse the multifaceted impact of digital technologies, alongside sophisticated sensors and data analysis, on the enhancement of intensive pig production systems. By combining these innovations, producers can effectively address environmental challenges, improve animal welfare standards, and contribute to a more sustainable pig production.

Sensor technologies in pig production

The word sensor comes from "sensus", which means "sense", and stands for devices that recognise and react to a stimuli or input signals from the environment.

Sensors such as cameras, microphones, thermometers and many others work on the principle of detecting environmental inputs such as heat, humidity, light, movement and similar phenomena (Table 1). The collected data is stored in external drives or sent from the sensors to the main processor, where it is combined with individual animal identifications, referenced observations and production data (Tzanidakis et al., 2021). The data is then integrated into algorithms to provide relevant information and alerts about the health, productivity and welfare of pigs. An algorithm is a specific set of defined commands, operations or formulas used to solve a specific issue. Programming algorithms are used in precision livestock breeding. These are computer procedures that tell the computer exactly which steps it must perform to solve the problem. The real value of an algorithm to farmers depends on its ability to convert data from the sensor into a biological result. The output variables are the output signals that are converted into readable data on the sensor screen that is visible to the producer. An example of a significant variable could be the percentage of time a pig spends lying down to determine the biological outcome of lameness, or the frequency of coughing to identify the biological outcome of possible respiratory disease (Pandey et al., 2021).

Types of sensors

Different types of sensors are used to ensure optimal conditions during the production cycle, which is critical for maintaining animal health and productivity (Halachmi et al., 2019). Environmental sensors play a crucial role by continuously monitoring conditions in real time. These environmental sensors can measure temperature, humidity, ammonia levels or air quality. Obtained data of these parameters have effect on pigs health, welfare and productivity. For instance, elevated ammonia levels and higher environmental temperatures can lead to respiratory problems and decreased growth rates of pigs (Pandey et al., 2021; Peng et al., 2022). To monitor individual behaviour, movement, feeding patterns and growth rates producers can use the animal-based sensors (Racewicz et al., 2021). Feeding behaviour and water consumption can provide information's on health problems even before clinical signs appear. This individualized approach allows for rapid interventions, contributing significantly to enhanced herd health management systems (Reza et al., 2024). Biosensors detect the physiological indicators of stress or early signs of possible health problems. They can be used to identify metabolites or changes in animal physiology indicative of health issues, allowing interventions in real time (Manassis et al., 2022; Nastasiyevic et al., 2025).

Table 1. Practical implementation, benefits, and limitations of different sensor technologies in intensive pig production

Sensor category	Specific sensors	Production area	Practical applications	Benefits	Limitations
Environmental sensors	Temperature, humidity, gas sensors (NH ₃), ventilation, light intensity, dust sensors	Climate control and environmental monitoring	Maintaining optimal climate, air quality, and environmental conditions	Improved animal welfare, increased productivity, reduced environmental footprint	Frequent calibration, sensor contamination, maintenance costs

Animal behavior sensors	Accelerometers , RFID tags, video tracking	Animal health and behavior monitoring	Behavioral analysis, early disease detection, improving welfare	Early intervention, better animal health, welfare improvement	High investment, durability concerns, complexity of data interpretation
Identification and tracking sensors	RFID ear tags, Bluetooth trackers, GPS trackers	Animal identification, inventory control, traceability	Identification, traceability, inventory management, group management	Enhanced traceability, improved animal management, regulatory compliance	Integration issues, upfront costs, system compatibility
Feeding and performance sensors	Automated weighing scales, feeding sensors, drinking sensors	Feed management, nutritional optimization, growth monitoring	Optimized feeding, performance monitoring, precise nutrition control	Increased growth rates, feed cost reduction, improved economic efficiency	Initial high cost, sensor reliability, data overload
Biosensors	Heart rate monitors, body temperature sensors, stress biomarker sensors	Physiological monitoring and disease detection	Early detection of diseases, stress monitoring, herd health management	Reduced antibiotic usage, proactive management, improved animal health	High price, limited availability, complexity, environmental sensitivity

Applications of sensors

The development of sensor technologies and their application in pig production contribute significantly to better production conditions. Health problems, the type of flooring, feeding and human manipulations are some of the factors that challenge ensuring good welfare condition and optimal pig production (Tzanidakis, 2021). The application of sensor in intensive pig production is complex but offers practical solutions to farmers for enhance management, health and animal welfare. Continuous monitoring of microclimate conditions prevents stress-induced production losses associated with unfavourable climatic conditions that may occur during the heat stress. Moreover, Elsayed et al. (2024) stated that maintaining optimal environmental conditions is one of the key factors for feed conversion efficiency in pigs. Schillings et al. (2021) stated that by measuring and managing microclimate factors like temperature and humidity in production facilities through sensor technology not only optimizes growth but also minimizes health risks associated with extreme conditions. Sensors also may be used for monitor individual parameters in pigs such as consumption of feed and water, activity of pigs and behaviour within the groups (Matthews et al., 2017). Tran and Thanh (2023) pig behaviours in pig farms is important from the aspect of pig health and welfare. The authors propose a deep learning method for automatically monitoring and detecting behaviour abnormalities that is based on behaviour patterns during the active periods. Integration of sensor technologies in pig production allows to the farmers to implement strategies that will detect changes in individual pig behaviour or environmental conditions which will lead to timely response from farmers.

Advantages and current limitations

Application of sensor technology in pig production has advanced pig production cyclus from the aspect of production efficiency and animal health and welfare. By using the different types of sensors on farms, farmers can obtain data that may be used for optimization of feeding practices, reducing the waste, and enhance productivity and profitability of the farms. Notably, advanced monitoring also allows farmers to identify the pig individual needs and effectively contributing to animal welfare. Despite these promising advantages there are also few limitations of implementations of sensor technology of pig farms (Arulmozhi et al., 2021). This mostly refers on high initial investment required for implementation, accuracy and reliability of obtained data. Also, integration of sensor technologies into existing farm structures may be challenge as traditional farm do not have necessary infrastructure necessary for these advanced systems. As a result, farmers may require additional training and support to fully use the capabilities of sensors in their operations (Schillings et al., 2021). Overcoming the limitations and challenges faced by current systems will be fundamental in shaping the future of automated and precision livestock farming.

Future trends of sensors in pig production (Schulze et al., 2019; Bao et al., 2024) will include the introduction of new technologies such as the integration of artificial intelligence, Internet of Things (IoT), and blockchain technologies. The impact of the these technologies is particularly reflected in the increase in productivity due to the improving decision-making processes during the production. The introduction of new technologies related to robotics and automation will lead to an improvement in overall efficiency on farms.

Conclusions

The use of digital sensor technologies has changed intensive pig production. The use of different types of sensors has a positive impact on the environment, animal health and welfare. Sensors in intensive pig production also have a positive impact on reducing waste, enhancing the feed efficiency and minimising the use of antibiotics. In addition, real-time monitoring and the data obtained can help farmers make precise management decisions and optimise production. Despite these advantages, there are some limitations to the application of these technologies, such as high investment costs, complex data interpretation and the need for specialised training for farmers. The introduction of digitalisation ensures competitive, efficient and sustainable pig production systems that meet both current and future challenges.

References

- Andronie M., Lăzăroiu G., Iatagan M., Uță C., Ștefănescu R., and Cocoșatu M. (2021). Artificial intelligence-based decision-making algorithms, internet of things sensing networks, and deep learning-assisted smart process management in cyber-physical production systems. *Electronics*. 10(20): 2497.
- Arulmozhi, E. Bhujel A., Moon B. E., and Kim H. T. (2021). The application of cameras in precision pig farming: An overview for swine-keeping professionals. *Animals*. 11(8): 2343.
- Bao Y., Llagostera P., and Plà-Aragonès L. M. (2024). Is Deep Learning useful for decision making in pig production?. *Internet of Things*. 101229.
- Gómez Y., Stygar A. H., Boumans I. J., Bokkers E. A., Pedersen L. J., Niemi J. K., and Llonch P. (2021). A systematic review on validated precision livestock farming technologies for pig production and its potential to assess animal welfare. *Frontiers in veterinary science*. 8: 660565.
- Halachmi I., Guarino M., Bewley J., and Pastell M. (2019). Smart animal agriculture: application of real-time sensors to improve animal well-being and production. *Annual review of animal biosciences*. 7(1): 403-42

- Manessis G., Gelasakis A. I., and Bossis I. (2022). Point-of-care diagnostics for farm animal diseases: from biosensors to integrated lab-on-chip devices. *Biosensors*. 12(7): 455.
- Matthews S. G., Miller A. L., Plötz T., and Kyriazakis I. (2017). Automated tracking to measure behavioural changes in pigs for health and welfare monitoring. *Scientific reports*. 7(1): 17582.
- Nastasijevic I., Kundacina I., Jaric S., Pavlovic Z., Radovic M., and Radonic, V. (2025). Recent Advances in Biosensor Technologies for Meat Production Chain. *Foods*. 14(5): 744.
- Pandey S., Kalwa U., Kong T., Guo B., Gauger P. C., Peters D. J., and Yoon K. J. (2021). Behavioral monitoring tool for pig farmers: Ear tag sensors, machine intelligence, and technology adoption roadmap. *Animals*. 11(9): 2665.
- Peng S., Zhu J., Liu Z., Hu B., Wang M., and Pu S. (2022). Prediction of ammonia concentration in a pig house based on machine learning models and environmental parameters. *Animals*. 13(1): 165.
- Racewicz P., Ludwiczak A., Skrzypczak E., Składanowska-Baryza J., Biesiada H., Nowak T., and Ślósarz P. (2021). Welfare health and productivity in commercial pig herds. *Animals*. 11(4): 1176.
- Rath K. C., Khang A., and Roy D. (2024). The role of Internet of Things (IoT) technology in Industry 4.0 economy. In *Advanced IoT technologies and applications in the industry 4.0 digital economy* (pp. 1-28). CRC Press.
- Reza M. N., Ali M. R., Kabir M. S. N., Karim M. R., Ahmed S., Kyoung H., and Chung S. O. (2024). Thermal imaging and computer vision technologies for the enhancement of pig husbandry: a review. *Journal of Animal Science and Technology*. 66(1): 31.
- Schillings J., Bennett R., and Rose D. C. (2021). Exploring the potential of precision livestock farming technologies to help address farm animal welfare. *Frontiers in Animal Science*. 2: 639678.
- Schulze M., Nitsche-Melkus E., Jakop U., Jung M., and Waberski D. (2019). New trends in production management in European pig AI centers. *Theriogenology*. 137: 88-92.
- Tran D. D., and Thanh N. D. (2023). Pig health abnormality detection based on behavior patterns in activity periods using deep learning. *International Journal of Advanced Computer Science and Applications*. 14(5).
- Tzanidakis, C., Simitzis, P., Arvanitis, K., Panagakis, P. (2021). An overview of the current trends in precision pig farming technologies. *Livestock Science*, 249, 104530.

ORIGINAL SCIENTIFIC PAPER

Mitochondrial DNA analysis of Posavina crested hen strains

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Summary

At HAPIH, in collaboration with HSUIPP and the Faculty of Agriculture, genetic analyses were carried out to determine the genetic status of Posavina crested hen and to evaluate their genetic distance from related and other commercial hen breeds and hybrids. The aim was to support the recognition of this population as an indigenous breed of hens in the Republic of Croatia.

The lowest genetic distance among Posavina strains was determined between the CS and SS strains, while the highest was between the GR and ZS strains. When comparing the Posavina strains with commercial breeds, the highest genetic distance was found between the ORC and GR strains of the Posavina hen, and the lowest between the NH breed and the CS strain. Analysis of mtDNA haplotypes revealed haplotypes that are common to different populations, as well as haplotypes specific to certain strains of the Posavina crested hen. A NeighborNet network was used to display the phylogenetic relationships between the strains of Posavina hens and commercial breeds of hens.

The analysis of the d-loop region of mtDNA showed a clear genetic differentiation among Posavina strains, and confirmed the genetic distinctiveness of Posavina crested hen relative to commercial breeds.

Key words: Posavina crested hen, mitochondrial DNA, haplotype analysis

Introduction

The first record of the existence of the Posavina crested hen dates back to 1996, when Šandor Horvat, in his book "Croatian Heritage Breeds" described chickens found in some backyards of Posavina, on the left bank of the Sava River, from the village of Veleševac to Lonja and Ivanić Grad, that stood out for their attractive colors and feathered cap behind the crest. The Posavina crested hen was later mentioned as an insufficiently known chicken breed in the "Green Book of Native Breeds of Croatia" (2011). In the chapter titled Extinct and Insufficiently Known Breeds, authors Roman Ozimec and Darko Petanjek stated that the Posavina crested hen originated from an old population of the heavier type of Zagorje-Styrian hens that were crossed with Orpington, Rhode Island, Plymouth Rock, New Hampshire, and Amrock breeds. In July 2019, the Zagreb County signed an agreement with the University of Zagreb, Faculty of Agriculture, to launch the project "Protection of the Posavina crested hen". After four years of work, a formal request for recognition of the Posavina crested hen as an indigenous breed of domestic animals was submitted. The breed officially received this status by a decision of the Ministry of Agriculture dated March 7, 2023.

Due to its high level of variability, mitochondrial DNA (mtDNA) is one of the most used genetic markers. Maternal inheritance of mtDNA allows tracing the maternal ancestry back to the time before domestication (Lenstra et al., 2012). Most studies have been conducted on the highly polymorphic D-loop region, which has contributed to the comprehension of mitochondrial haplogroups origin in domestic animals. Analyses of the mtDNA have been used

to determine the history of chicken domestication (Storey et al., 2007), to assess genetic variability within and between domestic chicken populations, and to compare them with wild populations (Kanginakudru et al. 2008).

The aim of this work was to determine the genetic status of the Posavina crested hen by sequencing the mtDNA D-loop region, to investigate genetic variability within the population, and to determine the phylogenetic relationship with certain commercial chicken breeds reared in the Republic of Croatia.

Material and methods

For the purpose of mtDNA analysis of Posavina crested hens, genomic DNA was isolated from blood samples collected from the following breeds or hybrids of chickens: Red Orpington (ORC) (14), Yellow Orpington (ORZ) (10), New Hampshire (NH) (22), Red Sasso (SASC) (20), and Black and White Sasso (SASG) (20). In addition, samples were obtained from five distinct strains of the Posavina crested hen: black and white (GR) (20); golden brown (ZS) (20); light grey (SS) (21); yellow white bordered (ZBO) (20); red spotted (CS) (20).

A 592 bp fragment of the mtDNA D-loop region was amplified (Nishibori et al., 2001). The purified PCR products were sequenced and the resulting sequences were manually reviewed in FinchTV Software 1.5.0 (Geospiza Inc.). For further analysis, 13 sequences from the GR strain, 10 sequences each from the remaining Posavina crested hens (ZS, SS, ZBO and CS), 6 sequences from the NH breed, 5 from ORC, 3 from ORZ, 5 from SASC, and 6 from SASG were selected. All sequences were trimmed to equal length of 358 bp, which corresponds the positions between 15672 and 16031 of the domestic chicken reference sequence of mitochondrial genome (Genbank accession number NC_053523.1).

Haplotype alignment was performed in MEGA X software (Kumar et al., 2018). A NeighborNet network based on the aligned haplotypes was constructed in SplitsTree4 program (Huson and Bryant, 2006), version 4.14.8. Complete sequence alignments were performed in Clustal Omega (Madeira et al., 2024), exported in Nexus format and further analyzed with PopArt software (Leigh and Bryant, 2015). The software was used to infer haplotype network from the alignment data using the Median joining network method.

Results and discussion

Genetic distances between the strains were calculated in the MEGA program (version X). The lowest genetic distance (0.00893) was observed between the red spotted (CS) and light gray (SS) strains, while the highest distance (0.02541) was found between the black and white (GR) and golden brown (ZS) strains.

Further, genetic distances between Posavina crested hen strains and commercial breeds of chickens Orpington (ORC and ORZ), Sasso (SASC and SASG), and New Hampshire (NH) were calculated, shown in Table 1.

Table 1. Genetic distances of Posavina crested hen and commercial breeds of chickens.

CS	CS	GR	NH	ORC	SASC	SS	ZBO	ZS
GR	0.01483	0						
NH	0.00912	0.01793	0					
ORC	0.02103	0.02805	0.02443	0				
SASC	0.01110	0.01706	0.01369	0.02396	0			
SS	0.00894	0.01789	0.01122	0.02305	0.01261	0		
ZBO	0.01187	0.01661	0.01427	0.02599	0.01531	0.01285	0	
ZS	0.01680	0.02542	0.02008	0.02318	0.02083	0.01817	0.02234	0

The highest genetic distance (0.02805) was observed between the red strain of Orpington (ORC) and the black and white strain (GR) of Posavina crested hen. In contrast, the lowest genetic distance (0.00912) was found between the New Hampshire breed (NH) and the red spotted (CS) strain of Posavina crested hen, which could indicate a historical contribution of the New Hampshire breed to the development of the red spotted strain.

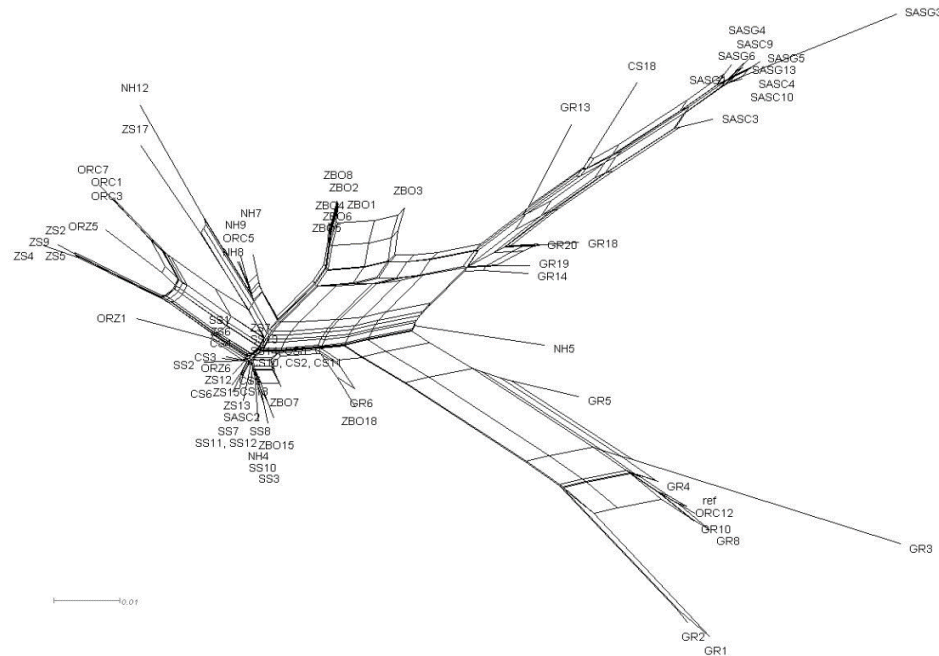


Figure 1. NeighborNet network of analyzed chicken breeds; the phylogenetic relationships of the analyzed sequences of Posavina crested hen strains and commercial chicken breeds: Orpington (ORC and ORZ), Sasso (SASC and SASG), and New Hampshire (NH). While most strains of the Posavina crested hen form clearly defined phylogenetic clades (ZBO, GR, ZS), the red-spotted (CS) and light grey (SS) strains are merged into one group, which corresponds to the low genetic distance between the two strains.

The NeighborNet network constructed in Splits Tree4 (version 4.14.6), Figure 1, shows the phylogenetic relationships among the analyzed mtDNA sequences of Posavina crested hen strains and commercial chicken breeds. While most strains of the Posavina crested hen form clearly defined phylogenetic clades (ZBO, GR, ZS), the red-spotted (CS) and light grey (SS) strains are merged into one group, which corresponds to the low genetic distance between the two strains. The GR, ZS, SS, ZBO, and CS samples are not grouped within a single cluster; instead, they are distributed across several branches of the network. GR samples are located in the lower right part of the network, mostly close to each other, though not all occupy the same branch. ZS samples are positioned on the left side of the network, relatively close to each other (particularly ZS2–ZS5–ZS9). They show signs of intra-strain relatedness but are clearly separated from GR samples, suggesting that different strains of the Posavina crested hen possess distinct mitochondrial lineages. ZBO samples are located in the upper central part of the network, between the GR and ZS clusters, but genetically closer to ZS. They exhibit moderate relatedness to other Posavina strains and may represent a transitional or intermediate group. SS samples are situated near the center of the network, within a dense area containing numerous connections. This may reflect a higher degree of recombination or heterogeneity, indicating that SS samples possess the greatest genetic diversity among all analyzed Posavina crested hen strains.

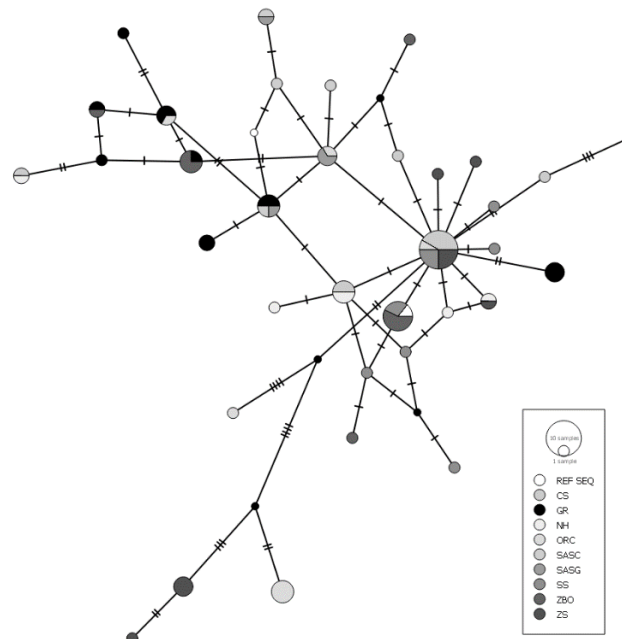


Figure 2. Median joining network; haplotypes relations between Posavina crested hen strains and commercial chicken breeds. Horizontal bars indicate the number of mutations between two adjacent haplotypes.

Among the commercial chicken breeds, Orpington and New Hampshire show the closest phylogenetic proximity to the Posavina crested hen strains, where Sasso breed chickens appear to be the most genetically distant. Nevertheless, none of the Posavina samples (GR, ZS, ZBO, SS, CS) overlap with commercial breeds, which implies that there is no introgression from commercial lines into the Posavina crested hen population, suggesting that the maternal lineage of this native breed has likely remained intact and preserved.

Haplotype analysis based on the Median joining network (Figure 2) revealed a total of 36 distinct haplotypes. Some haplotypes were found to be unique to a specific breed or strain, while others were shared across different chicken breeds. The largest haplogroup consisted of 13 sequences and was shared among three different Posavina crested hen strains (SS, CS and ZS) and Red Orpington chicken breed. Two haplotypes were shared between CS and NH, two between ZBO and SASG, and one between ZS and NH, which may indicate past introgression from commercial breeds.

Haplotype diversity of mtDNA in Posavina crested hen was found to be slightly higher than that observed in some indigenous Mediterranean chicken breeds (Ceccobelli et al, 2015), which could be due to the fact that Posavina crested hen breed consists of five phenotypically distinct strains. On the other hand, the level of variability was lower than that found in Asian chicken breeds, which are generally more genetically heterogeneous than European breeds (Cuc et al., 2011; Kanginakudru et al., 2008).

Conclusions

The analysis of the D-loop region of mtDNA showed indications of genetic structuring among strains within the population of Posavina crested hen, as well as the genetic distance of Posavina crested hen from commercial breeds. Smaller genetic distance observed between the New Hampshire and Orpington breeds and certain Posavina strains (CS and SS strain) may indicate the participation of commercial breeds in the breeding of those two strains.

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References

- Barać Z., Bedrica L.J., Čačić M., Dražić M., Dadić M., Ernoić M., Fury M., Horvath Š., Ivanković A., Janječić Z., Jeremić J., Kezić N., Marković D., Mioč B., Ozimec R., Petanjek D., Poljak F., Prpić Z., Sindičić M. (2011). Zelena knjiga izvornih pasmina Hrvatske; Ministarstvo zaštite okoliša i prirode, Državni zavod za zaštitu prirode, Hrvatska poljoprivredna agencija.
- Ceccobelli S., Di Lorenzo P., Lancioni H., Monteagudo Ibáñez L. V., Tejedor M. T., Castellini C., Landi V., Martinez Martinez A., Delgado Bermejo J.V., Vega Pla J.L., Leon Jurado J.m., Garcia N., Attard G., Grimal A., Stojanovic S., Kume K., Panella F., Weigend S., Lasagna E. (2015). Genetic diversity and phylogeographic structure of sixteen Mediterranean chicken breeds assessed with microsatellites and mitochondrial DNA. *Livestock Science*. 175: 27-36.
- Cuc N., Simianer H., Groeneveld L., Weigend S. (2011). Multiple Maternal Lineages of Vietnamese Local Chickens Inferred by Mitochondrial DNA D-loop Sequences. *Animal Bioscience*. 24(2): 155-161.
- Horvat Š. (1996): Hrvatske baštinske pasmine. Pokret prijatelja prirode „Lijepa naša“, Zagreb
- Huson D. H., Bryant D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular biology and evolution*. 23(2): 254–267.
- Kanginakudru S., Metta M., Jakati R. D., Nagaraju J. (2008). Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. *BMC evolutionary biology*. 8: 174.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*. 35: 1547-1549.
- Leigh J.W., Bryant D. (2015). PopART: Full-feature software for haplotype network construction. *Methods Ecol Evol* 6(9):1110–1116.
- Lenstra J. A., Groeneveld L. F., Eding H., Kantanen J., Williams J. L., Taberlet P., Nicolazzi, E. L., Sölkner J., Simianer H., Ciani E., Garcia J. F., Bruford M. W., Ajmone-Marsan P., Weigend S. (2012). Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. *Animal genetics*, 43(5), 483–502.
- Madeira F., Madhusoodanan N., Lee J., Eusebi A., Niewielska A., Tivey A. R. N., Lopez R., Butcher S. (2024). The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic acids research*, 52(W1), W521–W525.
- Nishibori M., Hayashi T., Tsudzuki M., Yamamoto Y., Yasue, H. (2001). Complete sequence of the Japanese quail (*Coturnix japonica*) mitochondrial genome and its genetic relationship with related species. *Animal genetics*. 32: 380-385.
- Storey, A. A., Ramírez, J. M., Quiroz, D., Burley, D. V., Addison, D. J., Walter, R., Anderson, A. J., Hunt, T. L., Athens, J. S., Huynen, L., Matisoo-Smith, E. A. (2007). Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *PNAS*. 104(25): 10335–10339.

ORIGINAL SCIENTIFIC PAPER

Physical quality parameters of brown bear (*Ursus arctos*) meat

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Summary

This study aimed to determine the pH, colour parameters, water-holding capacity and tenderness of brown bear meat and correlations between these parameters. Twelve samples of brown bear m. *semimembranosus* were collected during the 2023 autumn hunting season in the Croatian mountain region Gorski Kotar. The meat exhibited a darker coloration with a more yellow-brown hue and a less vibrant appearance, as reflected in the colour parameters: L* (28.57), a* (16.62), b* (3.82), hue angle (12.36), and chroma (16.94). The observed pH values were high (5.94), while thawing and cooking losses were 2.50% and 28.84%, respectively. The meat from the brown bear exhibited relatively high values of Warner-Bratzler Shear Force (55.30 N). A heat map analysis revealed a strong negative correlation between pH and hue ($p < 0.05$), as well as a moderate but statistically significant negative correlation between pH and thawing loss ($p < 0.05$). Further research with a larger sample size and consideration of additional factors affecting meat quality would be valuable.

Key words: *Ursus arctos*, pH, colour, water loss, meat

Introduction

Game meat has played a significant role in the human diet throughout history, and game hunting remains an important activity in many countries (Needham et al., 2023). In the last two decades, game meat has gained consumer interest due to its specific characteristics. In general, game meat is perceived as healthier due to its low fat and high protein content, better mineral composition and more favourable fatty acid composition compared to meat from domestic animals (Tomasevic et al., 2018). In addition to its nutritional value, game meat is also attractive to some consumers as a source of new exotic flavours and experiences. In Europe, especially in Central and Southern Europe, the most common game species are wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) (Needham et al., 2023). However, some species are less frequent, making their meat less available and a rare culinary experience, including beaver, brown bear or badger meat (Florek et al., 2017; Kelava Ugarković et al., 2020; Haščík et al., 2025). In 2023, the brown bear population in Croatia was estimated at around 450 animals, which are managed under the annual action plan. The hunting quota for brown bears in 2023 was set at 150 animals (Bišćan et al., 2023). Brown bear meat is traditionally consumed in Croatia in Gorski Kotar, a mountainous region that serves as a natural habitat for brown bears. The tradition of eating brown bear meat also exists in other parts of the world, e.g. in Quebec (Canada), several European countries (e.g. Romania) and certain regions of Asia (e.g. China and Siberia) (Kelava Ugarković et al., 2020). While the chemical and fatty acid composition of brown bear meat has been previously reported (Kelava Ugarković et al., 2020), the reports on its physical quality traits remain scarce. Therefore, the aim of this study was to determine the some of the physical meat quality parameters (pH, colour, thawing loss, cooking loss and tenderness) of brown bear meat from the Croatian mountain region of Gorski Kotar.

Material and methods

During the 2023 autumn hunting season for brown bear in Croatia (October 1 to December 15), twelve samples of *semimembranous* muscle weighing approximately 300 g were harvested in the Gorski Kotar region. Three samples originated from females and nine from males. Both females and males were on average 4 years old. The age was determined by analysing the first premolar teeth as described by Calvert and Ramsay (1998) and specified in the Action plan for managing brown bears in the Republic of Croatia (Bišćan et al., 2023). Meat samples were collected within 2 hours after evisceration. After collection, the samples were vacuum-packed and stored at -20°C until analysis. Prior to analysis, the frozen samples were weighed (JET 12002G/00, Mettler Toledo, Greifensee, Switzerland) and thawed on a polypropylene net in a plastic container in a refrigerator at 4 °C for 24 hours. After thawing, the samples were removed from the container, placed on a paper towel, blotted dry and weighed again. The thawing loss was calculated as follows:

$$\text{Thawing loss (\%)} = \frac{\text{weight of frozen sample} - \text{weight of thawed sample}}{\text{weight of frozen sample}} \times 100$$

Thawed muscle samples were also used to determine other physical meat quality parameters.

The pH of the meat was measured using a portable pH meter (S2-Food Kit, Mettler Toledo, Greifensee, Switzerland) and is expressed as the average of three consecutive measurements and ultimate pH value (pH_u). Meat colour was measured using a Chromameter CR-410 (Konica Minolta, Tokyo, Japan) with a measuring area of 50 mm and D65 illumination. Measurements were expressed in the CIE L*a*b* colour space. Meat colour was recorded after 45 minutes of blooming. The hue angle and chroma value were calculated as follows:

$$\text{Hue angle (}^\circ\text{)} = \tan^{-1}(b^*/a^*) \quad \text{Chroma value} = \sqrt{a^{*2} + b^{*2}}$$

Cooking loss was assessed using samples of *m. semimebranosus* approximately 50 mm in thickness and weighing 50 g, immersed in a preheated (80 °C) Sub Aqua Pro water bath (Grant Instruments Ltd., Cambridge, UK) and cooked until the internal temperature reached 75 °C. The temperature was measured using a digital thermometer. A Fantast digital meat thermometer with probe (IKEA, Sweden) was used to measure the temperature. After cooking, the samples were immersed in an ice water bath for 10 minutes and then stored in the refrigerator at +4 °C until completely cooled. The samples were then removed from the bags, blotted dry with paper towels and weighed. The cooking loss was calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{weight of sample before cooking} - \text{weight of sample after cooking}}{\text{weight of sample before cooking}} \times 100$$

Meat tenderness was determined using TA-XT Plus texture analyser (Stable Micro Systems Ltd., Godalming, UK). Samples used for cooking loss determination were also used for this analysis. Shear force was measured as the average of at least three subsamples on meat cores (1 cm diameter).

Statistical analysis was performed using SAS 9.4 (2013). The correlation heatmap was made in RStudio and expressed as Pearson correlation coefficients with significance $p < 0.05$, $p < 0.001$ and $p < 0.0001$.

Results and discussion

Descriptive statistics for pH, colour parameters, water holding capacity, and tenderness of brown bear meat are provided in Table 1. During *postmortem* changes in muscle, anaerobic glycolysis occurs, resulting in a formation of lactic acid and a consequent decrease of muscle pH (Chauhan and England, 2018). The extent and rate of the pH decline are important factors for evaluation of meat quality (Wang et al., 2021). The ultimate pH of meat depends primarily on the glycogen content in the muscle. The optimal ultimate pH of meat originating from

domestic meat animals is between 5.5 and 5.7. Values below or above optimal pH are attributed to acute or chronic *antemortem* stress events and glycogen depletion leading to undesirable meat characteristics (Carrasco-García et al., 2020). The pH of the meat depends on numerous factors, such as age, season, muscle fibre type, anatomical location of the muscle together with their interactions (Mo et al., 2023; Stanisiz et al., 2019).

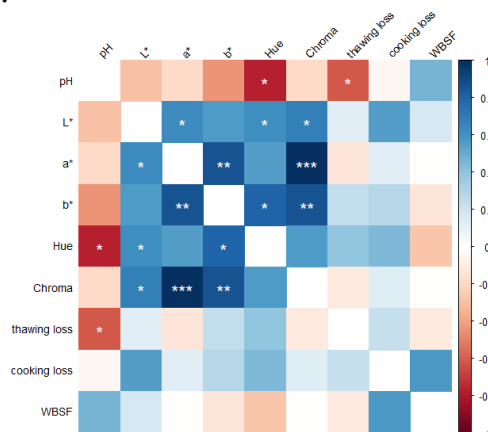
Table 1. Physical quality parameters of brown bear (*Ursus arctos*) meat

Parameter	Mean	SD	Median	Min	Max
pH	5.94	0.07	5.95	5.85	6.11
L*	28.57	2.20	28.74	25.09	33.05
a*	16.62	2.32	16.71	13.14	19.53
b*	3.82	1.14	4.04	2.34	5.36
Hue angle (°)	12.36	0.06	13.34	9.63	17.69
Chroma	16.94	2.57	17.20	13.13	19.94
Thawing loss (%)	2.50	2.20	1.67	0.32	7.31
Cooking loss (%)	28.84	10.11	29.18	14.04	44.31
WBSF (N)	55.30	21.42	49.32	28.56	89.07

SD= standard deviation; L*=lightness; a*=redness; b*=yellowness; WBSF = Warner Bratzler shear force

Brown bear meat has a higher pH value than livestock meat, but the evaluation of physical meat parameters for game meat cannot be the same as for domestic animals. Namely, game meat may have higher initial and final pH values than domestic animals due to different muscle fibre types (dominant slow oxidative vs. fast glycolytic) and lower glycogen content due to more intense movement (Samples et al., 2023). Higher ultimate pH values were reported for wild boar meat by Fabijanić et al. (2020), Palazzo et al. (2021) and Altissimi et al. (2023), and for large herbivorous ungulates by Calitz et al. (2020). However, it should be noted that in some of these studies fresh meat samples were analysed and in others frozen meat samples, which could have influenced the ultimate pH value. The determined L* value is lower than those reported for wild boar by Palazzo et al. (2021), Altissimi et al. (2023) and Tomljanović et al. (2022), with similar a* and especially lower b* values than those reported by the same authors. The meat of large game species is generally darker and has lower values for the colour parameters, which is due to higher myoglobin and iron content, abundant red muscle fibres and intense physical activity (Calitz et al., 2020). A more precise assessment of meat color, particularly redness intensity and saturation, can be achieved using hue and chroma values instead of L*, a*, and b*. Lower values of these parameters indicate a more yellow-brown appearance and less bright red meat (Lindahl et al., 2001). In the present study, very low hue and chroma values were found, indicating less bright red and more brown-yellow and dark meat. Altissimi et al. (2023) and Serrano et al. (2020) reported higher hue and chroma values for wild boar (>60 and around 20, respectively) and red deer meat (around 40 and 20, respectively). The hue value showed a strong negative correlation ($p < 0.05$) with the pH value (Figure 1). Results presented in Table 1 showed low thawing losses of brown bear meat, a similar value was determined by Calitz et al. (2020) and Laubscher (2009) for the five most common large African herbivorous ungulates, while Cifuni et al. (2014) determined higher values for fallow deer and wild boar. Cooking losses were similar to the results reported by Cifuni et al. (2014), but lower than those reported by Calitz et al. (2020) and Laubscher (2009). The thawing loss showed a moderate negative correlation ($p < 0.05$) with pH (Figure 1). This corresponds to previously reported correlations for kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*) meat, i.e. higher ultimate pH results in less water released from meat

(Laubscher, 2009). According to the meat tenderness scale for beef *longissimus dorsi* (Martinez et al., 2023), brown bear meat is tough (WBSF > 40 N). The WBSF of brown bear meat appears to be higher than that reported by Cifuni et al. (2014), Calitz et al. (2020) and Laubscher (2009) for wild ungulates. Although no correlation between ultimate pH and tenderness was found in the present study, it has been previously reported that higher ultimate pH can lead to permanent association of actin and myosin filaments, i.e. muscle shortening and reduced tenderness (Laubscher, 2009). It seems that the quality of game meat should not be judged and interpreted according to the same criteria as that of meat from domestic animals. In particular, the muscle structure and postmortem biochemistry of game meat appear to be different (Neethling et al., 2017), suggesting the need to develop specific criteria for assessing the quality of game meat (acceptable pH and colour).



* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$; WBSF=Warner Bratzler shear force

Figure 1. Correlation heatmap of brown bear physical meat parameters

Conclusions

Brown bear meat is part of the traditional cuisine in Croatia's mountain region and has not yet been studied in detail. Compared to the meat of domestic animals, brown bear meat has a higher ultimate pH, a darker, yellow-brown appearance, and reduced tenderness. This study provides one of the first insights into the physical quality characteristics of brown bear meat. Further research, including a larger sample size and different factors affecting these parameters, would be beneficial.

Acknowledgement

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References

- Altissimi C., Torregiani E., Cambiotti F. (2023). Wild boar captured in a large corral-style trap or hunted: preliminary comparison of meat quality traits. *Italian Journal of Food Safety* 12:4.
- Bišćan A., Budor I., Domazetović Z., Gospočić S., Grubešić M., Huber Đ., Jakšić Z., Jeremić J., Reljić S., Sindičić M., Šprem N., Žuglić T. (2023). Akcijski plan gospodarenja s medvjedom u Republici Hrvatskoj u 2023. godini. Ministarstvo poljoprivrede, Uprava šumarstva, lovstva i drvne industrije; Ministarstvo gospodarstva i održivog razvoja, Uprava za zaštitu prirode.
- Calitz K.J. (2020). Culling-associated stress and meat quality in ungulates. Master thesis. Stellenbosch University, South Africa. Available from: <https://scholar.sun.ac.za/items/a65f9cd5-5793-40b3-b7d7-8e1d7ccdb558>

- Calvert W., Ramsay M.A. (1998). Evaluation of Age Determination of Polar Bears by Counts of Cementum Growth Layer Groups. *Ursus* 10: 449–453.
- Carrasco-García A.A., Pardío-Sedas V.T., León-Banda G.G., Ahuja-Aguirre C., Paredes-Ramos P., Hernández-Cruz B.C., Murillo V.V. (2020). Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. *Asian-Australasian Journal of Animal Sciences*. 33(10):1656-1665.
- Cifuni G.F., Amici A., Contò M., Viola P., Failla S. (2014). Effects of the hunting method on meat quality from fallow deer and wild boar and preliminary studies for predicting lipid oxidation using visible reflectance spectra. *European Journal of Wildlife Research* 60: 519–526.
- Chauhan S.S., England E.M. (2018). Postmortem glycolysis and glycogenolysis: insights from species comparisons. *Meat Science*. 144:118-126.
- Fabijanić N., Šprem N., Konjević D., Kelava Ugarković N. (2020). Effect of sex on physical characteristics of wild boar meat. *Arhiv za higijenu rada i toksikologiju* 71 (1): 23.
- Florek M., Drozd L., Skąlecki P., Domaradzki P., Litwińczuk A., Tajchman K. (2017). Proximate composition and physicochemical properties of European beaver (*Castor fiber* L.) meat. *Meat Science*. 123: 8-12.
- Haščík P., Čech M., Bobko M., Korčok M., Jurčaga L. (2025). European badger (*Meles meles*) – potential novelty enhancement for meat products. *Future Foods* 11:100573.
- Hughe, J.M., Oiseth S.K., Purslow P.P., Warner R.D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*. 98:520–532.
- Laubscher L.L. (2009). The effect of different cropping methods on the meat quality of various game species. Master thesis. Stellenbosch University, South Africa.
- Lindahl G., Lundström K., Tornberg E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science*. 59:141–151.
- Kelava Ugarković N., Konjačić M., Malnar J., Tomljanović K., Šprem N., Ugarković D. (2020). Proximate Chemical Composition, Fatty Acid Profile, and Lipid Qualitative Indices of Brown Bear Meat. *Foods*. 10(1): 36.
- Martinez H.A., Miller R.K., Kerth C., Wasser B.E. (2023). Prediction of beef tenderness and juiciness using consumer and descriptive sensory attributes. *Meat Science* 109:292.
- Mo M., Zhang Z., Wang X., Shen W., Zhang L., Lin S. (2023). Molecular mechanisms underlying the impact of muscle fiber types on meat quality in livestock and poultry. *Frontiers in Veterinary Science* 10:1284551.
- Needham T., Bureš D., Černý J., Hoffman L.C. (2023). Overview of game meat utilisation challenges and opportunities: A European perspective. *Meat Science* 204:109284.
- Neethling N.E., Suman S.P., Sigge G.O., Hoffman L.C., Hunt M.C. (2017). Exogenous and Endogenous Factors Influencing Color of Fresh Meat from Ungulates. *Meat and Muscle Biology*. 1(1).
- Palazzo M., Tavaniello S., Petrecca V., Zejnelhoxha S., Wu M., Mucci R., Maiorano G. (2021). Quality and safety of meat from wild boar hunted in Molise region. *Italian Journal of Animal Science* 20(1): 1889-1898.
- RStudio Team (2019). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Available from: <http://www.rstudio.com/>
- Sampels S., Jonsson M., Sandgren M., Karlsson A., Segerkvist K.A. (2023). Sustainable Delicacy: Variation in Quality and Sensory Aspects in Wild Boar (*Sus scrofa*) Meat and Comparison to Pork Meat—A Case Study. *Foods*. 12(8): 1644.
- SAS Institute Inc. (2013) SAS OnlineDoc®, Version 9.4. SAS Institute Inc.

- Stanisz M., Ludwiczak A., Składanowska-Baryza J., Bykowska-Maciejewska M. (2019). The effect of age and ultimate pH value on selected quality traits of meat from wild boar. *Canadian Journal of Animal Science*. 99(2): 336-342.
- Serrano M.P., Maggolino A., Landete-Castillejos T., Pateiro M., Pérez Barbería J., Fierro Y., Domínguez R., Gallego L., García A., De Palo P., Lorenzo J.M. (2020). Quality of main types of hunted red deer meat obtained in Spain compared to farmed venison from New Zealand. *Scientific Reports*. 10:12157.
- Tomasevic I., Novakovic S., Solowiej B., Zdolec N., Skunca D., Krocko M., Nedomova S., Kolaj R., Aleksiev G., Djekic I. (2018). Consumer perceptions, attitudes and perceived quality of game meat in ten European countries. *Meat Science*. 142: 5–13.
- Tomljanović K., Grubešić M., Medić H., Potočnik H., Topolovčan T., Kelava Ugarković N., Marušić Radovčić N. (2022). The Impact of Premortality Stress on Some Quality Parameters of Roe Deer, Wild Boar, and Red Deer Meat. *Foods* 11(9):1275.
- Wang C., Matarneh S.K., Gerrard, D., Tan J. (2021). Modelling of energy metabolism and analysis of pH variations in postmortem muscle. *Meat Science*. 182: 108634.

ORIGINAL SCIENTIFIC PAPER

Fatty acid profile and lipid quality indices in wild hare (*Lepus europaeus*) meat

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*Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia (zlata.kralik@fazos.hr)***Summary**

The wild hare (*Lepus europaeus*) is an inhabitant of most hunting grounds in the Republic of Croatia. It is a game species with a high potential for the production of nutritionally valuable meat. The aim of the study was to analyze the fatty acid profile in the hind leg meat of wild hares and to calculate the qualitative and nutritional indices. The hares used for the study were hunted in the fall of 2023 in the area of the common hunting ground XIV/128, which is managed by the Jarebica Hunting Society, Antunovac. For the fatty acid analysis and index calculation, hind leg meat samples were taken from 15 male individuals after the hunt. The results of the fatty acid profile analysis show that the content of total saturated fatty acids (SFA) was 32.53%, monounsaturated fatty acids (MUFA) 14.06%, $\sum$ n-6 polyunsaturated fatty acids (n-6 PUFA) 44.02% and $\sum$ n-3 polyunsaturated fatty acids (n-3 PUFA) 9.39%. The qualitative lipid indices show the ratio $\sum$ PUFA/ $\sum$ SFA, which was 1.64 and the ratio $\sum$ n-6 PUFA/n-3 PUFA, which was 4.68. The nutritional indices calculated from the fatty acid data are: NVI (nutrition value index) = 1.31; AI (atherogenicity index) = 0.499; TI (thrombogenicity index) = 0.556 and HHI (hypo/hypercholesterolemic index) = 3.48. Wild hare hind leg meat is a good source of nutritionally valuable fats and can be a healthier alternative to other meats that people consume.

Keywords: wild hare; fatty acid profile; lipid quality indices**Introduction**

Wild hare (*Lepus europaeus*) meat has a high nutritional value and is distinguished by its characteristic texture, taste and color, which are different from those of big game and feathered game (Kumar et al., 2023). Wild hare meat differs in quality from meat of various domestic animals and also from farmed rabbit (*Oryctolagus cuniculus*). Unlike farmed rabbit, the availability of wild hare meat on the market is limited by the hunting season. Wild hare meat is reported to have a lower fat content than farmed rabbit meat (Cambero et al., 1991). Cobos et al. (1995) state that the protein content in the muscles of the hind leg of the wild hare averages 21.43% and the fat content is 2.45%. The same authors state that the linoleic fatty acid (LA) content in the hind leg of the hare is 23.41% and the α -linolenic acid (ALA) content is 8.90%. Rasinska et al. (2018) state that the protein content in the muscles of the hind legs of rabbits is 21.73% and the fat content is 3.02%. Dalle Zotte (2002) states that hare meat has a very low fat and cholesterol content, a high content of protein with essential amino acids, is rich in n-3 polyunsaturated fatty acids (n-3 PUFA) and is a significant source of B vitamins (B2, B3, B5, B6, B12) and minerals (phosphorus and selenium). Scientific evidence suggests that foods high in saturated fat and cholesterol are associated with increased levels of low-density lipoprotein (LDL) in the blood. This condition can lead to cardiovascular diseases (Hu et al., 2002). Forrester-Anderson et al. (2006) point out that due to its nutritional value, wild hare meat is better for consumption than chicken, pork, beef and lamb. The aim of the study was to analyze the fatty acid profile of wild hare hind leg meat and, based on the fatty acid profile, calculate lipid qualitative indices. The obtained data enable the assessment of the nutritional value of wild hare hind leg meat.

Material and methods

The collection of wild hare carcasses was carried out after the hunt in November 2023 in the common hunting area number XIV/128. The hunt was conducted by hunters from the hunting society "Jarebica" from Antunovac. A total of 15 hare carcasses were sampled, which, after skinning and evisceration of the intestines, were placed in refrigerated plastic boxes and transported to the Laboratory for the Quality of Animal Products at the Faculty of Agrobiotechnical Sciences Osijek. In the laboratory, the hare carcasses were cut into basic parts. A sample of the *Semimembranosus* muscle was taken from the hind leg and used for further analysis of the fatty acid profile. After sampling in the hunting grounds, the samples were placed in liquid nitrogen at -196°C and transported to the laboratory where they were transferred to a freezer at -80°C until further analysis. To determine the fatty acid profile, the samples were processed in a MARS 6 microwave device (CEM Corporation, Matthews, NC, USA) and the fatty acid profile was determined using a gas chromatograph (SCION 436-GC (SCION Instruments, Goes, The Netherlands)). The proportions of the individual fatty acids are expressed as a percentage of the total fatty acids in the lipids. Lipid quality indices (qualitative and nutritional) were calculated based on the results of the analysis of the fatty acid profile in the meat of the hind legs of wild hares. In his paper, Simopoulos (2008) provides an index for assessing the effects of the fatty acid profile of foods on cardiovascular health. He used the following formula for the calculation: $\text{PUFA/SFA} = (\sum \text{PUFA} / \sum \text{SFA})$. In addition to the above index, the index for the ratio $\sum \text{n-6 PUFA} / \sum \text{n-3 PUFA}$ is calculated (Das, 2006). The following formula is used to calculate this index: $\sum \text{n-6 PUFA} / \sum \text{n-3 PUFA} = (\text{C18:2n-6} + \text{C20:2n-6} + \text{C20:3n-6} + \text{C20:4n-6}) / (\text{C18:3n-3} + \text{C20:3n-3} + \text{C20:5n-3} + \text{C22:5n-3} + \text{C22:6n-3})$. Chen et al. (2016) developed a formula to calculate the nutritional value index $\text{NVI} = (\text{C18:0} + \text{C18:1n9}) / (\text{C16:0})$. According to the formulas provided by Omri et al. (2019), the atherogenic (AI) and thrombogenic (TI) indices are calculated: $\text{AI} = (\text{C:12:0} + 4 * \text{C14:0} + \text{C16:0} + \text{C18:0}) / (\text{UFA})$, $\text{TI} = (\text{C14:0} + \text{C16:0} + \text{C18:0}) / [(0.5 * \sum \text{MUFA}) + (0.5 * \sum \text{n-6 PUFA}) + (3 * \sum \text{n-3 PUFA}) + (\sum \text{n-3 PUFA} / \sum \text{n-6 PUFA})]$. Lower AI and TI values indicate better nutritional properties of the meat. Santos-Silva et al (2002) developed a formula to calculate the Hypo/Hypercholesterol Index. $\text{HHI} = (\text{C18:1n-9} + \sum \text{PUFA}) / (\text{C14:0} + \text{C16:0})$. The results obtained in the study were processed with the software package TIBCO Statistica® 14.0.0. (TIBCO Software Inc. 2020). The results are presented in tables in the form of descriptive statistics.

Results and discussion

Table 1 shows the profile of fatty acids in the meat of the hind leg of a wild hare. The results show that the total SFA content was 32.53% and the MUFA content was 14.06%. The most abundant saturated fatty acid was palmitic acid (17.23%) and of the monounsaturated fatty acids, oleic acid (8.47%). The most abundant n-6 PUFA fatty acid was linoleic acid (31.95%), followed by arachidonic acid at 10.83%. Overall, the proportion of n-6 PUFA fatty acids in wild hare leg meat was 44.02%. Of the n-3 PUFAs in thigh meat, α -linolenic acid (6.61%) and eicosapentaenoic acid (1.70%) were the most abundant, while the total n-3 PUFA content was 9.54%. Cobos et al. (1995) found that the linoleic acid content in the hind leg of a wild hare ranged from 17.94% to 27.48%, with an average of 23.41%. The arachidonic fatty acid content was 6.42%. They also state that the α -linolenic acid content ranged from 3.97% to 15.82%, with an average of 8.90%, and the eicosapentaenoic acid content was 0.72%. Their results are not consistent with ours, as the content of linoleic, arachidonic and eicosapentaenoic acids in their research is lower and the content of α -linolenic acid is higher compared to our results. Table 2 shows the values of lipid quality indicators in hare hind leg meat, which are an indicator of the health quality of lipids in the meat. The $\sum \text{PUFA} / \sum \text{SFA}$ ratio ranged from 1.50% to 1.80%, with an average of 1.65%, while the $\sum \text{n-6 PUFA} / \sum \text{n-3 PUFA}$ ratio ranged from 2.72% to 8.81%,

with an average of 4.61%. Mariamenatu and Abdu (2021) state that the ratio of PUFA n-6/PUFA n-3 in foods that people consume daily should be 4-5/1.

Table 1. Profile of fatty acids in wild hare (*Lepus europaeus*) hind leg meat (% of total fatty acids)

Fatty acid	min.	max.	$\bar{x}$	sd
Myristic (C14:0)	0.87	0.35	0.62	0.15
Pentadecanoic (C15:0)	0.13	0.25	0.18	0.04
Palmitic (C16:0)	15.5	18.83	17.23	0.96
Heptadecanoic (C17:0)	0.41	0.64	0.48	0.07
Stearic (C18:0)	12.87	15.41	14.02	0.82
ΣSFA	31.47	33.78	32.53	0.88
Palmitoleic (C16:1)	1.67	4.41	2.94	0.78
Heptadecenoic (C17:1)	2.23	2.99	2.65	0.18
Oleic (C18:1n9)	6.31	10.87	8.47	1.24
ΣMUFA	11.54	16.61	14.06	1.55
Linoleic (C18:2n6)	26.77	36.95	31.95	3.71
Eicosadienoic (C20:2n6)	0.32	0.48	0.43	0.04
Dihomo-gamma-linolenic (C20:3n-6)	0.64	1.07	0.81	0.12
Arachidonic (C20:4n6)	9.11	13.42	10.83	1.02
Σn-6 PUFA	38.47	50.84	44.02	4.26
α -Linolenic (C18:3n3)	3.42	9.81	6.41	2.08
Eicosatrienoic (C20:3n-3)	0.29	0.69	0.41	0.12
Eicosapentaenoic (C20:5n3)	0.97	3.32	1.70	0.82
Docosahexaenoic (C22:6n3)	0.65	1.06	0.82	0.11
Σn-3 PUFA	5.77	14.44	9.39	2.92

According to data reported in the literature and our results, the ratio of total PUFA n-6/PUFA n-3 in wild hare hind leg meat is favorable, as it fits within the values recommended by nutritionists.

Table 2. Health quality indicators of lipids in wild hare (*Lepus europaeus*) hind leg meat

Index		min.	max.	$\bar{x}$	sd
Qualitative	Σ PUFA/ Σ SFA	1.50	1.80	1.64	0.10
	Σ n-6 PUFA/ Σ n-3 PUFA	2.72	8.81	4.68	1.88
Nutritional	NVI	1.19	1.42	1.31	0.06
	AI	0.467	0.533	0.499	0.02
	TI	0.470	0.634	0.556	0.05
	HHI	3.10	4.01	3.48	0.27

NVI=nutritional value index; AI=atherogenic index; TI=thrombogenic index; HHI=hypo/hypercholesterol index

Simopoulos (2008) provides a calculation for the qualitative index Σ PUFA/ Σ SFA assuming that total PUFA can influence the reduction of low-density cholesterol and serum cholesterol, while total SFA can contribute to the increase of serum cholesterol. Therefore, this is an index whose higher value indicates that the food a person consumes has a better effect on health. Frunză et al. (2023) report the value of the Σ PUFA/ Σ SFA index in wild hare hind leg meat as 1.61. Their results are consistent with ours. The aforementioned authors report the values of NVI, AI, TI and HHI for wild hare hind leg meat as follows: 1.36, 0.72, 0.62 and 3.29. The values of NVI and HHI are consistent with ours, while the values of AI and TI in their study are somewhat higher.

Conclusions

According to the results of the fatty acid profile, qualitative and nutritional indices for assessing the nutritional value of food, we can point out that the meat of the wild hare hind leg from the common hunting ground number XIV/128, which we examined in this research, provides a favorable fatty acid composition for human consumption. Despite this, in most countries, including ours, wild hare is grown in hunting grounds mostly for hunting purposes. Future research could focus on analyzing the fatty acid profile of wild hare meat, including samples from different regions and hunting grounds, to determine potential variations in lipid quality indices.

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References

- Cambero M. I., de la Hoz L., Sanz B., Ordóñez J. A. (1991). Seasonal variations in lipid composition of Spanish wild rabbit (*Oryctolagus cuniculus*) meat. *Journal of the Science of Food and Agriculture*. 56(3): 351-362.
- Chen Y., Qiao Y., Xiao Y., Chen H., Zhao L., Huang M., Zhou G. (2016). Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers, and spent hens. *Asian-Australas. J. Anim. Sci.* 29(6): 855–864.
- Cobos A., de la Hoz L., Cambero M. I., Ordoñez J.A. (1995). Chemical and fatty acid composition of meat from Spanish wild rabbits and hares. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 200 (3): 182-185.
- Dalle Zotte A. (2002). Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock production science*, 75(1): 11-32.
- Das U.N. (2006). Essential fatty acids: Biochemistry, physiology and pathology. *Biotechnol. J.* 1: 420–439.
- Forrester-Anderson I.T., McNitt J., Way, R., Way, M. (2006). Fatty acid content of pasture-reared fryer rabbit meat. *Journal of Food and Analysis*, 19 (6-7): 715–719.
- Frunză G., Murariu O. C., Ciobanu M. M., Radu-Rusu R. M., Simeanu D., Boișteanu P. C. (2023). Meat quality in rabbit (*Oryctolagus cuniculus*) and hare (*Lepus europaeus pallas*)—A nutritional and technological perspective. *Agriculture*. 13(1), 126.
- Hu F.B., Bronner L., Willett W.C., Stampfer M.J., Rexrode K.M., Albert C.M., Hunter D., Manson J.E. (2002). Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *Jama*. 287(14): 1815-1821.
- Kumar S.A., Kim H.J., Jayasena D.D., Jo C. (2023). On-farm and processing factors affecting rabbit carcass and meat quality attributes. *Food Science of Animal Resources*. 43(2): 197-219.
- Mariamenatu A. H., Abdu E. M. (2021). Overconsumption of Omega-6 polyunsaturated fatty acids (PUFAs) versus deficiency of Omega-3 PUFAs in modern-day diets: the disturbing factor for their “balanced antagonistic metabolic functions” in the human body. *Journal of lipids*. 1: 8848161.
- Omri B., Chalghoumi R., Izzo L., Ritieni A., Lucarini M., Durazzo A., Abdouli H., Santini A. (2019). Effect of dietary incorporation of linseed alone or together with tomato-red pepper mix on laying hens’ egg yolk fatty acids profile and health lipid indexes. *Nutrients*. 11(4): 813.
- Rasinska E., Czarniecka-Skubina E., Rutkowska J. (2018). Fatty acid and lipid contents differentiation in cuts of rabbit meat. *CyTA-Journal of Food*, 16(1): 807-813.

- Santos-Silva J., Bessa R.J.B., Santos-Silva F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science*. 77 (2-3): 187–194.
- Simopoulos A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med.*, 233: 674–688.
- TIBCO® Data Sciences Workbench version 14.0.0.15 (©1984-2020 Tibco Software Inc.)

REVIEW ARTICLE

Bihevioral activity data for improving production of dairy cattle

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Summary

Monitoring and timely control of physiological processes in cows directly affect production and reproduction. Intensive farming has driven the development of monitoring systems for precise herd management. These systems vary in terms of sensor type, functionality, purpose, and the quality of processed data. Sensors enable monitoring of key activities like illness and estrus detection, rumination, feeding, group behavior, and calving prediction. The widespread use of pedometers has enabled research, improving data control and breeding programs. Besides reproductive and production data, selection requires genetic information. Integrating pedometer and genetic data supports innovative concepts that boost production efficiency and sustainability.

Key words: sensors, pedometers, dairy cattle, reproduction, production,

Introduction

Dairy production involves a range of technological processes that connect humans, cattle, and complex automated systems. Production parameters such as milk yield, fat and protein content, as well as the reproduction rate depend on the individual cow performance. Optimal nutrition, veterinary care, quality of housing conditions, and effective management are essential for maintaining productivity. In addition to providing optimal production conditions, monitoring animal behavior becomes more important factor in intensive farming systems. In these systems, cows undergo various physiological processes that must be promptly recognized and managed, posing significant challenges. The development of cow monitoring systems has enhanced efficiency in managing production and reproductive data. These systems vary in terms of group or individual approaches, as well as the purpose of the devices depending on targeted traits. Automated monitoring models consist of one or multiple devices, a data storage system, and software for interpreting the results. Devices can be designed as leg or neck collars, or ear or neck sensors, although applications to other body parts also exist but are less common. The advantages of these systems are numerous including individual or group monitoring, objective estrus detection and disease identification, improved synchronization of insemination, as well as the availability and storage of large amounts of data. Monitoring cow activity through tracking systems provides detailed insights into individual variability in traits measurable through these technologies. This variability can be the result of various factors, including genetic differences among animals, environmental influences, or their interaction. In addition to monitoring bihevioral activity variation, studying the genetic structure of these traits raised interest in scientific research. Breeding programs are based on genetic parameters such as heritability and genetic correlations mainly for economically important traits (Oliveira Junior et al., 2021). Identifying specific genomic sequences associated with bihevioral activity traits

represents a novel approach in modern livestock breeding, enabling the long-term optimization of production systems.

Systems for monitoring and tracking cows

Monitoring and tracking systems include technological solutions that provide continuous access to information on cow health, nutrition, reproduction, and overall status. Current tracking systems usually include: 1) individual sensors or a combination of sensors placed on each cow, including magnetometers, accelerometers, gyroscopes, compasses, pressure sensors, microphones, and global positioning systems (GPS); 2) devices for processing, storing, and distributing data collected by the sensors; 3) models or combinations of models that interpret animal behavior based on raw sensor data (Rahman et al., 2018). These monitoring systems have the ability to accurately track all processes related to production and reproduction. Nowadays, various sensors are available for classifying animal behavior, such as walking, ruminating, grazing, and resting (Neethirajan, 2020). The use of wearable sensors for cattle behavior is becoming an increasingly important tool in farm management and recently in breeding programs. Unlike traditional herd-based approaches, these technologies enable a stronger focus on improving individual welfare and efficiency (Rahman et al., 2018). Sensors are linked to the animal's identification number (ID) allowing collected data to be archived and analyzed in herd management software. Sensors can track various activities including detecting sick cows and estrus, monitoring rumination and feeding, analyzing group behavior, and predicting the onset of calving (Marques et al., 2024). Sensors can be placed on different parts of the animals, while the relevance and quality of the data depend on sensor type, control conditions, and manufacturer (Rahman et al., 2018). A study by Holman et al. (2011) suggests that activity monitoring devices placed on the leg can detect estrus more reliably than those mounted on neck collars. However, in grazing conditions, neck-mounted sensors have been shown to effectively identify estrus. Accelerometers are among the key devices used for monitoring cow activity. These sensors belong to the category of microelectromechanical (MEMS) systems and measure movement-based activity (Neethirajan and Kemp, 2021). One example is the SensOoR 3D accelerometer (Agis Innovations), which was developed to be attached to ear tags on cattle, allowing precise tracking of their activities. The sensor measures movements along three axes, enabling monitoring of head position and movement (Martiskainen et al., 2009). The system processes sensor data and classifies behavior into four categories: resting, activity, eating, and ruminating. This classification system is based on recognizing specific movement patterns, providing insights into the activity and behavior of animals, allowing more efficient monitoring and management (Bikker et al., 2014). According to the results of Bikker et al. (2014), it can be concluded that ear-mounted sensors can provide relevant data on rumination and resting patterns in dairy cows housed in free-stall barns. Ear sensors have microphones that can track the sounds of chewing and swallowing. Movements from the facial and neck muscles are transferred to the ears, allowing the sensors to detect micro-movements through algorithms designed to recognize rumination patterns. Additionally, these sensors show potential for monitoring feeding behavior in intensive commercial systems, though further research is needed to validate their effectiveness (Bikker et al., 2014). In addition to being attached to the ears, different types of accelerometers can be placed on the neck or legs, where they effectively track behavior with high sensitivity (Morrone et al., 2022). Cow activity and behavior can also be monitored using video and infrared cameras, which rely on temperature-sensing technology (Perez Marquez et al., 2019). However, a major challenge for monitoring systems in intensive cattle farms is the limited space available for movement, which reduces the effectiveness of pedometry (Palmer et al., 2010). Data sets generated through precision livestock farming, incorporating activity monitoring systems such as pedometers, accelerometers, thermal cameras, and video surveillance in combination with the development

of data science, are crucial for demonstrating animal welfare and improving genomic breeding values (Brito et al., 2020).

Implementation of digital systems in dairy cattle farming

Cattle farming systems present a specific challenge for farmers, as each individual animal represents a high-value investment. Overall production efficiency is influenced by a wide range of factors, which in turn affect herd profitability (Neethirajan Kemp, 2021.). In livestock sector, behavior analysis provides valuable insights into: 1) animal health; 2) food intake and satiety status; 3) heat periods and estrus occurrence (Rahman et al., 2018). Health status of the animal is usually determined by the animal behavior patterns (Shahriar et al., 2016). Behaviors such as feeding are indicators of feed consumption. The percentage of time spent in feeding related behaviors can be useful for understanding the relationship between the amount of feed intake and the availability of pasture, as well as for assessing animal preferences and their level of satiety (Greenwood et al., 2016). The periods of heat and signs of estrus can be detected through changes in activity, where the animal becomes restless. Identifying the estrus period indicates the optimal time for artificial insemination (Rahman et al., 2018). The identification of estrus is crucial in assessing the reproductive efficiency of dairy cows (Wang et al., 2020). Incorrect estrus recognition and false positive results in estrus detection can lead to missed opportunities or inaccurately timed artificial inseminations. The outcome of such decisions is poor reproduction, causing economic losses on dairy farms (Perez Marquez et al., 2019). Estrus is influenced by a large number of factors that affect its intensity and duration, leading to variation within the herd (Reith and Hoy, 2018). The ability to monitor the timing of fertile cycles in real-time is essential for effective herd management, while precise nutritional control plays a key role in maximizing milk production (Neethirajan and Kemp, 2021). Automated activity monitoring system in estrus detection vary in the variables they analyze, including step count, accelerated movement, rumination frequency, and lying duration (Morrone et al., 2022). Pedometers have proven to be an effective tool for estrus detection and are considered a promising method for predicting ovulation, ultimately improving fertility rates (Wang et al., 2020). In an experiment conducted by Valenza et al. (2012), a neck-mounted device successfully detected approximately 71% of pre-ovulatory phases, missing a total of 13% of recorded ovulations. Using the same devices, Aungier et al. (2012) detected 72% of pre-ovulatory follicular phases, with the remaining results classified as false positives. Cows are highly sensitive animals, and in intensive farming systems, diseases often arise that negatively impact production. Another significant issue in dairy cattle farming is lameness, which is widely recognized as a major concern. Clinical lameness leads to financial losses for farmers due to reduced production, delayed estrus, and various indirect costs. Early detection and subsequent treatment shorten the time needed for complete recovery and return to production, thereby reducing financial losses for the farmer (Mazrier et al., 2006). Most cases of lameness related to the hoof, excluding trauma-induced injuries, result from subclinical laminitis, which includes conditions such as abscesses and double sole (Van Nuffel et al., 2016). Pedometers, along with other monitoring systems, are commonly used to detect lameness in dairy and beef cattle. In a study conducted by Morrone et al. (2022), seven variables were used to diagnose lameness: walking speed, beats per minute, milk yield, lactation number, milk flow rate, and milk electrical conductivity. Feeding and rumination are among the most important activities for ruminants, as these behaviors occupy most of their daily routine. Monitoring these activities provides a more accurate insight into animal health and productivity.

Conclusion

Precision livestock farming systems have developed models for monitoring and tracking animal activity. These models are capable of tracking major physiological activities and behaviors in real time. Data collected from automated devices are transmitted to specialized programs for behavior and activity interpretation. Tracking models vary in terms of sensor type, functionality, communication, and attachment method. Pedometer devices, due to their effectiveness in monitoring production and reproductive processes, have significantly contributed to advancements in livestock technology. However, a major drawback of these systems is the high initial investment cost and expensive maintenance. Modern breeding programs require not only technological solutions but also the integration of genetic data. Combining information from monitoring systems with genetic data from genotyped individuals enhances breeding selection programs, creating a more precise and efficient approach to livestock management.

References

- Aungier, S.P.M., Roche, J.F., Sheehy, M., Crowe, M.A. (2012). Effects of management and health on the use of activity monitoring for estrus detection in dairy cows. *Journal of Dairy Science*, 95(5): 2452-66.
- Bikker, J.P., van Laar, H., Rump, P., Doorenbos, J., van Meurs, K., Griffioen, G.M., Dijkstra, J. (2014). Technical Note: Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. *Journal of Dairy Science*, 97(5): 2974-9.
- Brito, L.F., Oliveira, H.R., McConn, B.R., Schinckel, A.P., Arrazola, A., Marchant-Forde, J.N., Johnson, J.S. (2020). Large-Scale Phenotyping of Livestock Welfare in Commercial Production Systems: A New Frontier in Animal Breeding. *Frontiers in Genetics*, 31, 11:793.
- Greenwood, P., Bishop-Hurley, G., Gonzalez, L.A., Ingham, A.B. (2016). Development and application of a livestock phenomics platform to enhance productivity and efficiency at pasture. *Animal Production Science*, 56(8): 1299-1311.
- Holman, A., Thompson, J., Routly, J.E., Cameron, J., Jones, D.N., Grove-White, D., Smith, R.F., Dobson, H. (2011). Comparison of oestrus detection methods in dairy cattle. *Veterinary Record*, 9, 169(2): 47.
- Marques, J.C.S., Burnett, T.A., Denis-Robichaud, J., Madureira, A.M.L., Cerri, R.L.A. (2024). Validation of a leg-mounted pedometer for the measurement of steps in lactating Holstein cows. *JDS Communications*, 5(1): 67-71.
- Martiskainen, P., Jarvinen, M., Skon, J.P., Tiirikainen, J., Kolehmainen, M., Manonen, J. (2009). Cow behaviour pattern recognition using a three-dimensional accelerometer and support vector machines. *Applied Animal Behaviour Science*, 119(1-2): 32-38.
- Mazrier, H., Tal, S., Aizinbud, E., Bargai, U. (2006). A field investigation of the use of the pedometer for the early detection of lameness in cattle. *Canadian Veterinary Journal*, 47(9): 883-6.
- Morrone, S., Dimauro, C., Gambella, F., Cappai, M.G. (2022). Industry 4.0 and Precision Livestock Farming (PLF): An up to Date Overview across Animal Productions. *Sensors*, 7, 22(12): 4319.
- Neethirajan, S. (2020.) The role of sensors, big data and machine learning in modern animal farming. *Sensing and Bio-Sensing Research*, 29:100367.
- Neethirajan, S., Kemp, B. (2021). Digital Livestock Farming. *Sensing and Bio-Sensing Research*, 32(10).
- Oliveira Junior, G.A., Schenkel, F.S., Alcantara, L., Houlahan, K., Lynch, C., Baes CF. (2021). Estimated genetic parameters for all genetically evaluated traits in Canadian Holsteins. *Journal of Dairy Science*, 104(8): 9002-9015.

- Palmer, A.M., Olmos, G., Boyle, L.A., Mee, J.F. (2010). Estrus detection and estrus characteristic in housed and pastured Holsten-Friesian cows. *Theriogenology*, 15, 74(2): 255-64.
- Perez Marquez, H.J., Ambrose, D.J., Schaefer, A.L., Cook, N.J., Bench, C.J. (2019). Infrared thermography and behavioral biometrics associated with estrus indicator and ovulation in estrus-synchronised dairy cows housed in tiestalls. *Journal of Dairy Science*, 102(5): 4427-4440.
- Rahman, A., Smith, D., Little, B., Ingham, A. B., Greenwood, P. L., & Bishop-Hurley, G. J. (2018). Cattle behaviour classification from collar, halter, and ear tag sensors. *Information Processing in Agriculture*, 5(1), 124-133. doi.org/10.1016/j.inpa.2017.10.001.
- Reith, S., Hoy, S. (2018). Behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle. *Animal*, 12: 398–407.
- Shahriar, S., Smith, D., Rahman, A., Freeman, M., Hills, J., Rawnsley, R., Henry, D., Bishop-Hurley, G. (2016). Detecting heat event in dairy cows using accelerometers and unsupervised learning, 129: 20-21.
- Valenza, A., Giordano, J.O., Lopes Jr, G., Vincenti, L., Amundson, M.C., Fricke, P.M. (2012). Assessment of an accelerometer system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows. *Journal of Dairy Science*, 95(12):7115-27. doi:10.3168/jds.2012-5639.
- Van Nuffel, A., Van De Gucht, T., Saeys, W., Sonck, B., Opsomer, G., Vangeyte, J., Mertens, K.C., De Ketelaere, B., Van Weyenberg, S. (2016). Environmental and cow-related factors affect cow locomotion and can cause misclassification in lameness detection systems. *Animal*, 10(9):1533-41.
- Wang, S., Zhang, H., Tian, H., Chen, X., Li, S., Lu, Y., Li, L., Wang, D. (2020). Alterations in vaginal temperature during the estrous cycle in dairy cows detected by a new intravaginal device-a pilot study. *Tropical Animal Health and Production*, 52(5): 2265-2271.

REVIEW ARTICLE

GWAS analysis in Holstein cattle: current knowledge and future directions

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Summary

Genomic selection, combined with traditional selection methods, improves the efficiency of animal selection and allows for a more precise assessment of their production potential. The Genome-Wide Association Studies (GWAS) method is crucial for analysing the association of genetic markers with milk yield traits, whereby increasing the sample size and marker density improves the accuracy of the genomic association. GWAS analyses have identified key genes associated with milk production in cattle, as well as traits such as productive life, age at first calving and disease resistance in Holstein cows. In addition, genes associated with mastitis, one of the major health problems in Holstein cows, together with other health traits, represent potential targets for further research.

Key words: selection, genomics, GWAS, Holstein cattle, milk production

Introduction

Milk has been valued as a high-quality food of animal origin since ancient times, and cow's milk is the most widely used food in the human diet, accounting for 81% of global milk production (Solarczyk et al., 2024). The dominant breed in milk production is the Holstein-Friesian cattle, the most widespread dairy breed in the world, mainly due to its high milk production. In addition to high milk production, milk quality is another key factor in cattle breeding programs (Liu et al., 2020). Profitability and quality of dairy cattle production also depends on the health and fertility of cows and their productive lifespan, and improving traits such as calving ease and conformation reduces the herd's elimination rate and improves the production. (Abo-Ismael et al., 2017, Liang et al., 2024). Selection and breeding programs are crucial for achieving and improving the production traits of dairy cattle (Ozdemir et al., 2024). Since the traits related to milk production are quantitative, they are influenced by the additive genetic effect and environmental factors, so traditional selection methods have proven to be ineffective (Liu et al., 2020). While traditional selection methods remain in use, advances in genetics and genomics have transformed dairy cattle breeding by enabling much greater selection accuracy. Genomic tools allow breeders to identify animals with superior genetic potential at a very early age, often before any phenotypic traits are expressed, thereby shortening the generation interval and accelerating genetic gain.

Brief history of genotyping

Genome analysis is used to assess the potential of domestic animal production. This analysis involves genotyping using DNA markers distributed throughout the genome. A genetic marker is a marker based on a DNA polymorphism caused by a mutation (Peka and Balatsky, 2024). The addition of DNA information allows for a more accurate assessment of breeding values of breeding animals and a more precise selection. Selection at the DNA and quantitative trait loci

(QTL) levels accelerates genetic progress by identifying key genes responsible for milk production (Ozdemir et al., 2024). Although the use of DNA markers to improve genetic progress in livestock breeding was proposed several decades ago, their application was limited for along time due to the high cost of genotyping and the complexity of calculating breeding values. Today, these obstacles have been completely overcome, so that genomic evaluation is officially accepted and widely used in dairy farming due to its advantages over traditional selection methods (Palombo et al., 2020). The first GWAS results were already published in 2005 and 2006, but the starting point is the Wellcome Trust Case Control Consortium (WTCCC) document from 2007 (Wellcome Trust Case Control Consortium, 2007). This case-control study in humans aimed to identify variants associated with common diseases and represented a major breakthrough at the time. GWAS analyses a number of genetic variants in the genome to determine the associations between genomic variants and specific traits and aims to identify which genomic locations are linearly associated with complex traits in the population. GWAS analyses are based on the concept of genetic linkage disequilibrium or LD, i.e. the non-random association of alleles at different loci, and a particular focus is on assessing the association between common single nucleotide polymorphisms or SNPs. LD facilitates the identification of genomic regions associated with specific traits through markers that are correlated with causal variants. Therefore, the pattern and extent of LD in a population have impact on the accuracy and effectiveness of detecting significant genetic associations (Visscher et al., 2012). In dairy cattle genomics, a various SNP panels (Illumina Bovine SNP50K, GGP Bovine 100K etc.) of different densities are commonly used for analysis (Abo-Ismael et al., 2017). The sample size in GWAS analyses is crucial for the accuracy of the results. Increasing the sample size in combination with advanced multivariate analysis methods (e.g. Multivariate Linear Mixed Models or mvLLM; Multi-Trait Mixed Model or MTMM; Multi-Trait Bayesian Models etc.) has improved the precision of estimates, which has led to a wider acceptance of polygenic predictors in all research disciplines (Abdellaoui et al., 2023). In addition to increasing sample size, increasing marker density from low to mid density in cattle has also contributed to the accuracy of genomic linkage and provided better insight into the genetic architecture of dairy traits in cows (Liu et al., 2020).

GWAS and genetic markers in selection for milk yield and longevity traits

The adoption of GWAS has enabled genomic research to successfully identify several key genes responsible for milk production in cattle (Chen et al., 2018). GWAS analysis has also used to investigate the association between genetic factors and longevity as an important factor for productivity in Holstein cows in the USA (Wiggans and Carrilo, 2022). Two chromosomal regions with additive effects were associated with productive lifespan in Holstein breed. The SLC4A4-GC-NPFFR2 (*SGN*) region on chromosome 6, which has a significant pleiotropic effect on a total of four traits: milk yield, number of somatic cells, fertility and productive life, and a large region on chromosome 10 that affects several immune-related functions such as the development, homeostasis, and differentiation of T cells and B cells (Liang et al., 2024).

Age at first calving is a relatively new reproductive trait used in genomic analyses of Holstein cows in the USA (Wiggans and Carrilo, 2022). This trait affects the longevity and productivity of dairy cows, particularly in terms of total milk yield over their lifetime production. GWAS has been used to identify genes and chromosomal regions that influence this trait. Significant (p -value $< 10^{-8}$) additive effects were identified on three chromosomes: in the 7.86–8.12 Mb region on chromosome 15, 27.07–27.48 Mb region and 31.25–32.11 Mb region on chromosome 19, and in the 26.92–32.60 Mb region on chromosome 23. In these regions, two genes are associated with sex hormones, and their known biological functions may be relevant to age at first calving. These are a sex hormone-binding globulin gene (*SHBG*) and a progesterone receptor gene (*PGR*). Significant SNP variants in their proximity and their known biological

function indicate a possible link between these reproductive hormones with age at first calving in Holstein cows (Prakapenka et al., 2023). The diacylglycerol O-acyltransferase 1 (*DGAT1*) gene discovered by GWAS is one of the key genes influencing milk production in cows (Liu et al., 2020). The gene is located on the 14th autosomal chromosome of cattle in the centromere region. It encodes diacylglycerol-O-transferase, an enzyme essential for triglyceride synthesis, lipid absorption, and adipose tissue development. The non-conservative polymorphism K232A, caused by an A-A to G-C substitution in exon 8, leads to a lysine-to-alanine change and significantly affects milk fat and protein content (Mahmoudi and Rashidi, 2023). It is possible to use *DGAT1* as a genetic marker for the selection of milk composition traits, especially in Holstein breed (Ozdemir et al., 2024.). In addition to this gene, kappa casein gene (*CSN3*) can be used as a good marker for milk quality (protein and fat content) (Liu et al., 2020) and the prolactin gene (*PRL*) as a marker for milk quantity (Ozdemir et al., 2024). Within the *DGAT1* gene, a SNP was discovered that showed a significant association with bone quality in Holstein cows, as well as with rump width and conformation of the animal. Bone quality is an important trait that influences the functional longevity of cows, indicating a possible link between the *DGAT1* gene and functional longevity in Holstein cows (Abo-Ismael et al., 2017). In addition to this gene, which has been the most studied, several other genes identified by GWAS influence milk production in Holstein cows. Chen et al., (2018) confirmed the association of the: DLG associated protein 1 (*DLGAP1*), adaptor related protein complex 2 subunit beta 1 (*AP2B1*), inositol 1,4,5-trisphosphate receptor type 2 (*ITPR2*) and thrombospondin 4 (*THBS4*) genes with milk yield, and the rho guanine nucleotide exchange factor 4 (*ARHGEF4*), tudor domain containing 1 (*TDRD1*) and kinesin family member 19 (*KIF19*) genes are currently assumed to be associated with milk production (Lung et al., 2019). The ATP-binding cassette subfamily G member 2 gene (*ABCG2*) located on 6th autosomal chromosome of cattle, has been identified as an important genetic factor influencing milk production traits. It is associated with both the yield and composition of milk, as well as with the excretion efficiency of certain pharmaceuticals into milk. The Y581S polymorphism within this gene plays a significant role in modulating milk quality by affecting its fat and protein content (Peka and Balatsky, 2024). The growth hormone receptor gene (*GHR*) is crucial for postnatal growth. It significantly affects metabolism, and it is also associated with meat quality in cattle, and participates in the regulation of milk production and fertility in cows (Dettori et al., 2018). Studies have shown that *GHR* affects milk yield, protein and fat content, as well as the overall physical condition of cows. Among several identified SNPs in this gene, the F279Y missense polymorphism in exon 8 is considered to have a direct impact on both milk quality and quantity. This effect has been observed not only in Holstein cows, but also in Ayrshire and Jersey breeds (Peka and Balatsky, 2024). The *PRL* gene, which is located on chromosome 23 showed a significant association with fatty acids in milk. The mechanism by which *PRL* influences the fatty acid composition of milk is not yet fully understood, but it appears to involve signaling pathways correlation with signal transducers and transcriptional activators. The leptin (*LEP*) gene, located on chromosome 4, is associated with nine fatty acids in milk, and in the presence of the *PRL* gene, fat synthesis is enhanced, suggesting that these two genes together influence milk quality (Pegolo et al., 2016). Do et al. (2017) identified the *PRL* gene as a possible candidate for influencing lactation persistence in Holstein cows. The casein beta (*CSN2*) regulates the synthesis of all casein fractions except κ -casein, and its relevance in selection programs stems from its distinct characteristics and strong association with specific DNA markers. Allelic variants of beta-casein represent quality traits as targeted mutations can influence the type of milk product, such as A1 milk (Kulibaba et al., 2024). A GWAS analysis was also performed in the Holstein breed to identify genes that influence the occurrence of mastitis in cows. Significant SNPs were identified on chromosomes 14, 15, 22 and 29, with the following genes being associated with the immune response and inflammatory processes: deoxyribonuclease

1L3 (*DNASE1L3*), solute carrier family 36 member 4 (*SLC36A4*), armadillo repeat containing 1 (*ARMC1*), phosphodiesterase 7A (*PDE7A*), matrix metalloproteinase 13 (*MMP13*) and CD44 molecule (*CD44*). These genes represent potential targets for further research, which should focus on comparing their expression levels in cows affected by mastitis and in healthy individuals for better insight, since mastitis is one of the main health problems in milk production (Ashja et al., 2024).

Conclusions

GWAS results have made a significant contribution to Holstein cattle breeding by identifying genes linked to production and longevity traits, leading to improvements in milk yield and composition, fertility, and overall lifespan. More recent GWAS studies, aimed to identify genes that influence disease resistance in cattle, particularly those affecting udder health, with the goal of reducing of mastitis incidence. Identifying these genes and incorporating them into selection programs can improve animal health and lower treatment costs. GWAS results, combined with genomic selection, provide deeper insights and enable faster genetic progress by allowing breeders to select the best animals for breeding even before they produce offspring. In most cases, the majority of genetic variation is effectively captured by GBLUP models, which assume small, additive effects across the whole genome. However, for certain traits influenced by major genes or large-effect loci, incorporating specific SNPs as fixed effects detected by GWAS in evaluations can enhance prediction accuracy and biological interpretation. In addition, the estimated breeding values are more accurate than the breeding values obtained using traditional selection methods. The combination of GWAS and genomic selection is a promising strategy in modern cattle breeding to achieve sustainability and productivity.

References

- Abdellaoui A., Yengo L., Verweij K.J.H., Visscher P.M. (2023). 15 years of GWAS discovery: Realizing the promise. *American Journal of Human Genetics*, 2, 110(2): 179-194.
- Abo-Ismael M.K., Brito L.F., Miller S.P., Sargolzaei M., Grossi D.A., Moore S.S., Plastow G., Stothard P., Nayeri S., Schenkel F.S. (2017). Genome-wide association studies and genomic prediction of breeding values for calving performance and body conformation traits in Holstein cattle. *Genetics Selection Evolution*, 7, 49(1): 82.
- Ashja A., Zorc M., Dovc P. (2024). Genome-Wide Association Study for Milk Somatic Cell Score in Holstein Friesian Cows in Slovenia. *Animals*, 19, 14(18): 2713.
- Chen Z., Yao Y., Ma P., Wang Q., Pan Y. (2018). Haplotype-based genome-wide association study identifies loci and candidate genes for milk yield in Holsteins. *PLoS One*, 15, 13(2).
- Dettori M. L., Pazzola M., Paschino P., Amills M., Vacca G. M. (2018). Association between the GHR, GHRHR, and IGF1 gene polymorphisms and milk yield and quality traits in Sarda sheep. *Journal of dairy science*, 101(11): 9978–9986.
- Do D.N., Bissonnette N., Lacasse P., Miglior F., Sargolzaei M., Zhao X., Ibeagha-Awemu E.M. (2017). Genome-wide association analysis and pathways enrichment for lactation persistency in Canadian Holstein cattle. *Journal of Dairy Science*, 100(3): 1955-1970.
- Iung L.H.S., Petrini J., Ramírez-Díaz J., Salvian M., Rovadoscki G.A., Pilonetto F., Dauria B.D., Machado P.F., Coutinho L.L., Wiggans G.R., Mourão G.B. (2019). Genome-wide association study for milk production traits in a Brazilian Holstein population. *Journal of Dairy Science*, 102(6): 5305-5314.
- Kulibaba R.O., Liashenko Y.V., Sakhatyskiy M.I. (2024). Polymorphism of CSN2 and TNF- α Genes in the Population of Holstein Cattle Bred in Ukraine. *Cytology and Genetics*, 58: 29–38.

- Liang Z., Prakapenka D., Zaabza H.B., VanRaden P.M., Van Tassell C.P., Da Y. (2024). A million-cow genome-wide association study of productive life in U.S. Holstein cows. *Genetics Selection Evolution*, 26, 56(1): 67.
- Liu L., Zhou J., Chen C. J., Zhang J., Wen W., Tian J., Zhang Z., Gu Y. (2020). GWAS-Based Identification Of New Loci for Milk Yield, Fat, and Protein in Holstein Cattle. *Animals*, 10(11): 2048.
- Mahmoudi P., Rashidi A. (2023). Strong evidence for association between K232A polymorphism of the DGAT1 gene and milk fat and protein contents: A meta-analysis. *Journal of dairy science*, 106(4): 2573–2587.
- Ozdemir M., Motmain Z., Ekinci K., Saygil E. (2024). Associations Between BLG, CSN3, DGAT1, GH, PIT1, and PRL Gene Polymorphisms and Milk Production Traits in Holstein Dairy Cows: A Meta-Analysis. *Biochemical Genetics*.
- Palombo V., Pegolo S., Conte G., Cesarani A., Macciotta N. P. P., Stefanon B., D’Andrea, M. (2020). Genomic prediction for latent variables related to milk fatty acid composition in Holstein, Simmental and Brown Swiss dairy cattle breeds. *Journal of Animal Breeding and Genetics*.
- Pegolo S., Cecchinato A., Mele M., Conte G., Schiavon S., Bittante G. (2016). Effects of candidate gene polymorphisms on the detailed fatty acids profile determined by gas chromatography in bovine milk. *Journal of Dairy Science*, 99(6): 4558-4573.
- Peka M., Balatsky V. (2024). Bioinformatic approach to identifying causative missense polymorphisms in animal genomes. *BMC Genomics*, 19, 25(1): 1226.
- Prakapenka D., Liang Z., Da Y. (2023). Genome-Wide Association Study of Age at First Calving in U.S. Holstein Cows. *International Journal of Molecular Sciences*, 12, 24(8): 7109.
- Solarczyk P., Slószarz J., Gołębiewski M., Natalello A., Musati M., Luciano G., Priolo A., Puppel K. (2024). The Influence of Crossbreeding on the Composition of Protein and Fat Fractions in Milk: A Comparison Between Purebred Polish Holstein Friesian and Polish Holstein Friesian × Swedish Red Cows. *Nutrients*. 16(21): 3634.
- Visscher P.M., Brown M.A., McCarthy M.I., Yang J. (2012). Five years of GWAS discovery. *American Journal of Human Genetics* 13;90(1):7-24.
- Wellcome Trust Case Control Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, 447(7145): 661–678.
- Wiggans G. R., Carrillo J. A. (2022). Genomic selection in United States dairy cattle. *Frontiers in genetics*, 13, 994466.

REVIEW ARTICLE

Precision cattle farming as a response to modern challenges

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Summary

Global population growth has significantly increased the demand for animal products, driving the intensification of farm production. Farmers face the challenge of producing sufficient amounts of food while managing limited natural resources. Major challenges in cattle production include climate change, lack of skilled labor, price competitiveness, animal welfare, and the environmental impact of production. Additionally, managing farm processes is becoming increasingly complex, particularly due to the challenges of timely detection of diseases and estrus in large herds, as well as difficulties in monitoring individual nutritional statuses. The response to modern challenges is in the application of new technologies and artificial intelligence, which has consequently led to the development of precision livestock farming. This modern practice enhances decision-making in production management, disease detection, nutrition optimization, and business planning. The implementation of new technologies in cattle farming has become inevitable, as traditional monitoring methods are often inefficient- being time-consuming, limit data collection, prone to subjective assessments, and susceptible to bias. In conclusion, the adoption of new technologies enhances farm profitability, improves economic and environmental sustainability, and provides better animal welfare.

Key words: cattle, farm management, milk production, technology, artificial intelligence

Introduction

Cattle production today faces numerous challenges. The continuous growth of the global population has led to an increasing demand for animal products. According to UN projections from 2019, the global population could reach 10.9 billion people by the end of this century. Fears of potential food shortages have led to the intensification of agricultural production, both in crop farming and livestock breeding. Historically, animal husbandry has been decentralized, with farms managed by individuals; however, this is no longer the case today. One of the greatest global challenges is achieving the high production levels demanded by the market while managing limited land, water, and other natural resources (Neethirajan, 2020). Additionally, climate change presents significant obstacles across all sectors of livestock production, including cattle farming. A lack of labor, coupled with insufficient expertise, presents another significant challenge (Yaseer and Chen, 2021). Price competitiveness in the market, animal welfare concerns, and the environmental impact of cattle production are additional factors that producers must consider (Mijić et al., 2024). Livestock farming, especially cattle breeding, significantly contributes to global greenhouse gas emissions, making it increasingly important to develop strategies for improving feeding efficiency and reducing the negative environmental impact of cattle production (Sonea et al., 2023). Due to the increased demand for animal products in the global market, producers have inevitably shifted their focus toward high-yield breeds, leading to increased production per animal. However, these high-performance animals are much more sensitive, more susceptible to disease, and

require technologically advanced farm conditions alongside precise and balanced nutrition. A key challenge for farm managers is the inability to detect diseases and estrus on time due to the increased number of animals on the farm, as well as the inability to monitor the nutritional status of individual animals effectively. In other words, managing large herds becomes more difficult, with animal welfare and milking supervision being especially important in dairy farming (Yaseer and Chen, 2021). Modern technologies, supported by various sensors and artificial intelligence, offer numerous opportunities for analysis and decision-making that were traditionally carried out by humans. These advancements enable production monitoring at the herd or group level, early identification of sick cows or cows in estrus, employee training, feeding optimization, and business planning (De Vries et al., 2023). This approach, known as precision livestock farming, represents the future of the agro-industry, enabling more efficient operations, reducing losses, and improving animal welfare.

Development of precision animal husbandry

The idea of precision agriculture was first introduced in the early 1990s in the United States (Morrone et al., 2022). Initially, it referred to technological advancements in the crop farming sector, while concepts of precision livestock farming were developed later. Artificial intelligence (AI) has since become a key tool across various industries. Although significant advancements with AI technologies have already been made in engineering, pharmaceuticals, and medicine, their integration into agriculture began more prominently in the late 2010s (Miyazawa et al., 2022). Artificial intelligence refers to the ability of a digital computer or a computer-controlled robot to perform tasks requiring intelligence, such as reasoning, learning, and problem-solving. Thanks to advancements in hardware and software, AI is being applied in dairy farms, enabling the processing of large amounts of data and the automation of repetitive tasks. This technology improves working conditions and reduces the need for manual labor (De Vries et al., 2023). Based on these insights, precision livestock farming has emerged as an innovative approach that utilizes digital technologies such as smart sensors, advanced control systems, intelligent robotics, the Internet of Things (IoT), big data analytics, and machine learning. These technologies automate the animal breeding process, making it more efficient, economically viable, and environmentally sustainable (Yaseer and Chen, 2021). The potential of precision livestock farming lies in its ability to provide early warnings, enabling farm managers to respond promptly to the first signs of impaired animal health or welfare. Implementing this approach requires interdisciplinary collaboration among experts in animal production, veterinary medicine, engineering, and data analytics. Such collaboration facilitates the development of efficient monitoring systems that improve animal welfare (Morrone et al., 2022). About a decade ago, most farmers did not have access to modern technologies such as fast internet, smartphones, and high-performance computers. Today, these technologies are widely available, providing new opportunities for improving farm management (Neethirajan, 2020). For the effective implementation of precision livestock farming, it is essential to collect large amounts of diverse data. This includes information about local weather conditions, air quality, animal sound signals, movement patterns, behavior, and production data. Real-time data collection is enabled by various sensors, creating a solid foundation for decision-making and production optimization (Neethirajan, 2020).

Available precision technologies in cattle farming

A sensor is defined as a device that measures or detects biological, chemical, physical, or mechanical properties, or a combination of these, and records and collects data for interpretation. Sensors can be classified in various ways (Neethirajan, 2020). In cattle breeding, various types of sensors are used, such as temperature, visual and audio sensors, and those designed for precision milking and feeding. Sensors can be categorized as invasive and non-

invasive, as well as stationary and wearable (Yaseer and Chen, 2021). Invasive sensors are implanted into the animal's body or ingested, which can negatively impact welfare and cause health problems such as infections and stress, thereby reducing the accuracy of the collected data. Therefore, non-invasive sensors are usually preferred for monitoring animal welfare, including remote sensors such as visual and thermal cameras, microphones, and wearable sensors attached to the animal (Neethirajan, 2022). Environmental sensors are used to collect data within facilities, such as concentrations of CO₂ and NH₃ gases, which is crucial for ventilation control, given that cattle produce large amounts of these gases (Yaseer and Chen, 2021). By using video cameras, it is possible to track not only the movement of animals but also their emotional state by analyzing facial expressions and the movements of specific body parts, such as eyes, ears, snout, and tail (Neethirajan, 2022). Lameness is one of the most common health problems in modern cattle farming, traditionally assessed through visual scoring. Research by Van Heretm et al. (2017) suggests that the optimal time interval between two consecutive cows passing through the recording should be at least 35 seconds. On average 79.1% of cows were automatically scored via video analysis-based system at least five times a week, with the highest accuracy in lameness detection achieved for the angle related to the curvature of the back in the hip joint area. Thermal (infrared) cameras can measure the peripheral temperature of the entire body or specific parts of the animal (Neethirajan, 2022). A change in temperature, unless related to seasonal effects (e.g., higher temperatures in summer), may indicate the presence of disease or estrus. Vocalizations, especially those indicating negative emotional states, are often involuntary, making them reliable indicators of an animal's current emotional states (Neethirajan, 2022). Microphones installed in stalls can continuously record sound, providing additional behavioral insights. Accelerometers embedded in wearable devices attached to the animal, such as pedometers, enable the collection of three-dimensional movement patterns. By analyzing this data, valuable insights into behavior, health, and welfare can be obtained. However, wearable sensors must be designed to withstand harsh farm environment (e.g. mud, dust, and mechanical stress) and to avoid interference with the animal's natural behaviour. Poor attachment or sensor damage caused by chewing or crushing remains a challenge (Neethirajan, 2022). The accuracy of data collected by accelerometers is, on average, 87% (Williams et al., 2018). Studies have demonstrated that changes in step count, measured by a pedometer, can serve as an indicator for detecting estrus (Hojo et al., 2018). Data collected from a single type of sensor is often not sufficient for precise measurement, therefore, it is crucial to apply a multimodal approach, which has not yet been fully implemented in livestock farming. Additionally, animal activity data collected by sensors is often not integrated in the same software program as production records, complicating historical data analysis. Therefore, it is necessary to establish a robust hardware infrastructure for reliable collection of multimodal sensor data, along with an efficient cloud-server architecture for storage and processing (Neethirajan, 2022; De Vries et al., 2023). Sometimes, drawing meaningful conclusions from sensor data can be difficult. That difficulty can be improved by applying machine learning methods, because offer significant potential for improving accuracy and predictive capabilities (Yaseer and Chen, 2021).

Machine learning

Machine learning is a branch of artificial intelligence that focuses on the development of algorithms capable of learning from data and making decisions without explicit programming (Miyazawa et al., 2022). In livestock farming, the most commonly used machine learning algorithms include decision trees, random forests, support vector machines (SVM), k-means clustering, k-nearest neighbor (KNN), and multilayer perceptron (MLP) (Yaseer and Chen, 2021). Artificial neural networks are increasingly present in data analysis within animal husbandry. These computer systems, developed within the framework of artificial intelligence

research, are mostly implemented as software simulations running on conventional computers. Numerous studies have shown that artificial neural networks outperform traditional linear or classical statistical methods in predictive accuracy (Dongre and Gandhi, 2016). The results of using machine learning have yielded significant advancements. For example, random forest algorithms are used to predict postpartum diseases in cows based on pre-calving data. Along with random forest, boosted tree models help in detecting dystocia in heifers and cows by predicting different levels of calving difficulties. Support vector machine (SVM) identifies areas of elevated facial temperature, which may indicate stress or the presence of respiratory diseases. Random forest models often provide the best results in analyzing cow behavior, including grazing, ruminating, walking, and resting patterns. In lameness detecting, the KNN algorithm has shown the best results, while SVM and random forest are also commonly used. Furthermore, KNN can be used to classify condition states or BCS by analyzing body condition features extracted from different body regions. The application of machine learning enhances the precision of health monitoring, welfare management, and the productivity of dairy cows (Yaseer and Chen, 2021). With the help of these technologies, precise feeding can be implemented as well, optimizing efficiency, reducing feed costs, and increasing the profitability and economic sustainability of the system (Sonea et al., 2023). Data sets generated by animal precision tracking technologies are essential for selective breeding (Brito et al., 2020). However, along with all the benefits of these technologies, there is also the risk of cyber-attacks, leading to serious consequences for the entire management system. Therefore, technological solutions such as strong data encryption, cloud storage, and advanced analytical tools could help in managing and securing large amounts of data (Neethirajan, 2023).

Conclusion

Due to the challenges faced by modern cattle farming, the implementation of advanced technologies into production systems has become inevitable. The availability of the internet and the ease of system installation further facilitate their application. The use of these technologies brings numerous advantages. Specifically, it simplifies animal monitoring, enabling timely detection of diseases and estrus, which reduces treatment costs and increases reproductive efficiency. Monitoring of feeding patterns and adjusting nutritional plans according to the animal's specific needs help lower feeding costs, minimize food waste, and reduce harmful gas emissions. These improvements not only enhance productivity and profitability but also have a positive impact on the environment. Additionally, collecting large amounts of production data allows for more precise selection and improvement of the herd's genetic potential. Using these technologies, farm managers can increase the profitability and sustainability of their operations while simultaneously improving animal welfare and reducing the negative environmental impact of cattle farming.

References

- Brito, L.F., Oliveira, H.R., McConn, B.R., Schinckel, A.P., Arrazola, A., Marchant-Forde, J.N., Johnson, J.S. (2020). Large-Scale Phenotyping of Livestock Welfare in Commercial Production Systems: A New Frontier in Animal Breeding. *Frontiers in Genetics*. 11:793.
- De Vries, A., Bliznyuk, N., Pinedo, P. (2023). Invited Review: Examples and opportunities for artificial intelligence (AI) in dairy farms. *Applied Animal Science*. 39(1): 14-22.
- Dongre, V., Gandhi, R.S. (2016). Applications of artificial neural networks for enhanced livestock productivity: A review. *Indian Journal of Animal Sciences*. 86 : 1232-1237.

- Hojo, T., Sakatani, M., Takenouchi, N. (2018). Efficiency of a pedometer device for detecting estrus in standing heat and silent heat in Japanese Black cattle. *Animal Science Journal*. 9(8): 1067-1072.
- Mijić, P., Bobić, T., Baban, M., Domaćinović, M. (2024). Transformacije mljekarskoga sektora: izazovi i alternative. *Krmiva*. 66(2): 91-106.
- Miyazawa, T., Hiratsuka, Y., Toda, M., Hatakeyama, N., Ozawa, H., Abe, C., Cheng, T.Y., Matsushima, Y., Miyawaki, Y., Ashida, K., Iimura, J., Tsuda, T., Bushita, H., Tomonobu, K., Ohta, S., Chung, H., Omae, Y., Yamamoto, T., Morinaga, M., Ochi, H., Nakada, H., Otsuka, K., Miyazawa, T. (2022). Artificial intelligence in food science and nutrition: a narrative review. *Nutrition Reviews*. 7, 80(12).
- Morrone, S., Dimauro, C., Gambella, F., Cappai, M.G. (2022). Industry 4.0 and Precision Livestock Farming (PLF): An up to Date Overview across Animal Productions. *Sensors (Basel)*. 22(12): 4319.
- Neethirajan, S. (2020). The role of sensors, big data and machine learning in modern animal farming. *Sensing and Bio-Sensing Research*. 29: 100367.
- Neethirajan, S. (2022). Affective State Recognition in Livestock-Artificial Intelligence Approaches. *Animals*. 12(6): 759. doi: 10.3390/ani12060759.
- Neethirajan, S. (2023). Artificial Intelligence and Sensor Technologies in Dairy Livestock Export: Charting a Digital Transformation. *Sensors (Basel, Switzerland)*, 23(16): 7045.
- Sonea, C., Gheorghe-Irimia, R.A., Tapaloaga, D., Gurau, M.R., Udrea, L., Tapaloaga, P.R. (2023). Optimizing Animal Nutrition and Sustainability Through Precision Feeding: A Mini Review of Emerging Strategies and Technologies. *Annals of "Valahia" University of Târgoviște. Agriculture*. Valahia University of Targoviste, 15(12): 7-11.
- United Nations. *World Population Prospects (2019). Highlights*; United Nations: New York, NY, USA, 2019.
- Van Hertem, T., Schlageter Tello, A., Viazzi, S., Steensels, M., Bahr, C., Romanini, C. E. B., Berckmans, D. (2017). Implementation of an automatic 3D vision monitor for dairy cow locomotion in a commercial farm. *Biosystems Engineering*. 173: 166–175
- Williams, J., Ntallaris, T., Routly, J.E., Jones, D.N., Cameron, J., Holman-Coates, A., Smith, R.F., Humblot, P., Dobson, H. (2018). Association of production diseases with motor activity-sensing devices and milk progesterone concentrations in dairy cows. *Theriogenology*. 118: 57-62.
- Yaseer, A., Chen, H. (2021). A Review of Sensors and Machine Learning in Animal Farming. 2021 IEEE 11th Annual International Conference on CYBER Technology in Automation, Control, and Intelligent Systems (CYBER). 747-752. Jiaxing, China.

PRELIMINARY COMMUNICATION

Melissopalynological and sensory properties of ivy honey (*Hedera helix* L.)

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Summary

The aim of this study was to determine the pollen spectrum and sensory properties of ivy honey. The research was conducted on 10 ivy honey samples collected from the beekeepers in 2023. Samples were analyzed using qualitative melissopalynological analysis. The sensory evaluation of the collected honey samples was performed by a panel of three sensory assessors. The proportion of ivy pollen grains ranged from 51% to 98%. Pollen from 9 botanical families was recorded. It's aroma found to be herbal, reminiscent of ivy flowers, chemical, and highly intense. The taste was rated as persistent, with weak sweetness and acidity. The findings of this study contribute to the characterization of ivy honey, given the scarce available scientific data on this honey type.

Key words: *Hedera helix*, honey, melissopalynology, sensory properties

Introduction

Common ivy (*Hedera helix* L.) is an evergreen woody climber belonging to the Araliaceae family (Juss. 1789). It is naturally distributed across Western Europe, Turkey, and the Middle East, from where it has spread to Western Asia and other parts of the world. In Croatia, it is widely distributed as a climbing plant that thrives in sunlit, warm, and shaded habitats, with a particular preference for fertile, fresh, and calcareous soils (Kremer et al., 2021). Young leaves are typically covered with fine hairs, whereas mature leaves are glabrous and leathery. The flowers are actinomorphic, yellow-green, hermaphroditic, fragrant, and arranged in semi-globular umbellate inflorescences (Figure 1.a). Ivy is highly attractive to honey bees due to its abundant nectar and pollen supply (Kremer et al., 2021; Franjić and Škvorc, 2010).

Melissopalynological analysis is commonly used to determine both the botanical and geographical origin of honey. The determination of honey's botanical origin is based on the identification and quantification of pollen grains and other microscopic particles present in honey. Several factors can influence pollen presence in honey. One of these factors is plant morphology and physiology. Some plant species produce very little pollen or have sterile stamens that do not generate pollen, as seen in certain *Citrus* species (e.g., Satsuma mandarin). Two types of contamination can affect the presence of pollen in honey. The first occurs within the hive during honey production and pollen incorporation, where pollen collecting foragers physically contaminate the nectar in uncapped honey cells with pollen from the plants they have visited. The second type of contamination arises during technological processing when pollen unintentionally enters honey during uncapping and frame preparation for extraction. Furthermore, the filtration of nectar within the bee's body also influences the pollen content. During nectar collection and honey processing, pollen is efficiently separated in the bee's honey sac via the proventriculus, which functions as a filtration and food flow regulator into the midgut. The degree of pollen removal depends on the retention time of nectar in the honey sac, the amount of nectar being processed, pollen grain size, and exine structure. Larger and spiny pollen grains are more easily removed. Based on these factors, pollen in honey can be classified as normally represented, underrepresented (relatively low pollen content compared to nectar

volume), or overrepresented (relatively high pollen content compared to nectar volume) (Prđun, 2017).

The most important sensory properties of honey are color, aroma, and taste. Sensory analysis, in combination with physicochemical and pollen analysis, is commonly used to determine the botanical origin of honey. The color of honey primarily originates from the nectar source collected by bees and ranges from colorless to yellow, dark red, and almost black (Brudzynski, 2023). Upon crystallization, honey becomes lighter due to the formation of white glucose crystals; however, it darkens over time during storage, a process that is accelerated when honey is kept at higher temperatures (Vahčić and Matković, 2009). Volatile and semi-volatile aromatic organic compounds found in plant nectar are primarily responsible for the aromatic properties of honey. Unifloral honey varieties exhibit characteristic sensory aroma profiles compared to multifloral honey (Cuevas-Glory et al., 2007). Preliminary studies carried out in Croatia in the last decade revealed an appearance of new or insufficiently described rare unifloral honey, such as alder buckthorn, wild cherry and Christ's thorn honey (Svečnjak et al., 2024). This indicates a need for new insights into such honey types. Therefore, this study aimed to investigate the melissopalynological and sensory properties of rare ivy honey in order to complement its characterization.

Material and methods

The study was conducted on ten ($n=10$) ivy honey samples (Fig. 1.b) collected directly from the beekeepers in Croatia during the fall of 2023. All samples were stored in glass jars and kept in a cool, dark environment to preserve their properties, and all analyses were conducted within three months. Qualitative melissopalynological analysis was performed according to the method described by Von der Ohe et al. (2004). Pollen identification and quantification were carried out using a Carl Zeiss Axio light microscope (Jena, Germany) at magnifications ranging from $400\times$ to $1000\times$. The classification was based on a reference collection of native pollen preparations (internal collection of the Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, University of Zagreb Faculty of Agriculture), and relevant literature (Ricciardelli D'Albore, 1998; Von der Ohe and Von der Ohe, 2003). Pollen grains were analyzed in terms of shape, color, and size, as well as exine structure and the number and position of germination pores. The sensory analysis, aimed at assessing the visual, olfactory, and gustatory characteristics of the honey samples, was conducted by a panel of three trained and experienced sensory evaluators. Sensory analysis was carried out to provide a preliminary description of organoleptic properties of ivy honey.



Figure 1.a) Ivy flower (*Hedera helix* L.); b) samples of ivy honey

Results and discussion

The occurrence and frequency of plant species within each honey sample were calculated from the total (absolute) number of pollen grains present in it. Relative frequencies were divided into four groups: $> 45\%$ predominant pollen, $15\text{--}45\%$ secondary pollen, $3\text{--}15\%$ important minor pollen and $< 3\%$ minor pollen. Based on the qualitative melissopalynological analysis (Table 1), it was found that all 10 samples were unifloral as they contained $> 45\%$ of ivy pollen grains

which meet the criteria for uniflorality according to the national legislation (Ordinance on the quality of unifloral honey, NN 122/09). The range of uniflorality varied from 51% to 98%. Pollen from plants of 9 botanical families was recorded. In 8/10 samples, pollen from the Asteraceae family was found, in 4/10 from the Apiaceae family, and in 3/10 from the Fabaceae family.

Table 1. Results of the melissopalynological analysis of ivy honey (n=10)

Sample	Pollen content		
	(> 45 %)	(16 - 45 %)	(3 - 15 %) (< 3 %)
1. <i>H. helix</i> 87 %			Rhamnaceae 2 %, Apiaceae 2 %, <i>Trifolium</i> spp. 2 %, undetermined 2 %, <i>Cornus sanguinea</i> 1 %, Asteraceae (<i>Taraxacum</i> form) 1 %, Lamiaceae 1 %
2. <i>H. helix</i> 87 %		Apiaceae 8 %	<i>Castanea sativa</i> 2 %, undetermined 2 %, Rhamnaceae 1 %
3. <i>H. helix</i> 83 %		Asteraceae 4 %, <i>Castanea sativa</i> 4 %, <i>Helianthus annuus</i> 3 %	undetermined 2 %, Fabaceae (<i>Robinia</i> form) 2 %, Poaceae 1 %, Apiaceae 1 %
4. <i>H. helix</i> 92 %		Asteraceae 4 %	undetermined 2 %, <i>Cornus sanguinea</i> 1 %, Liliaceae 1 %
5. <i>H. helix</i> 96 %			undetermined 2 %, <i>Amorpha fruticosa</i> 1 %, Asteraceae 1 %
6. <i>H. helix</i> 51 %	<i>F. alnus</i> 21 %	Asteraceae 13 %, Fabaceae 5 %, undetermined 5 %, <i>Trifolium</i> spp. 3 %	<i>Cornus sanguinea</i> 2 %, Lamiaceae (<i>Mentha</i> form) 1 %
7. <i>H. helix</i> 88 %		<i>Asparagus</i> spp. 4 %, <i>Satureja</i> spp. 3 %	undetermined 2 %, <i>Rhamnus</i> spp. 2 %, Brassicaceae 1 %
8. <i>H. helix</i> 90 %		Asteraceae 4 %, undetermined 3 %	Apiaceae 2 %, Boraginaceae 1 %
9. <i>H. helix</i> 87 %		Asteraceae 6 %	Fabaceae 2 %, undetermined 2 %, <i>Trifolium</i> spp. 1 %, <i>Cornus sanguinea</i> 1 %, <i>Centaurea jacea</i> 1 %
10. <i>H. helix</i> 98 %			undetermined 1 %, Liliaceae 1 %

In the accompanying group (16-45%), the pollen from alder buckthorn (*F. alnus*) accounted for 21%. In the important minor pollen (3-15%) and minor pollen (<3%) groups, pollen from *C. sativa*, *H. annuus*, *Trifolium* spp., *Asparagus* spp., and *Satureja* spp. was found, as well as pollen from *C. sanguinea*, *A. fruticosa*, and *C. jacea*. The presence of alder buckthorn pollen (*F. alnus*) in the accompanying group indicates that the bees collected nectar from other plants that flowered several months before ivy. This residual pollen, along with the presence of other plants such as *C. sativa* and *H. annuus*, confirms that bees gather nectar from various sources over a longer time frame, before ivy becomes the dominant source in autumn. These data help to understand the seasonal dynamics of plant species and their availability to bees. The pollen of the most frequent plant species found in ivy honey is shown in Figure 2.

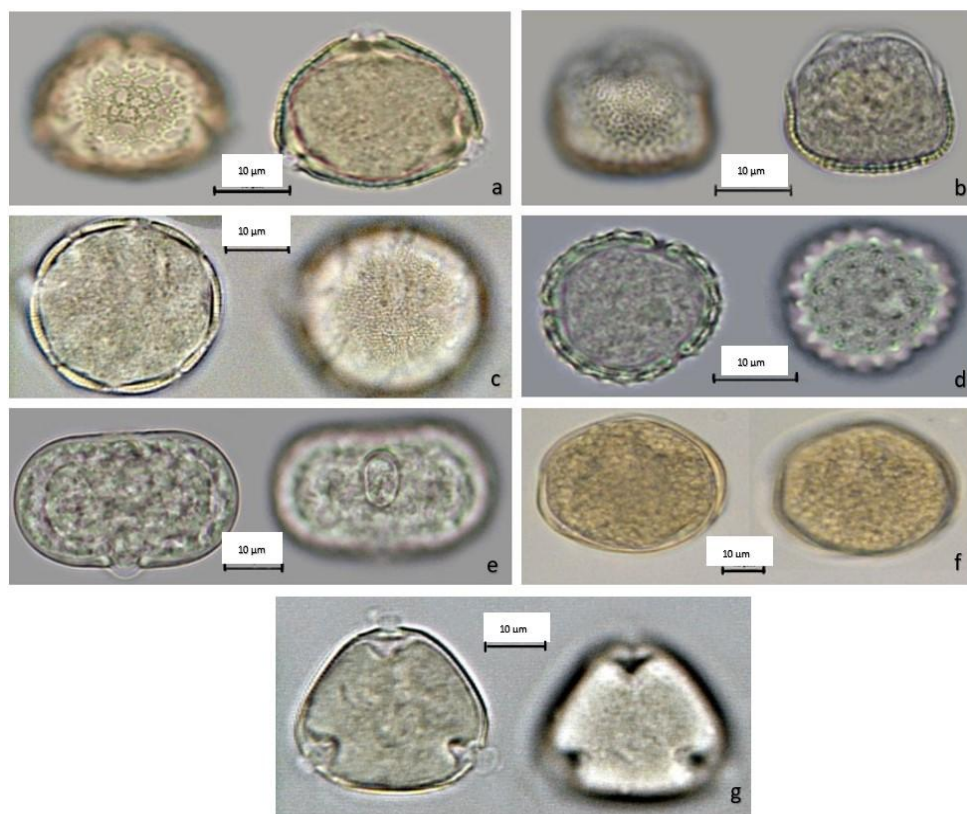


Figure 2. Pollen grains of ivy – *H. helix* (a), asparagus – *Asparagus* spp. (b), savory – *Satureja* spp. (c), plants from the Asteraceae family (d) and Apiaceae family (e), dogwood – *C. sanguinea*, (f) and buckthorn – *F. alnus* (g)

Since there is no data available in the scientific literature regarding the pollen spectrum of ivy honey, these results were compared with research conducted on autumn honey varieties from Spain. In their study, Boi et al. (2013) published the results of qualitative and quantitative melissopalynological analysis on 25 autumn honey samples. The pollen analysis identified 54 species of pollen belonging to 29 botanical families. In all samples, only two species were found: carob tree (*Ceratonia siliqua* L.) and heather (*Erica multiflora* L.). In total 17 samples were classified as unifloral honey, 10 as carob tree honey, 6 as heather honey, and only 1 as unifloral ivy honey (*H. helix*), while the remaining 8 samples were identified as multifloral. It was found that, along with ivy pollen grains, ivy honey contains a significant share of pollen from both heather and carob tree.

The physicochemical properties of ivy honey were investigated in our previous study (Flanjak et al., 2024), where, in addition to the mentioned parameters, the color of the honey was also determined, characterized as light amber (46-77 mPfund). On the other hand, Ciulu et al. (2016) stated in their study that ivy honey can vary from extra light amber to dark amber, with an average value of 100 mm on the Pfund scale, classifying it as dark honey. After evaluation of the sensory properties of investigated ivy honey samples, it can be concluded that ivy honey is prone to fast crystallization with fine crystals, which gives it a delicate texture. It's aroma is pronounced, herbal, reminiscent of ivy flowers, with a strong chemical note. The taste is persistent, with weak sweetness and a absent acidity. The aroma is intense and persistent, similar to its scent (Table 2).

Table 2. Sensory characteristics of ivy honey (n= 10)

Visual assessment	Colour intensity: light amber
	Colour tone: light brown in liquid state, and light (milky-yellow, beige) in crystallized state
Olfactory assessment	Intensity of odour: moderate
Tasting assessment	Description: aromatic, chemical, reminiscent of ivy flowers
	Sweetness: weak
	Acidity: weak
	Bitterness: absent
	Intensity of aroma: highly persistent
	Description of aroma: herbal, chemical, warm tones, refreshing
Physical characteristics	Persistence/aftertaste: medium, gradually develops
	Crystallization rate: fast, pasty mass with fine crystals

Conclusions

Based on the preliminary results of melissopalynological and sensory analysis of ivy honey, it was found that its uniflorality (% of ivy pollen grains) ranged from 51% to 98%. In addition to ivy pollen, pollen from 9 other plant families and 10 species was identified. After determining the sensory properties, it can be concluded that ivy honey is prone to rapid crystallization with fine crystals, which gives it a delicate texture. Its aroma is pronounced, herbal, reminiscent of ivy flowers, with a strong chemical note. The taste is persistent, with moderate sweetness and absent acidity. The results of this research will contribute to the characterization of ivy honey as the occurrence of this honey in a unifloral form is extremely rare, and there is almost no available data about it in scientific and professional literature.

References

- Boi M., Llorens J. A., Cortés L., Lladó G., Llorens L. (2013). Palynological and chemical volatile components of typically autumnal honeys of the western Mediterranean. *Grana*. 52(2): 93-105.
- Brudzynski K. (2023). Unexpected Value of Honey Color for Prediction of a Non-Enzymatic H₂O₂ Production and Honey Antibacterial Activity: A Perspective.
- Ciulu M., Piana F., Spano N., Sanna G. (2016). Preliminary analytical characterization of new beehive products from Italy: the case of ivy honey. 8èmes Journées Franco-Italiennes de Chimie-8° Giornate Italo-Francesi di Chimica. Avignon
- Cuevas-Glory L. F., Pino A. J., Santiago S. L., Sauri-Duch E.(2007). Analytical, Nutritional and Clinical Methods. A review of volatile analytical methods for determining the botanical origin of honey. *Food Chemistry*. 103: 1032-1043.
- Flanjak I., Bubalo D., Prđun S., Svečnjak L., Bilić Rajs B. (2024). Contribution to the description of rare unifloral honeys: ivy (*Hedera helix* L.) honey. 6th International Symposium on Bee Products and Annual Meeting of International Honey Commission-Book of abstracts, pp. 56-57.
- Franjić J., Škvorc Ž. (2010). Šumsko drveće i grmlje Hrvatske. Sveučilište u Zagrebu, Šumarski fakultet.
- Kremer D., Brajković J., Bubalo D. (2021). Medonosno bilje regije Gacke. Hrvatska gospodarska komora.
- Ordinance on the quality of unifloral honey (2009). Ministry of agriculture, fisheries and rural development, Official Gazette NN 122/09)

- Prđun S. (2017). Skupljačka aktivnost pčelinje zajednice na paši i sastav nektara i meda unšijske mandarine (*Citrus unshiu* Marc.). Doktorski rad. Sveučilište u Zagrebu, Agronomski fakultet.
- Ricciardelli d'Albore, G. (1998). Mediterranean melissopalynology; Università degli Studi di Perugia, Facoltà di Agraria, Istituto di Entomologia Agraria: Perugia, Italy.
- Svečnjak L., Bubalo D., Flanjak I., Bilić Rajs B., Prđun S. (2024). New unifloral honey types with high electrical conductivity: challenges related to incompatibility with the EU legislation on honey. 6th International Symposium on Bee Products and Annual Meeting of International Honey Commission. Book of abstracts, pp 31-32.
- Vahčić N., Matković D. (2009). Kemijske, fizikalne i senzorske značajke meda. Available from: www.pcelinjak.hr.
- Von der Ohe, K., Von der Ohe, W. (2000). Celle's mellisopalynological Collection; Niedersächsisches Landesinstitut für Bienenkunde: Celle, Germany.
- Von der Ohe W., Persano Oddo L., Piana L., Morlot M., Martin P. (2004). Harmonised methods of melissopalynological analysis, *Apidologie*, 35, special issue. 18-25.

ORIGINAL SCIENTIFIC PAPER

The analysis of morphological characteristics honey bees from breeding centers in the Bosnia and Herzegovina

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Summary

The aim of the study was to determine, through the analysis of the morphological characteristics of the cubital index, the area of the corbicular, and the length of the spur, if there is an influence of other honey bee subspecies on the indigenous Carniolan honey bee *Apis mellifera carnica* Polman 1879, from the Republic of Srpska, Bosnia and Herzegovina. The obtained research results indicate the existence of certain differences in the morphological characteristics measured on worker bees at registered breeding centers in the Republic of Srpska, Bosnia and Herzegovina. Further selective breeding work should continue the preservation of the indigenous Carniolan honey bee at our breeding centers, which should be achieved through continuous morphometrical control of the queens that serve as mother colonies at the breeding centers.

Key words: analysis, morphometric characteristic, honey bee, selection

Introduction

The determination of morphological characteristics is a mathematical-statistical method that studies variability among individual bees and is used as an auxiliary method in calculating the average values of certain parameters in beekeeping selection. Alpatov (1948), Goetze, G. (1959), Ruttner (1988) introduces the value of the cubital index as one of the relative values for determining the subspecies of honeybee. Across the broader area of the European continent, the honeybee species is spread with several of its subspecies, among which the most prominent are: dark European honey bee (*Apis mellifera mellifera*), Carniolan honey bee (*Apis mellifera carnica*), Caucasian honey bee (*Apis mellifera caucasia*), and Italian honey bee (*Apis mellifera ligustica*). Carniolan honey bee is primarily spread in the southeastern Europe and the Balkans. This bee breed is well-covered with short gray hairs all over its body, which is why it has also received the additional name "gray." To preserve the purity of the breed, a specific system for evaluating external characteristics is required, and the aforementioned four breeds of the honeybee differ from each other based on external signs (morphological and morphometric characteristics), as well as biological traits. Variability between individual bees in terms of phenotype is determined through morphometric analysis. Among the most commonly used morphometric characteristics are: the color of the rings, the width of the tomentum, the length of the hairs on the fifth tergite, the length of the proboscis or tongue, the cubital index, and the size of the corbicular. Bees with a longer proboscis have the ability to collect nectar from plants that have deep tubular cups, where they produce nectar, such as red clover. Kulinčević et al. (1997) reported 6.4 -6.8 mm proboscis length in honey bee from Banat. Nakrst (2008) showed that median length of the proboscis of *A. m. carnica* is 6.54 mm (n = 1387), which is longer,

compared to some other sub-species like *A. m. mellifera* (≈ 6 mm) and *A. m. ligustica* (6.3–6.6 mm), but shorter compared to *A. m. caucasia* (≈ 7 mm). The cubital index is the ratio of sides a and b of the third cubital cell on the front part of the worker bees' wings. The lower vein of the third cubital cell is divided into two parts, a and b. The average cubital index for the gray bee is 2.7, and it ranges from 2.3 to 3.0 (Brandorf et al., 2019). Poklukar (1992) determined that the surface area of the corbicula in the gray bee race is 2.647 mm². The aim of this research was to determine if there is an influence of other honey bee subspecies on the indigenous Carniolan bee in study area, through the analysis of the morphological characteristics of cubital index, area of the corbicula, and length of the spur.

Material and methods

The research was conducted in 2023, and worker honey bee samples were taken from 10 registered breeding centers for queen production in the Republic of Srpska, Bosnia and Herzegovina. The measurements were carried out in the Entomology Laboratory of the Faculty of Agriculture, University of Banja Luka. One sample consisted of 5 to 10 worker bees from each of the 10 breeding centers, with 24 samples taken per center, totaling 240 samples. The samples were preserved in 70% alcohol at a temperature of -5°C. The bees were dried, and dissection was performed using dissection tools, including scalpels and tweezers. After dissection, the tongue, right forewing and third hind leg were separated, and preparations for further morphological research were analyzed using an Olympus SZX 10 microscope (magnification power of 10 to 60 times) with an attached software QuickPHOTO CAMERA 3.2. The cubital index represents the ratio between sides a and b of the third cubital cell on the forewing of a worker bee. The length of the proboscis was obtained by measuring the distance from the submentum, across the mentum, to the tip of the tongue, and the sum of these segments gives the total proboscis length. The surface area of the tibia was determined by measuring its length and width. The obtained data were presented using standard descriptive measures, arithmetic mean, and standard deviation ($\bar{x} \pm s_{\bar{x}}$). Comparison of the examined data groups was made using general linear models. The statistical significance of the obtained differences was set at $p \leq 0.001$. This significance threshold was selected due to the historical importation of several thousand *Apis meda* queen bees into Bosnia and Herzegovina. The objective of this study was to assess whether this introduction has had a measurable impact on the current apicultural practices in Bosnia and Herzegovina and neighboring.

Results and discussion

The range of value of cubital index in our study ranged from 2.3 to 3.0, which is characteristic of the Carniolan bee breed, with the average value of the cubital index for the Carniolan bee being 2.7 (Rutnner, 1988; Gregorc et al., 2010). The minimum value of the cubital index (2.489) was recorded at the Zvornik 2 breeding center, while the highest value (2.863) was recorded at the Rudo breeding center. There is a significant difference between the values of cubital index from all the breeding centers $F(df_1, df_2) = 11.72, p < 0.001$. Mladenovic et al. (2006) in their research conducted in Serbia determined that the size of the cubital index of bees ranged from 2.55 to 2.89. Trozic (2006), studying the cubital index of bees from the Una-Sana Canton in the Federation of Bosnia and Herzegovina, found that the average value of the cubital index is 2.63.

Table 1. Value of the Cubital index

Breeding centers (location)	Cubital index [mm]		
	$\bar{x}$	$\pm$	SD
Derventa	2.608	$\pm$	0.39
Zvornik 1	2.817	$\pm$	0.34
Rudo	2.863	$\pm$	0.34
Sokolac	2.736	$\pm$	0.28
Srbac	2.571	$\pm$	0.27
Petrovo	2.692	$\pm$	0.30
K. Dubica	2.534	$\pm$	0.36
Banja Luka	2.632	$\pm$	0.49
Zvornik 2	2.489	$\pm$	0.51
Milici	2.578	$\pm$	0.50
$F_{\text{repr.}}, p_{\text{repr.}}$	11.72**, $p < 0.001$		
$LSD_{0,05}$	0.099		

The obtained values for the surface area of the tibia of the hind leg indicate that the largest tibia surface area was found in bees from the Derventa breeding center, at 2.939 mm², while the smallest was found in bees from the Milici breeding center, at 2.556 mm². Statistical analysis of the obtained results revealed a significant statistical difference between all the breeding centers in the study $F(df1, df2) = 123.71^{**}$, $p < 0.001$.

Tabela 2. The value of the tibia (shin) surface area.

Breeding centers (location)	The surface area of the corbicula [mm]		
	$\bar{x}$	$\pm$	SD
Derventa	2.939	$\pm$	0.22
Zvornik 1	2.834	$\pm$	0.05
Rudo	2.794	$\pm$	0.08
Sokolac	2.776	$\pm$	0.05
Srbac	2.724	$\pm$	0.05
Petrovo	2.765	$\pm$	0.04
K. Dubica	2.701	$\pm$	0.11
Banja Luka	2.665	$\pm$	0.10
Zvornik 2	2.631	$\pm$	0.10
Milici	2.556	$\pm$	0.12
$F_{\text{repr.}}, p_{\text{repr.}}$	123.71**, $p < 0.001$		
$LSD_{0,05}$	0.027		

According to Stevanovic (2002), research conducted in Serbia indicates that the average surface area of the tibia (shin) was 2.82 mm². According to Aliagic (2005), the values for the tibia surface area, measured on a sample of 300 bees from six localities in the Una-Sana Canton of

the Federation of Bosnia and Herzegovina, resulted in an average tibia surface area of $x = 2.626 \text{ mm}^2$. Identical values were confirmed by the research of Stanimirovic et al. (2000) and Poklukar et al. (1994).

The longest spur (tongue) length was found in bees from the Zvornik 1 breeding center, measuring 7.186 mm, while the shortest proboscis length was found in bees from the Milici breeding center, measuring 6.077 mm. Statistical analysis of the obtained results revealed a highly significant difference between the breeding centers $F (df1, df2) = 73.83^{**}$, $p < 0.001$, while the recorded tongue length values did not deviate from those characteristic of the Carniolan bee, taking into account the standard deviation values.

Table 3. Value of the Length of the Bee's Tongue (Spur)

Breeding centers (location)	Length of the Bee's Tongue [mm]		
	$\bar{x}$	$\pm$	SD
Derventa	6.236	$\pm$	0.968
Zvornik 1	7.186	$\pm$	0.494
Rudo	6.707	$\pm$	0.642
Sokolac	7.183	$\pm$	0.246
Srbac	6.958	$\pm$	0.433
Petrovo	7.144	$\pm$	0.193
K. Dubica	7.014	$\pm$	0.289
Banja Luka	6.440	$\pm$	0.535
Zvornik 2	6.761	$\pm$	0.445
Milići	6.077	$\pm$	0.457
$F_{\text{repr.}, \text{prepr.}}$	73.83**, $p < 0.001$		
$LSD_{0,05}$	0.131		

According to the values for the length of the tongue (proboscis) of the bee (Ruttner, 1988), the range length of the Carniolan bee's tongue is 6.4 - 6.8 mm, with variations from 6.0 mm to 7.0 mm. Rados et al. (2008), determining the morphological characteristics of bees from three localities in the Vojvodina region, concluded that the average tongue length was 6.4 mm. Additionally, Mladenovic et al. (2008), measuring bees from six localities in southern Serbia, concluded that the average tongue length was 6.59 mm. Similar results were found by Trozic (2006), measuring the tongue length of bees from the Una-Sana Canton, where the average tongue length was 6.56 mm, with variations from 6.3 mm to 7.0 mm.

Conclusions

Based on the research results, it was found that the value of the cubital index of the bees ranged from 2.48 to 2.86, with an average value of 2.65. The values for the surface area of the tibia (shin) of the bee samples ranged from 2.55 to 2.93 mm², with an average value of 2.73 mm². The length of the proboscis of the bees in this study ranged from 6.0 to 7.1 mm, with an average value of 6.7 mm. Further selective breeding work and the application of modern selection methods (controlled mating) should continue, with full support from the relevant ministries and scientific institutions. The process of preserving the native Carniolan bee breed at the breeding centers in the Republic of Srpska, Bosnia and Herzegovina, should be maintained, and this approach should take on a regional character.

References

- Alijagić S. (2005). Morfometrijske karakteristike medonosne pčele *apis mellifera*, Sarajevo.
- Alpatov V. V. (1948). Porody medonosnoi pchely, Moskva: Izd-vo Moskovskogo obshchestva ispytatelei prirody.
- Brandorf A.Z., Rodrigues M. (2019). The Origin of the European Bees and their Intraspecific Biodiversity, Phylogenetics of Bees.
- Goetze G. (1959). Die Bedeutung des Fluegelgeaeders fuer die zuechterische Bedeutung der Honigbiene, Zeitschrift fuer Bienenforschung 4: 141-148.
- Gregorc A., Lokar Vesna (2010). Selection criteria in an apiary of carniolan honey bee (*apis mellifera carnica*) colonies for queen rearing, Journal of Central European Agriculture. 11(4):401-408.
- Kulinčević J., Krunić M., Stanisavljević LJ. (1997). Medonosne i druge vrste domestifikovanih pčela kao genetički, ekološki i privredni resursi Jugoslavije. Savremena poljoprivreda, Vol. 46 br. 1-2: 257-268.
- Mladenović M. (2006). Medna produktivnost nekih selekcijskih linija matica u Srbiji, XIV naučno savjetovanje sa međunarodnim učešćem „Zaštita i proizvodnja domaće pčele i meda“, 12-12. Februar, Beograd, 7-13.
- Mladenović M., Simeonova V. (2008). Morfometrijske karakteristike pčela reprotanica medonosne pčele na jugu Srbije, XVI Naučno savjetovanje sa međunarodnim učešćem, Zemun, 1-7.
- Nakrst M. (2008). Morphological characterization of honeybee (*Apis mellifera carnica*) workers and queens in queen rearing apiaries. Graduation thesis. (68 p), University of Maribor.
- Poklukar J. (1992). Genetski parametri površine tibije domaće pčele (*Apis mellifera carnica*) i proizvodnja meda, Disertacija, Agronomski fakultet Sveučilišta u Zagrebu.
- Poklukar J., Kezić N., Pechhacker H. (1994). Cubital index and behavioural characteristic of the honey bee *Apis mellifera carnica*, Apidologie. 5 (25): 494-495.
- Radoš R., Mladenović M. (2008). Morfometrijske karakteristike žute medonosne pčele u Vojvodini, XVI naučno savjetovanje „Kvalitetom i selekcijom u pčelarstvu ka Evropi“, 9-10. Februar 2008., Poljoprivredni fakultet Beograd, Zbornik plenarnih naučnih radova.
- Ruttner F. (1988). Biogeography and Taxonomy of Honeybees. Springer-Verlag, Berlin.
- Stanimirović Z., Soldatović B., Vučinić M. (2000). Biologija pčela, Medicinska knjiga Beograd.
- Stevanović J. (2002). Istraživanja morfometrijske i hromozomske varijabilnosti u funkciji očuvanja diverziteta kranjske medonosne pčele (*Apis mellifera carnica* Pollman, 1879) na teritoriji Srbije, Magistarska teza, Univerzitet u Beogradu, Veterinarski fakultet.
- Trožić-Borovac S. (2006). Karakteristike medonosne pčele (*Apis mellifera* L.) na području Unsko-Sanskog kantona, Šumarski fakultet, Sarajevo.

Session 3

Agroecology and Regenerative Agriculture

ORIGINAL SCIENTIFIC PAPER

Evaluation of phytoremediation potential of plants from *Asteraceae* family

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A field study was conducted to evaluate the phytoremediation potential of several medicinal plants from the *Asteraceae* family, including costmary, tansy, yarrow, wormwood, and tarragon. The studied species demonstrated notable tolerance to heavy metal stress and were capable of growing on highly contaminated soils. Wormwood was identified as a potential accumulator of Cd and Pb, and an excluder of Zn, yarrow - a potential Cd accumulator and an excluder for Pb and Zn, tansy - an indicator plant for Cd and an excluder for Pb and Zn, costmary and tarragon - excluder for all three metals. The concentrations of heavy metals in the essential oils remained below the maximum permissible levels, indicating their suitability for use in pharmaceutical, cosmetic, and related industries.

Key words: essential oils, heavy metals, phytoremediation, polluted soils

Introduction

Soil pollution is a major environmental and human health concern worldwide. In Europe alone, there are more than 2 million potentially contaminated sites (Liedekerke et al., 2014), the vast majority of which are polluted with heavy metals (Antoniadis et al., 2017). One promising remediation approach is the use of plants to extract heavy metals from contaminated soils, a process known as phytoremediation. This emerging technology offers several advantages, including in situ application, low cost, and environmental sustainability (Raveau et al., 2021). Medicinal and aromatic plants (MAPs) represent a promising group for phytoremediation applications. These plants are predominantly cultivated for their by-products - especially essential oils - thus minimizing the risk of introducing heavy metals into the food chain. Essential oils are high-value commodities used extensively in perfumery, cosmetics, medicine, and increasingly in plant protection products, providing potential economic returns from the biomass produced (Raveau et al., 2020). However, the effects of heavy metal contamination on plant growth, essential oil yield and composition, and the capacity of these species to accumulate metals are still under investigation. Current research remains limited, especially regarding their long-term phytoremediation potential. This study aimed to conduct a comparative investigation to (i) determine the concentration and distribution (organs of accumulation) of Pb, Zn, and Cd in the vegetative and reproductive parts of selected *Asteraceae* medicinal plants - namely, costmary, tansy, yarrow, wormwood, and tarragon - and in their essential oil products, and (ii) assess their potential for use in the phytoremediation of heavy metal-contaminated soils.

Material and methods

The experiment was conducted on an agricultural field contaminated with Zn, Pb, and Cd, located approximately 0.5 km from the source of pollution - the Non-Ferrous Metal Works (NFMW) near Plovdiv, Bulgaria. Seedlings of the selected medicinal plants - costmary (*Tanacetum balsamita* L.), tansy (*Tanacetum vulgare* L.), yarrow (*Achillea millefolium* L.),

wormwood (*Artemisia absinthium* L.), and tarragon (*Artemisia dracunculus* L.) - were purchased and planted in the spring. In the second and third years following planting, samples were collected during the flowering stage. The samples were then dried at room temperature to achieve air-dry mass. Essential oils from the aromatic plants were extracted under laboratory conditions by steam distillation for 2 hours using a Clevenger-type apparatus. The pseudo-total metal content in the soil was determined according to ISO 11466. The available (mobile) fractions of heavy metals were extracted using a DTPA solution following ISO 14870. Concentrations of Pb, Zn, and Cd in plant organs (roots, stems, leaves, flowers) and essential oils were measured after microwave-assisted digestion. Quantitative analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES, Jobin Yvon Emission – JY 38 S, France). The digestion procedure and ICP-OES accuracy were validated with a certified reference material - apple leaves (SRM 1515, National Institute of Standards and Technology, NIST).

Results and discussion

Soils

The total concentrations of Zn, Pb, and Cd in the contaminated soils were significantly elevated, ranging from 2423.9 to 3196.1 mg kg⁻¹ for Zn, 2509.1 to 2966.9 mg kg⁻¹ for Pb, and 64.3 to 92.0 mg kg⁻¹ for Cd. These values far exceed the maximum permissible concentrations (MPCs) for agricultural soils - 400 mg kg⁻¹ for Zn, 100 mg kg⁻¹ for Pb, and 3.0 mg kg⁻¹ for Cd (Table 1). Table 1 also presents the concentrations of mobile (bioavailable) forms of these metals, extracted using DTPA. The data indicate that cadmium exhibited the highest mobility, with its mobile forms accounting for 57.2–67.3% of the total Cd content in soil. Lead followed with a mobile fraction of 33.8–42.0%, while zinc had the lowest mobility, with only 9.8–10.9% of its total content present in mobile form.

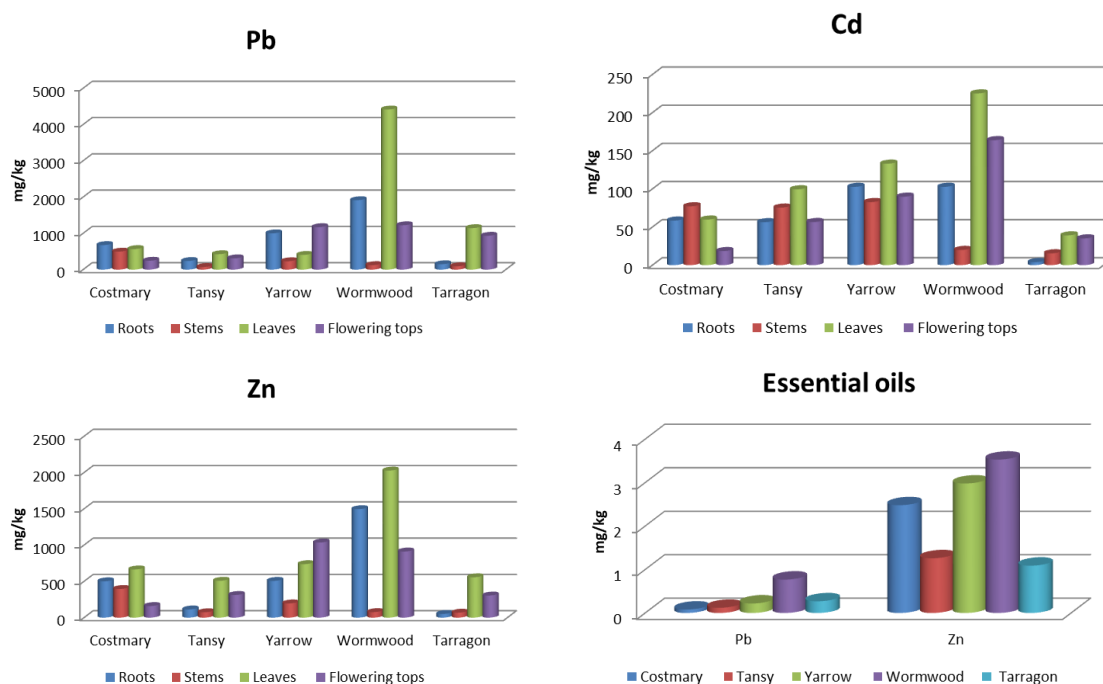
Table 1. Total content and DTPA-extractable Pb, Zn, Cd (mg kg⁻¹) in soils sampled from NFMW-Plovdiv

Distance from NFMW	pH	Pb, mg kg ⁻¹ x±sd		Zn, mg kg ⁻¹ x±sd		Cd, mg kg ⁻¹ x±sd	
		Total	DTPA	Total	DTPA	Total	DTPA
0.5 km from NFMW (S1)	7.6	2966.9 ±24.5	1244.7 ±13.2	3196.1 ±13.5	339.1 ±1.8	92.0 ±0.8	61.9 ±0.5
0.5 km from NFMW (S2)	7.5	2509.1 ±6.5	849.1 ±0.5	2423.9 ±6.3	237.8 ±1.2	64.3 ±0.6	36.8 ±0.3
MAC	>7.4	100		400		3.0	

x- average value (mg kg⁻¹) from 5 repetitions; sd - standard deviation; MAC (maximum allowable concentrations)

Medicinal and aromatic plants

The concentrations of Pb, Cd, and Zn in the vegetative and reproductive organs of the studied plants are presented in Graph 1. Pb content in the roots ranged from 148.8 mg kg⁻¹ in tarragon to 1913.0 mg kg⁻¹ in wormwood. Cd concentrations in the roots were lowest in tarragon (3.92 mg kg⁻¹) and highest in yarrow and wormwood (102.6 and 102.7 mg kg⁻¹, respectively). Zn content in the roots varied from 50.9 mg kg⁻¹ in tarragon to 1,495.2 mg kg⁻¹ in wormwood.



Graph 1. Content of Pb, Zn and Cd (mg/kg) in tested plants

Pb concentrations in stems ranged from 56.5 mg kg⁻¹ in tarragon to 495.1 mg kg⁻¹ in costmary, while in leaves, Pb levels ranged from 405.6 mg kg⁻¹ in yarrow to a maximum of 4,414.4 mg kg⁻¹ in wormwood (Graph 1). Cd concentrations in stems ranged from 1.3 mg kg⁻¹ in tarragon to 82.8 mg kg⁻¹ in yarrow, while in leaves they varied between 9.8 mg kg⁻¹ and 225.1 mg kg⁻¹, with wormwood showing the highest levels. Zn concentrations in stems ranged from 68.5 to 395.1 mg kg⁻¹, while leaf concentrations varied between 508.0 mg kg⁻¹ and 2026.6 mg kg⁻¹. Imelouane et al. (2011) reported elevated levels of Pb (1036.1 mg kg⁻¹), Cd (20.6 mg kg⁻¹), and Zn (798.1 mg kg⁻¹) in wormwood leaves, attributed to road traffic emissions. In contrast, the present findings exceed the levels reported by Chizzola (2012) and Badea (2015), who noted relatively low metal accumulation in similar plants. The results contradict Murtic et al. (2019), who found that yarrow had a limited capacity for heavy metal uptake and accumulation. Pb concentrations in flowers ranged from 244.1 mg kg⁻¹ in costmary to 1223.3 mg kg⁻¹ in wormwood. Cd content ranged from 18.4 to 163.9 mg kg⁻¹, with maximum levels again found in wormwood. Zn content ranged from 159.9 mg kg⁻¹ in costmary to 911.5 mg kg⁻¹ in wormwood and 1035.3 mg kg⁻¹ in yarrow. The content of heavy metals in essential oils extracted from the studied plants was significantly lower than in their aerial parts and flowers. This suggests that heavy metals do not readily transfer into the oil phase during distillation. Measured concentrations were: Pb: 0.08 mg kg⁻¹ in costmary to 0.77 mg kg⁻¹ in wormwood; Zn: 1.09 mg kg⁻¹ in tarragon to 3.53 mg kg⁻¹ in wormwood; Cd: below the quantification limits of the analytical method. These values are well below the regulatory maximum permissible limits for heavy metals in essential oils (5 mg kg⁻¹ for Pb and 1 mg kg⁻¹ for Cd, Council of Europe, 2021), indicating that the oils meet the criteria for environmentally safe products. The results are in agreement with Angelova et al. (2015), who reported minimal heavy metal transfer into essential oils, regardless of soil contamination levels.

Bioaccumulation factor (BCF) and translocation factor (TF)

To assess the phytoremediation potential of the studied species, the bioconcentration factor (BCF) and translocation factor (TF) were calculated according to the following equations:

$$TF = C_{\text{shoots}} / C_{\text{roots}}$$

where C_{shoots} represent the element contents (in mg kg^{-1}) in the plant shoots, and C_{roots} shows the element concentration in the roots (in mg kg^{-1})

$$BCF = C_{\text{plant parts}} / C_{\text{soil}}$$

where $C_{\text{plant parts}}$ represent the element contents (in mg kg^{-1}) in the plant organs (roots, stems, leaves, seeds), and C_{soil} (in mg kg^{-1}) shows the element concentration in the corresponding soils.

Table 2. Bioaccumulation factor (BCF) and Translocation factor (TF)

	BCFroots			BCFleaves			TF		
	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn
Costmary	0.23	0.64	0.16	0.19	0.65	0.21	0.84	1.02	1.33
Tansy	0.08	0.61	0.04	0.14	1.08	0.16	1.79	1.77	4.50
Yarrow	0.40	1.65	0.21	0.16	2.14	0.30	0.41	1.30	1.46
Wormwood	0.76	1.65	0.62	1.76	3.61	0.84	2.31	2.19	1.36
Tarragon	0.06	0.06	0.02	0.46	0.63	0.23	7.69	9.96	10.93

The results presented in Table 2 reveal key differences in the translocation and accumulation efficiency of Pb, Cd, and Zn among the studied plant species. TF values for Pb ranged from 0.41 in costmary to 7.69 in tarragon. TF for Cd and Zn values were consistently > 1 across all species. BCFroots for Pb ranged from 0.06 in tarragon to 0.76 in wormwood; all values were < 1 , suggesting excluder behavior; for Cd - from 0.06 in tarragon to 1.65 in yarrow and wormwood. BCF > 1 for Cd was only observed in yarrow and wormwood, indicating their potential as Cd accumulators; for Zn - from 0.02 in tarragon to 0.62 in wormwood; all species showed values < 1 , indicating Zn exclusion at the root level. BCFleaves for Pb ranged from 0.14 in tansy to 1.76 in wormwood. Only wormwood exhibited BCF > 1 , indicating Pb accumulation in leaves; for Cd - from 0.63 in tarragon to 3.61 in wormwood. BCF > 1 was found in tansy (1.08), yarrow (2.14), and wormwood (3.61), identifying them as potential Cd accumulators; for Zn - all species had BCF values < 1 for Zn in leaves, confirming Zn exclusion from leaf tissues.

Conclusions

Based on the results obtained, the following important conclusions can be drawn:

1. Heavy metal tolerance and phytoremediation potential: Costmary, tansy, yarrow, wormwood, and tarragon demonstrated high tolerance to elevated heavy metal concentrations in soils (Zn: 2423.9–3196.1 mg kg^{-1} ; Pb: 2509.1–2988.9 mg kg^{-1} ; Cd: 64.3–92.0 mg kg^{-1}). These species are suitable for cultivation on heavily contaminated sites and show potential for application in phytoremediation strategies.
2. Phytoremediation classification: Wormwood - Accumulator of Cd and Pb; Excluder for Zn; Yarrow - Accumulator of Cd; Excluder for Pb and Zn; Tansy - Indicator for Cd; Excluder for Pb and Zn; Costmary and Tarragon - Excluders for Pb, Cd, and Zn
3. Safety and economic feasibility of essential oil use: The Pb, Cd, and Zn concentrations in the essential oils extracted from these species were significantly below the maximum permissible limits. Therefore, these oils are suitable for use in pharmaceutical, cosmetic,

and other industrial applications. Furthermore, essential oil production from plants grown in contaminated areas offers a sustainable strategy to offset phytoremediation costs by adding economic value.

Acknowledgement

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References

- Angelova V.R., Grekov D.F., Kisyov V.K., Ivanov K.I. (2015). Potential of Lavender (*Lavandula vera* L.) for phytoremediation of soils contaminated with heavy metals. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 9: 465-472.
- Antoniadis V., Levizou E., Shaheen S.M., Ok Y.S., Sebastian A., Baum C., Prasad M.N.V., Wenzel W.W., Rinklebe J. (2017). Trace elements in the soil-plant interface: phytoavailability, translocation, and phytoremediation—a review. *Earth-Science Reviews*, 171: 621-645.
- Badea D. N. (2015). Determination of potentially toxic heavy metals (Pb, Hg, Cd) in popular medicinal herbs in the coal power plant area. *Rev. Chim.* Vol 66, 11321136.
- Council of Europe. (2021). *European Pharmacopoeia 10th Edition* (with supplements 10.6-10.7-10.8). Strasbourg.
- Chizzola R. (2012). Metallic mineral elements and heavy metals in medicinal plants. *Medicinal and aromatic plant science and biotechnology*, 6: 39-53.
- Imelouane B., Tahri M., Elbastrioui M., Aouinti F., Elbachiri A. (2011). Mineral Contents of Some Medicinal and Aromatic Plants Growing in Eastern Morocco. *Journal of Materials and Environmental Science*, 2:104-111.
- ISO. (1995). *Soil quality - Extraction of trace elements soluble in aqua regia*, [ISO 11466: 1995]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2001). *Soil Quality- Extraction of Trace Elements by Buffered DTPA Solution*. [ISO 14780:2001]. Geneva, Switzerland: International Organization for Standardization.
- Liedekerke M., Prokop G., Rabl-Berger S., Kibblewhite M., Louwagie G. (2014). Progress in the management of contaminated sites in Europe. European Commission, Joint Research Centre, and Institute for Environment and Sustainability. (Luxembourg: Publications Office).
- Murtic S., Jurkovic S.J., Basic E., Hekic E. (2019). Assessment of wild plants for phytoremediation of heavy metals in soils surrounding the thermal power station. *Agronomy Research*, 17: 234-244.
- Raveau R., Fontaine J., Bert V., Perlein A., Tisserant B., Ferrant P., Lounès-Hadj Sahraoui A. (2021). In situ cultivation of aromatic plant species for the phytomanagement of anaged-trace element polluted soil: Plant biomass improvement options and techno-economic assessment of the essential oil production channel. *Science of the Total Environment*, 789: 147944. Available from: <http://www.sciencedirect.com/>
- Raveau R., Fontaine J., Lounès-Hadj Sahraoui A. (2020). Essential oils as potential alternative biocontrol products against plant pathogens and weeds: a review. *Foods*, 9: 365. <https://doi.org/10.3390/foods9030365>.

ORIGINAL SCIENTIFIC PAPER

Conservation tillage effects on weed population of maize

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Summary

The experiment conducted in Križevci (16°33'32" E, 46°01'38" N, 141 m) in 2021 aimed to investigate the impact of conservation tillage on maize weediness. The tillage treatments included conventional tillage (CT), deep conservation tillage (CTD), and shallow conservation tillage (CTS). Weed assessments were performed at V7 and R5 stage of maize growth and included: weed density (D), weed biomass (B), number of weed species (SN), weed coverage (C) and Shannon-Wiener index (H'). The highest B (8.18 g m⁻²), SN (3.00 m⁻²) and H' (0.77) in V7 growth stage were recorded on CTS. CTS treatment resulted in the highest values ($P < 0.05$) of weediness parameters in R5 growth stage with the exception of weed density (D). Shallow conservation tillage (CTS) increased maize weediness with increasing weed diversity.

Key words: soil conservation, tillage, weed species, maize weediness

Introduction

In conditions of increasingly adverse weather conditions due to present climate changes and the orientation of agricultural production towards sustainability, conservation agriculture is becoming one of the fundamental principles in modern agricultural production. The concept of conservation agriculture is based on proper crop rotation, soil cultivation without plowing and constant soil coverage with plant residues of minimum 30% (Jug et al., 2025). Conservation tillage positively affects numerous soil properties by increasing its fertility, water conservation, yield stability while reducing costs and simultaneously increasing biodiversity (Palm et al., 2013, Pittelkow et al., 2015). Despite the numerous advantages of conservation tillage, the widespread and general application of such tillage systems is still sometimes limited by certain factors that often relate to expected changes in the level of weed infestation of field crops (Derrouch et al., 2020). With the availability of numerous measures for sustainable weed management, weeds continue to represent a limiting factor in agricultural production, and conservation tillage certainly changes the composition of the weed flora and the level of weed infestation, but not necessarily with negative consequences for the crop in terms of yield reduction (Brozović et al., 2023). Tillage can have both positive and negative effects on weed occurrence. Thus, annual and biennial weeds can be destroyed by tillage, which can also stimulate the germination and emergence of new weed species. The results of numerous studies indicate different changes in weed infestation in conservation reduced tillage systems (Nichols et al., 2015, Winkler et al., 2023, Travlos et al., 2018). By introducing conservation tillage systems, weed communities adapt to new conditions, which means a change in the composition of the weed flora in a particular agroecological area in which conservation tillage systems are introduced. One of the significant characteristics of weed plants is their high and rapid adaptability to new environmental conditions (Sosnoskie et al., 2006). Therefore, the aim of this work was to determine the impact of different conservation tillage systems on maize weediness.

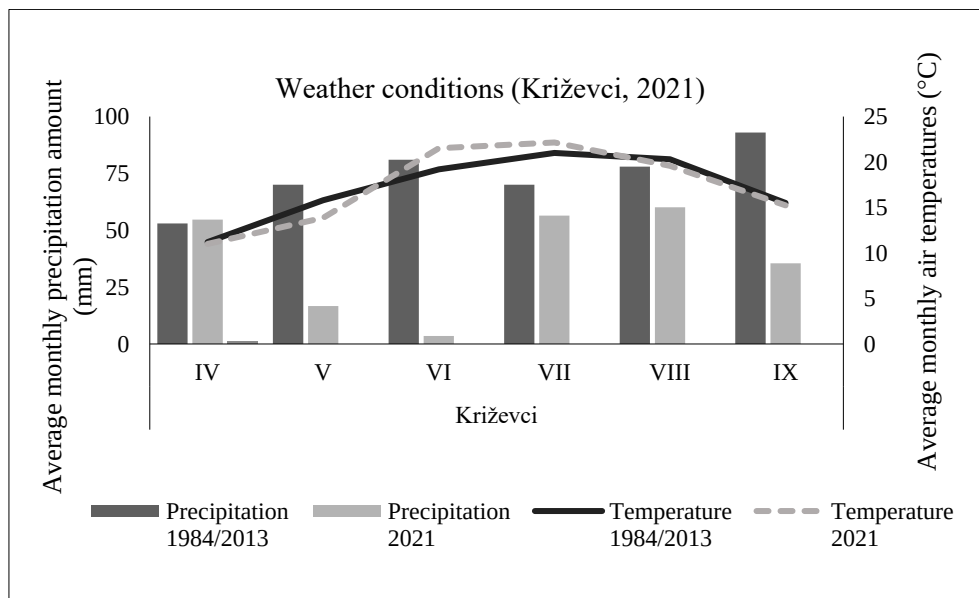
Material and methods

A field experiment with conservation tillage systems was conducted in Križevci (16°33'32" E, 46°01'38" N, 141 m) in 2021. The soil tillage treatments included: conventional tillage (CT), which involved plowing to a depth of 30 cm; deep conservation tillage (CTD), which consisted of loosening the soil to 30 cm with at least 30% of the surface covered by crop residues; and shallow conservation tillage (CTS), which involved loosening the soil to a depth of 10 cm with a minimum of 50% soil surface coverage by crop residues. Plowing was conducted using the KUHN Multi-master 121 plow, and soil loosening was carried out with the Maschio Artiglio Magnum 300/7 loosener in autumn 2020. Pre-sowing soil preparation for maize planting involved a single pass with the spike-tooth harrow and hollow roller (Agromerkur). A weed survey was conducted twice during the maize growing season, focusing on the following parameters: weed density (D), aboveground weed biomass (B), weed coverage (C), weed species identification, and weed species number (SN). Weed density, defined as the total number of weeds per unit area, was determined by counting all classified weed species in a randomly selected 0.25 m² area, with four repetitions per plot. Total weed coverage was visually assessed (Vitta et al. 1996). within the same sampling area. For the biomass evaluation, weeds were clipped at ground level, separated by species, and dried at 60°C for 48 hours to measure dry aboveground biomass. The first weed sampling occurred during the V7 maize growth stage (seven fully developed leaves with visible collars) at June 22 2021. The second weed observation was made during the R5 maize growth stage (dent stage), September 20 2021. The Shannon-Wiener index (H') was used to assess the biodiversity of weed flora. This index takes into account the abundance and distribution of individual species and allows for a comparison of biodiversity between different tillage systems. Weed control was applied using a HARDI Commander 3300 L sprayer through all treatments uniformly. Glyphosate (360 g/L) was applied as a pre-sowing treatment at a rate of 1.5 L/ha. A post-emergence application of Tembotrione (44 g/L) was made at a rate of 2.25 L/ha when the maize reached the V4 growth stage. The impact of various tillage systems on weed density, weed biomass, weed coverage, weed species diversity was assessed using a factorial ANOVA design, with soil tillage as factor. Mean values found to be significant based on the F-test were compared using the LSD test at a significance level of $p < 0.05$. The ANOVA analysis was conducted using the Statistica software package, version 14.0.0 (TIBCO Software Inc., Palo Alto, CA, USA).

Results and discussion

Throughout the maize growing season, crop development was markedly influenced by unfavorable agroclimatic conditions, particularly a significant precipitation deficit and atypical air temperature patterns. During May, recorded precipitation was approximately 75% lower than the multi-year average (1984 – 2013), while in June only 3.5 mm of rainfall was registered, indicating pronounced drought conditions. These adverse weather patterns persisted until the end of the growing season, with a continuous lack of rainfall contributing to increased drought stress on the crop. In terms of temperature, deviations from the long-term average were also observed. April and May were characterized by slightly below-average air temperatures, whereas in June and July, average temperatures exceeded the long-term mean. This temperature rise, combined with limited precipitation, further exacerbated soil moisture depletion, adversely affecting maize growth and associated weed dynamics under different tillage systems. The typical composition of the maize weed flora in the studied agroecological areas was observed during the experiment (Knežević et al., 2003), where six weed species were identified in selected treatments, with the dominance of the annual grass *Setaria viridis* (L.) P. Beauv. (green foxtail) and the perennial broadleaf weed *Symphytum officinale* (L.) (common comfrey). In addition to the listed species, the following

weeds were also present: *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass), *Oxalis fontana* Bunge (common yellow-sorrel), *Calystegia sepium* (L.) R.Br. (hedge bindweed), and *Cirsium arvense* (L.) Scop. (Canada thistle).



Graph 1. Weather conditions during investigation (Križevci, 2021)

Tillage had a notable impact on maize weediness throughout the growing season, except for weed coverage (C) during the V7 maize growth stage and weed density in R5. The highest weed density (D) and weed coverage (C) in the first sampling were recorded on the conventional tillage system (ST) with the lowest number of weed species (Table 1) and Shannon Weiner index at the same time (H') in comparison to conservation tillage systems. The above indicates a greater dominance of a single species and less diversity on conventional tillage system which is consistent with Brozović et al. (2023). Higher weed density on conventional tillage is confirmed by Shrestha et al. (2022). According to Chauhan et al. (2006) reduced weediness in conservation tillage systems in the earlier stages of maize development is a consequence of the suppressive effect of crop residues on weed emergence and early growth development, while later in the vegetation, crop residues can stimulate weed growth through water conservation and nutrient release. The suppressive effect of crop residues on weed growth is especially important in the early stages of maize development due to pronounced competition for growth resources (light, water, nutrient).

Table 1. Maize weediness in V7 growth stage

Tillage (T)	D (m ⁻²)	B (g m ⁻²)	SN (m ⁻²)	C (%)	H'
CT	87.33 ^a	3.21 ^b	1.33 ^b	25.67	0.19 ^b
CTD	39.33 ^b	2.55 ^b	2.67 ^a	11.33	0.29 ^b
CTS	24.00 ^b	8.18 ^a	3.00 ^a	22.33	0.77 ^a
F(T)	*	*	*	n.s.	*

T-tillage, CT-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, D-weed density, B-weed biomass, SN- species number, C-weed coverage, F(T)- F test for tillage, H' -Shannon Weiner index. Mean values with the same letters are not statistically different at $P < 0.05$

The effect of soil tillage on weed density varies, as confirmed by Ruisi et al. (2015). This is also evident in the current research, where the impact of soil tillage on weed density (D) was not observed in the second opservation (R5) while weed biomass (B), weed coverage (C) and H' showed noticable rise on conservation tillage systems among two sampling (Table 2). The treatment with the highest weediness in terms of tested parameters was CTS (shallow conservation tillage) with statistically significant differences ($p < 0.05$) established compared to other tillage treatments with exception of weed density (Table 2) which is in line with Winkler et al. (2023) and Travlos et al. (2018). Recorded weed biomass on CTS treatment in R5 growing phase (Table 2) was over five times grater compared to first sampling (Table 1). Hofmeijer (2019) also demonstrated significantly higher weed biomass in reduced tillage compared to conventional tillage, with a gradual increase from the beginning of the growing season to the later stages. Additionally, previous research has confirmed the increase in both weed coverage and the number of weed species (Armengot et al., 2016, Mulugeta et al., 2001) in reduced tillage systems. The above was confirmed by this research with the highest weed species number (SN) and weed coverage (C) values in R5 maize growth stage followed with the highest value of Shannon-Wiener index (H') on shallow conservation tillage system (CTS).

Table 2. Maize weediness in R5 growth stage

Tillage (T)	D (m^{-2})	B (g m^{-2})	SN (m^{-2})	C (%)	H'
CT	36.67	7.22 ^b	1.33 ^c	12.33 ^b	0.14 ^b
CTD	37.67	17.35 ^b	2.33 ^b	18.67 ^b	0.41 ^b
CTS	21.33	42.59 ^a	4.00 ^a	50.67 ^a	8.30 ^a
F(T)	n.s.	*	*	*	*

T-tillage, CT-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, D-weed density, B-weed biomass, SN- species number, C-weed coverage, F(T)- F test for tillage, H' -Shannon Weiner index. Mean values with the same letters are not statistically different at $P < 0.05$

Conclusions

Conservation tillage systems influenced changes in the level of weed infestation in maize compared to the conventional tillage system. The investigated weed infestation parameters differed with respect to the determined values of the total number of weeds per unit area, weed biomass, number of weed species and Shannon-Wiener index. Reducing the depth of soil cultivation and increasing the amount of crop residues on the soil surface led to an increase in the weed biomass, weed species number and weed coverage. Conservation tillage systems in this study resulted in an average increase in the Shannon-Wiener index which indicates a positive impact of such tillage systems on biodiversity. Conservation tillage systems led to an average increase in the tested parameters of weed infestation in maize and further research should certainly include more parameters such as agroecological conditions and ultimately the achieved yield of maize.

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References

- Armengot L., Blanco-Moreno J., Bàrberi P., Bocci G., Carlesi S., Aendekerk R. (2016). Tillage as a driver of change in weed communities: A functional perspective. *Agriculture, Ecosystems & Environment*. (222): 276–285.
- Brozović B., Jug I., Đurđević B., Ravlić, M., Vukadinović, V., Rojnica I., Jug D. (2023). Initial Weed and Maize Response to Conservation Tillage and Liming in Different Agroecological Conditions. *Agronomy*. (13): 1116.
- Chauhan B.S., Gill G.S., Prseton C. (2006). Tillage systems effects on weed ecology, herbicide activity and persistence: A review. *Aust. J. Exp. Agric.* (46): 1557–1570.
- Derrouch D., Chauvel B., Felten E., Dessaint F. (2020). Weed Management in the Transition to Conservation Agriculture: Farmers' Response. *Agronomy*. (10): 843.
- Hofmeijer M.A.J., Krauss M., Berner A., Peigné J., Mäder P., Armengot L. (2019). Effects of Reduced Tillage on Weed Pressure, Nitrogen Availability and Winter Wheat Yields under Organic Management. *Agronomy* (9): 180.
- Jug D., Jug I., Brozović B., Šeremešić, S., Dolijanović Ž., Zsembeli J., Ujj A., Marjanovic J., Smutny V., Dušková, S. et al. (2025). Conservation Soil Tillage: Bridging Science and Farmer Expectations—An Overview from Southern to Northern Europe. *Agriculture*. (15): 260.
- Knežević M., Đurkić M., Knežević I., Lončarić Z. (2003). Effects of pre- and post-emergence weed control on weed population and maize yield in different tillage systems. *Plant Soil Environ*. (49): 223–229.
- Mulgeta D., Stoltenberg D.E., Boerboom C. M. (2001). Weed Species-Area Relationships as Influenced by Tillage. *Weed Sci*. (49): 217–223.
- Nichols V., Verhulst N., Cox R., Govaerts B. (2013). Weed dynamics and conservation agriculture principles: A review. *Field Crop. Res.* (183): 56–68.
- Palm C., Blanco-Canqui H., De Clerck F., Gatere P. (2013). Conservation agriculture and ecosystem services: An overview. *Agriculture, Ecosystems & Environment*. 187: 87–105.
- Pittelkow C.M., Linquist B.A., Lundy M.E., Liang X., Van Groenigen K.J., Lee J., Gestel N., Six J., Venterea R.T., Van Kessel C. (2015). When does no-till yield more? A global meta-analysis. *Field Crops Res.* (183): 156–168.
- Ruisi P., Frangipane B., Amato G., Badagliacca G., Di Miceli G., Plaia A., Giambalvo D. (2015). Weed seedbank size and composition in a long-term tillage and crop sequence experiment. *Weed Res.* (55): 320–328.
- Shrestha A., Knežević S.Z., Roy R.C., Ball-Coelho B.R., Swanton C.J. (2022). Effect of tillage, cover crop and crop rotation on the composition of weed flora in a sandy soil. *Weed Res.* (42): 76–87.
- Sosnoskie L.M., Herms C.P., Cardina J. (2018). Weed seedbank community composition in a 35-yr-old tillage and rotation experiment. *Weed Sci.* (54): 263–273.
- Tibco Software Inc., Statistica (data analysis software system) 2020, version 14. <http://tibco.com>
- Travlos I.S., Cheimona N., Roussis I., Bilalis D.J. (2018). Weed-Species Abundance and Diversity Indices in Relation to Tillage Systems and Fertilization. *Frontiers in Environmental Science*. (6): 1-10.
- Vitta J.I., Quantilla C.F. Canopy measurements as predictors of weed competition. *Weed Sci*. 1996, 44, 511–516.
- Winkler J., Dvořák J., Hosa J., Martínez Barroso P., Vavřková M.D. (2023). Impact of Conservation Tillage Technologies on the Biological Relevance of Weeds. *Land*. (12): 121.

ORIGINAL SCIENTIFIC PAPER

Scientific challenges in quantifying and verifying GHG emissions in agriculture

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(idjekic@agrif.bg.ac.rs)***Summary**

Calculating greenhouse gas emissions in agriculture consists of four stages: (i) collecting the data; (ii) defining emission factors; (iii) calculation and (iv) interpretation. The main objective was to quantify scientific challenges related to GHG emissions in agriculture. The main challenges were evaluated by employing the Failure Mode and Effect Analysis method. Challenges associated with the first stage are related to the occurrence of limitations within the defined boundaries of the food organization and/or food being studied, as well as problems related to the quality of collected data. The main criterion behind emission factors is to use adequate values from trustful databases. In the calculation stage, selection of functional units is of utmost importance. Interpretation stage has two main challenges – sensitivity and comparability of the results. The main challenge with the risk of 80, is the use of different, sometimes incomparable functional units, that disable benchmarking across the same agricultural sector misleading carbon footprint results. Verification also brings its challenges in terms of how it's performed and by whom. This research brings to attention the need to combat any greenwashing associated with food production.

Key words: climate change, carbon footprint, food chain.

Introduction

Living in the period of climate change, researchers worldwide seek for two types of answers: what are the causes of such a phenomenon and what are potential mitigation measures. The Intergovernmental Panel on Climate Change emphasizes that human activities covering the entire industry joint with agriculture are recognized as contributors to climate change (IPCC, 2022; Leal Filho et al., 2022). However, a stumbling stone is the estimation of the anthropogenic contribution compared to natural processes such as volcanic eruptions, ocean currents, the Earth's orbital changes and solar variations (Đekić, 2009).

Bearing in mind that agriculture and the entire food production chain have an interaction with the climate change, some activities are directed at limiting the increase of temperature to 1.5 °C (UN, 2015) while others are focused on mitigating measures of climate change affecting food production (Doelman et al., 2019). Challenges in food production comprise of producing enough food for feeding people and understanding the interaction between climate change and food production throughout the food supply chain. To better understand the impact of food production on climate change in terms of greenhouse gas (GHG) emissions, it is common to calculate the “carbon footprint”. Depending on the approach, carbon footprint may be calculated on company level or on product level. Company-based approach calculates GHG emissions in line with methodologies outlined in ISO 14064-1 (ISO, 2018a) or GHG protocols (GHG, 2015). Product-based approach calculates GHG emissions in line with methodologies outlined in ISO 14067 (ISO, 2018b) and ISO 14040 (ISO, 2006).

In company-based calculations, it is important to define categories (total six categories in ISO 14064-1) / scopes (total three scopes in GHG Protocols) that will be included in the calculations. When the calculation is focused on specific food, it is important to clearly define the system boundaries or subsystem within the food supply chain. A typical food supply chain consists of four subsystems: primary production, food processing, retail and households (Djekic et al., 2018).

The main aim of this paper is to elaborate the main scientific challenges in quantifying and verifying GHG emissions in agriculture at both company and product levels.

Material and methods

To estimate specific scientific challenges, Failure Mode and Effects Analysis (FMEA) is a method that can be used as a risk-based tool (Xiao et al., 2011). It easily captures potential failures / nonconformities, lightens their causes and enables effects analysis (IEC, 2016). FMEA requires classification of potential failures and evaluation of risks (Papadopoulos et al., 2011). To evaluate failures when calculating carbon footprint, Table 1 outlines the main phenomena that can have failures associated with different stages of the process.

Table 1. Inventory of phenomena associated with carbon footprints

Carbon footprint studies	Phenomenon
Collecting the data	Setting categories / scope and boundaries
	Use of questionnaires
	Quality of data
Defining emission factors	Access to databases
	Use of correct emission factors
Calculation	Functional unit choice
	Conversion of units
Interpretation	Potential for benchmarking
	Sensitivity analysis

The FMEA risk priority number (RPN) comprises three factors (IEC 2016):

$$RPN = S \times O \times D \quad (1)$$

where symbols are: (S) severity; (O) occurrence; and (D) detection. Risks are associated with difficulties or failures resulting in inadequate GHG emission calculations. Table 2 outlines weighting factors that were modified in line with previous research of Djekic et al. (2019).

Table 2. Rating scale of the three factors - Severity, Occurrence and Detection

Severity		
Rank	Consequence	Description
1	None	No failure(s)
2	Minor	Failure(s) associated with results for one inventory / emission factor
3	Low	Failure(s) associated with results of one subsystem / one category
4	Major	Failure(s) associated with results within more than one subsystem / category
5	Severe	Failure(s) associated with results for the organization / throughout the entire life-cycle
Occurrence		
Rank	Probability	Description
1	Very unlikely	Minimal probability of occurrence of failure(s) as a result of <i>force majeure</i>
2	Unlikely	Occurrence of failure(s) only as a result of misuse of software
3	Possible	Occurrence of failure(s) only because of errors in man-made calculations/estimations
4	High probability	Occurrence of failure(s) will occur only for certain type of products / organizations
5	Certain	Occurrence of failure(s) in each subsystem of the entire life-cycle or in each category for organizations
Detection		
Rank	Criteria	Description
1	Very high	Failure(s) associated with results is easily detected
2	High	Failure(s) associated with results is detected during inventory phase
3	Low	Failure(s) associated with results is detected during calculation phase
4	Remote	Failure(s) associated with results is detected during interpretation phase
5	Never	No possibility of identifying failure(s)

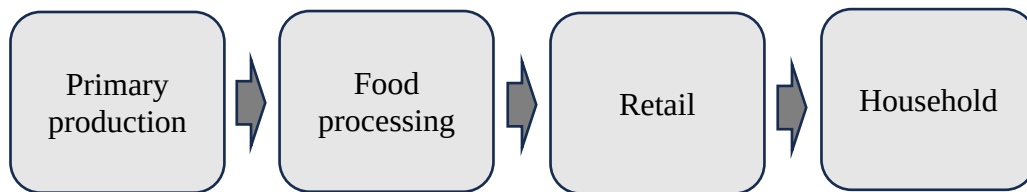
Results and discussion

Table 3 depicts results from the FMEA analysis highlighting phenomena with highest risks, when performing any type of carbon footprint calculations.

Table 3. FMEA of carbon footprint

No	Stage	Phenomenon	Failure	Potential Model Effect?	Severity (S)	Occurrence (O)	Detection (D)	Risk
1	Collection of data	Setting categories / scope and boundaries	Limitations within boundaries	Important data not included	5	4	2	40
			Limitations within subsystems / categories	Important data not included	4	4	2	32
2		Use of questionnaires	Too long questionnaire (subject doesn't fill all the data)	Inadequate data	4	4	2	32
			Generic questionnaire for all types of companies / food	Inadequate data	4	4	2	32
3		Quality of data	Incorrect data collected	Inadequate data	4	3	2	24
			Generic data collected	Inadequate data	4	4	2	48
4	Defining emission factors	Access to databases	Use of generic open databases	Effect on carbon footprint calculation	2	4	3	24
			Use of old (out-of-date) databases		2	4	3	24
5		Use of emission factors	Improper emission factors		2	3	3	18
6	Calculation	Functional unit choice	Different functional units in the supply chain / life cycle	Effect on carbon footprint calculation	4	4	3	48
7		Conversion of units	Calculation mistake		2	3	3	18
8	Interpretation	Potential for benchmarking	Incomparable functional unit	Misleading carbon footprint results	5	4	4	80
9		Sensitivity analysis	High level of input uncertainties		4	3	4	48

Within the first stage 'collection of data', one of the most important activities is to clearly define categories (for organization) and/or scope and boundaries (for life cycle assessment of a product), i.e. what will be included from the perspective of the supply chain / life cycle, Figure 1.

**Figure 1.** Generic food supply chain with four main actors

A food supply chain is typically a very complex food system with four main actors: primary producers, food processors, retailers and consumers (Djekic and Tomasevic, 2020; Djekic et al., 2018). Depending on the food system in focus (from organizational or product perspective), the actors differ. In meat production more actors may be introduced such as - livestock farms (with or without considering feed producers), slaughterhouses, meat processors, retailers and consumers (Djekic and Tomasevic, 2016). In agricultural systems, it's important to consider if the whole plant cycle is covered or not, and in the case of perennial crops, how many years are analyzed (Djekic et al., 2018). Depending on the geographic perspective, there are "local", "regional" and/or "global" supply chains (Gamboa et al., 2016). Finally, short food supply chains promoted in the EU agricultural policy have only two actors – producer and consumer (Malak-Rawlikowska et al., 2019).

Potential limitations in the possibility of collecting data from different actors and part of the supply chain / life cycle directly influence the results. This brings additional issues as follows: (a) collection methods (on-site / off-site visits); (b) assumptions causing variability and impreciseness; (c) exclusion of different processes / actors within system boundaries (i.e. exclusion of transportation activities between actors in the supply chain, generalization of waste management scenarios, etc.), (Djekic et al., 2019).

When it comes to the quality of data, it is important to emphasize that incorrect / generic data will affect further calculations results in misleading carbon footprint. Typical formula for calculating carbon footprint is displayed in equation 1:

$$CP = \sum_{i=1}^n Q_i * EF_i \quad /1/$$

Where CP – carbon footprint is the sum of the GHG emissions of the entire inventory included in the scope and boundary (supply chain / life cycle); EF – emission factor of an “i” inventory expressed as CO_{2e} (carbon dioxide equivalent) and Q – quantity of an “i” inventory.

Based on the data collected from the actors it is important to capture emission factors of the entire list of inventories collected. Additional issues that arise are sources of the emission factors that can be found in different carbon footprint and/or life cycle assessment databases, as follows: who collects these emission factors in the databases, is the database open or not, how often is it updated, does it provide emission factors for a specific inventory associated with the country of origin or not, etc. This causes potential mistakes and affects carbon footprint calculation.

Use of inconsistent or incomparable functional units poses a great risk (Djekic et al., 2019). To additionally explain the risk, we can observe a meat supply chain and identify three different functional units: one kg of livestock; one kg of carcass and/or one kg of meat or meat product (Djekic and Tomasevic, 2016). When it comes to dairy products, functional units may be 1 L of raw but also 1 kg of dairy products (Djekic et al., 2014). Additional functional units developed in the food sectors are associated with nutritional values such as: Energy (KJ / cal); Protein / digestible protein (g); Total fat / saturated fat / trans-fat (g); Carbohydrates / sugars (g) (Djekic et al., 2019).

To finalize the challenges, sensitivity analysis and emphasis on the input data and their influence on the final results are needed to gain trust in the results, using different statistical tools and models (Groen et al., 2017; Lewandowska et al., 2004).

Conclusions

Considering all these challenges in calculating GHG emissions, a logical question arises: “can we trust these data?”. The answers are both “yes and no”.

If the calculation is externally verified, the answer is “yes” as two main types of verifications exist. If a company wishes to share the carbon footprint of its emission to its customers / consumers (in line with ISO 14064-1 / ISO 14067), then third-party verification is performed by certification bodies in line with ISO 14064-3 (ISO, 2019). If a scholar wishes to publish data associated with life-cycle assessment of a food product throughout a food supply chain, then through a peer-review process, these data when published, become trustful sources for emission factor databases.

Why can the answer be “no”? Depending on the calculation approach, food technology and all the above-mentioned challenges, the results for same type of products can differ several times, misleading consumers worldwide. This implies a risk of inadequate interpretation of the results, drawing wrong and imprecise conclusions through the food chain affecting all actors from the farm to the fork and beyond including consumers. As a result, we are witnessing various pressures on the food chain accusing this sector of climate change with unclear and ambiguous statements. Therefor a more transparent approach is needed to combat any type of greenwashing associated with promoting and/or undermining “greener” food production.

References

- Đekić I. (2009). Upravljanje zaštitom životne sredine u proizvodnji hrane (1st Ed.). Beograd, Srbija: Poljoprivredni fakultet Univerziteta u Beogradu.
- Djekic I., Miocinovic J., Tomasevic I., Smigic N., Tomic N. (2014). Environmental life-cycle assessment of various dairy products. *Journal of Cleaner Production* 68(0):64-72.
- Djekic I., Pojić M., Tonda A., Putnik P., Bursać Kovačević D., Režek-Jambrak A., Tomasevic I. (2019). Scientific challenges in performing life-cycle assessment in the food supply chain. *Foods* 8(8):301.
- Djekic I., Sanjuán N., Clemente G., Jambrak A.R., Djukić-Vuković A., Brodnjak U.V., Pop E., Thomopoulos R., Tonda A. (2018). Review on environmental models in the food chain - Current status and future perspectives. *Journal of Cleaner Production* 176:1012-1025.
- Djekic I., Tomasevic I. (2016). Environmental impacts of the meat chain – Current status and future perspectives. *Trends in Food Science & Technology* 54:94-102.
- Djekic I., Tomasevic I. (2020). Role of Sustainable Quality in the Food Chain. In W Leal Filho, AM Azul, L Brandli, PG Özuyar & T Wall (Eds.), *Zero Hunger* (pp. 1-10). Cham: Springer International Publishing.
- Doelman JC, Stehfest E, Tabeau A, van Meijl H (2019) Making the Paris agreement climate targets consistent with food security objectives. *Global Food Security* 23:93-103.
- Gamboa G, Kovacic Z, Di Mazzo M, Mingorría S, Gomiero T, Rivera-Ferré M, Giampietro M (2016) The complexity of food systems: Defining relevant attributes and indicators for the evaluation of food supply chains in Spain. *Sustainability* 8(6):515.
- GHG. (2015). The Greenhouse Gas Protocol. In *A Corporate Accounting and Reporting Standard*. Amsterdam, The Netherlands: World Resources Institute and World Business Council for Sustainable Development.
- Groen E.A., Bokkers E.A.M., Heijungs R., de Boer I.J.M. (2017). Methods for global sensitivity analysis in life cycle assessment. *The International Journal of Life Cycle Assessment* 22(7):1125-1137.
- IEC. (2016). Failure modes and effects analysis (FMEA and FMECA). [IEC 60812:2016(E)]. Geneva, Switzerland: Commission Electrotechnique Internationale.
- IPCC. (2022). Summary for Policymakers. In. Hayama, Kanagawa, Japan: *Intergovernmental Panel on Climate Change*.
- ISO. (2006). Environmental management — Life cycle assessment — Principles and framework. [ISO 14040:2006(E)]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2018a). Greenhouse gases - Part 1: Specification with guidance at the organization level for quantification and reporting of greenhouse gas emissions and removals. [ISO 14064-1:2018(E)]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2018b). Greenhouse gases - Carbon footprint of products - Requirements and guidelines for quantification. [ISO 14067:2018(E)]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2019). Greenhouse gases - Part 3: Specification with guidance for the verification and validation of greenhouse gas statements. [ISO 14064-3:2019(E)]. Geneva, Switzerland: International Organization for Standardization.
- Leal Filho W., Setti A.F.F., Azeiteiro U.M., Lokupitiya E., Donkor F.K., Etim N.N., Matandirotya N., Olooto F.M., Sharifi A., Nagy G.J., Djekic I. (2022). An overview of the interactions between food production and climate change. *Science of The Total Environment* 838:156438.
- Lewandowska A., Foltynowicz Z., Podlesny A. (2004). Comparative lca of industrial objects part 1: lca data quality assurance — sensitivity analysis and pedigree matrix. *The International Journal of Life Cycle Assessment* 9(2):86-89.
- Malak-Rawlikowska A., Majewski E., Wąs A., Borgen S.O., Csillag P., Donati M., Freeman R., Hoàng V., Lecoer J-L, Mancini M.C. (2019). Measuring the economic, environmental, and social sustainability of short food supply chains. *Sustainability* 11(15):4004.
- Papadopoulos Y., Walker M., Parker D., Rüdé E., Hamann R., Uhlig A., Grätz U., Lien R. (2011). Engineering failure analysis and design optimisation with HiP-HOPS. *Engineering Failure Analysis* 18(2):590-608.
- UN. (2015). Adoption of the Paris Agreement. In FCoC Change (Ed.), *Report No. FCCC/CP/2015/L.9/Rev.1*. Paris, France: United Nations.
- Xiao N., Huang H-Z, Li Y., He L., Jin T. (2011). Multiple failure modes analysis and weighted risk priority number evaluation in FMEA. *Engineering Failure Analysis* 18(4):1162-1170.

ORIGINAL SCIENTIFIC PAPER

Potential Of *Gracilaria gracilis* For Improving Leptosol Quality And Mitigating Climate Change Impacts On Viticulture In Dalmatia

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Summary

Viticulture, vital in the Mediterranean, faces climate change threats, with altered precipitation and temperature impacting soil, yields, and quality. Dalmatia, a Croatian region covered in large part by shallow Leptosol type soil, is especially vulnerable to droughts and heat shifts, with this soil being particularly prone to erosion and poor water retention. This condensed review explores the potential of macroalgae, particularly *Gracilaria gracilis*, as a biofertilizer to improve soil health. Rich in nitrogen, phosphorus, and potassium, it can boost organic matter, microbial activity, water retention, and nutrient cycling, enhancing grapevine resilience. While promising, further experimental studies are needed to assess long-term impacts on Dalmatian vineyards.

Keywords: Climate Change, *Gracilaria gracilis*, Soil Quality

Introduction

Dalmatia is a coastal region in southeastern Croatia stretching from the Zrmanja River in the north to Konavle near the Montenegrin border in the south, encompassing a narrow mainland strip, Adriatic islands, and a mountainous hinterland extending toward the Dinaric Alps. This area relies heavily on viticulture to support the local economy and preserve the Mediterranean cultural heritage. According to the latest data, 5556 ha of land in Dalmatia is covered with vineyards with 35 million grapevines and 10802 registered producers rely on viticulture as their source of income (APPRR Vinogradarski Registar, 2023). Due to the expected effects of climate change, which could lead to 70% of the world's wine-growing regions becoming unsuitable for growing vines, this agricultural activity is at great risk (van Leeuwen et al., 2024). The risk is even higher in Dalmatia due to the characteristics of the Leptosol type soil which makes up about 20% of the soil in the Mediterranean region and is predominant type of soil on the Dalmatian Coast (Allam et al., 2020; Soil Bureau Network, n.d.; Tadić et al., 2014). It is a shallow, well-drained type of soil typically found on hard rock or calcareous parent materials (Ebelhar et al., 2008). The Leptosol soil has low water-holding capacity, which can benefit viticulture by encouraging grapevine roots to explore deeper soil layers for nutrients and water, improving grape quality and resilience to environmental stresses. Its well-drained nature also prevents waterlogging, reducing the risk of root diseases. However, this can be challenging in the context of climate change, as reduced water retention may make the soil more vulnerable to drought conditions. (Allam et al., 2020, Smart et al., 2006). To sustain vineyards on Leptosol, adaptation strategies such as drought-resistant grape varieties, improved irrigation management, soil amendments, and innovative approaches will be crucial in mitigating climate change effects (Jindo et al., 2022; Naulleau et al., 2021). One of the solutions could be introduction of macroalgae-derived biofertilizers. Since the red macroalga *Gracilaria gracilis* was shown to be one of the best candidates for cultivation, due to its resilience to salinity and temperature fluctuations and the ease of vegetative reproduction (Mouedden et al., 2024), thus allowing the production of

large biomass quantities, the aim of this paper was to evaluate its potential for altering the challenging properties of Leptosol and mitigating the effects of climate change on viticulture in the Dalmatian area, as it still hasn't been applied for this purpose.

Climate change effect on the Mediterranean soil and viticulture

In recent years, the Mediterranean basin has been increasingly experiencing the effects of climate change characterized by rising temperatures, shifting rainfall patterns, and more frequent extreme weather events (del Pozo et al., 2019), which poses a risk to the region's soil and agricultural productivity. Vineyards, vital to the Mediterranean's agricultural and cultural identity, are highly vulnerable to climate change, particularly soil erosion and desertification. Rising temperatures and irregular precipitation disrupt soil moisture and vegetation cover, increasing susceptibility to wind and water erosion. This threatens vineyards, especially on sloping terrains, as the loss of fertile topsoil reduces nutrient availability and water retention, jeopardizing grapevine health and yield. Erosion may also expose rocky subsoils, complicating grape cultivation without significant interventions (Rodrigo-Comino et al., 2018). The region has also seen a decline in soil organic matter content due to higher temperatures and changes in land use practices. Organic matter is crucial for maintaining soil fertility, structure and water holding capacity, and its loss can significantly affect soil health (Telo da Gama, 2023). The fertility of the soil in the vineyards is closely linked to the quality of the grapes, as poor soil fertility can lead to unbalanced vine growth, sub-optimal grape ripening and a reduced flavor profile, which affects the economic value of wine production (Hannah et al., 2013). In addition, the aggravation of drought and increased evaporation contribute to soil salinization, especially in low-lying coastal areas and regions that rely on irrigation. Increased salinity can inhibit plant growth, reduce crop yields and degrade soil structure, making it less able to retain water and nutrients (van Leeuwen et al., 2024; Rodrigo-Comino et al., 2018). Another critical issue is the disruption of microbial communities which play a key role in nutrient cycling, carbon sequestration, maintaining soil health and affect the availability of key nutrients like nitrogen and phosphorus (Classen et al., 2015). Additionally, altered microbial activity may influence the chemical composition of grapes and the resulting wine (Zarraonaindia et al., 2015). Mitigating and adapting to these impacts requires a multifaceted approach, including sustainable land management, reducing greenhouse gas emissions and addressing water scarcity to protect Mediterranean soils and ensure agricultural viability (FAO, 2019). Innovative solutions, such as the use of algal biostimulants, offer promising potential for improving soil health and plant resilience (Kholssi et al., 2022). Advances in cultivation and extraction techniques make macroalgae a promising solution for sustainable soil management and climate-resilient agriculture. Furthermore, due to the resilience of the *G. gracilis* to the environmental challenges during cultivation, this specie represents an excellent potential candidate for cultivation in the in integrated multitrophic aquaculture offering an eco-friendly biomass source through nutrient recycling and aquaculture by-products (Gamito et al., 2020).

Potential effects of *Gracilaria gracilis* on Leptosol quality and grapevine resilience

G. gracilis is a red macroalga whose main constituents include polysaccharides with gel-forming and water-binding properties, proteins, minerals, polyphenolic compounds and essential fatty acids, all of which contribute to its nutritional and bioactive potential (Francavilla et al., 2013). The polysaccharides from *G. gracilis*, namely agar (Yahyaoui et al., 2024), can act as natural soil conditioners by stabilizing soil aggregates, improving porosity and increasing water retention capacity, which are crucial functions in soils such as Leptosol (Akbar et al., 2024; Jang et al., 2024). Apart from the effects on soil characteristics, agar from *G. gracilis* also showed positive effects on kale seed germination which were

higher than in other three red seaweeds included in the study showing the potential for multifaceted roles of this alga (Pacheco et al., 2021). Furthermore, the organic matter contributed by the algal biomass can act like a sponge and improve moisture retention in the upper layers of Leptosol (Kholssi et al., 2022). Moreover, studies on related species of the *Gracilaria* genus suggest that these algae can regulate soil pH (Morales Aparicio et al., 2023) and reduce the carbon-to-nitrogen ratio (Ammar et al., 2022), enriching soils with essential nutrients such as nitrogen. It was shown that use of *Gracilaria* sp. biomass positively affects the soil quality in terms of carbon and nitrogen content with the highest results recorded after six months of use on clay loam soils, which are generally more fertile and have better water retention than Leptosols (Babatsikou, 2011). These results offer valuable insights, however, due to the shallow profile and limited fertility of Leptosols, further site-specific trials are necessary to validate these assumptions. Phytohormones such as auxins, cytokinins and gibberellins, which play an important role in promoting plant growth and stimulating root development, are also present in considerable quantities. In some species of the genus *Gracilaria*, the content of these phytohormones exceeds that in commercial organic fertilizers (Tsaniya et al., 2021). In addition, *G. gracilis* showed stimulation of auxin production in tomato plants by promoting biofilm production of auxin-promoting bacterial strains and also increased shoot length and biomass production in tomato plants, especially at early developmental stages, indicating potential for achieving same effects in grapevines (Rodrigues Dos Santos, 2023). Moreover, the bioactive molecules found in *G. gracilis*, especially polyphenols (Neves et al., 2023), may affect auxin transport and play its role in defense signaling of plants (Ahmed et al., 2020), further supporting the beneficial effects. The bioactive compounds in *Gracilaria* sp. can also support microbial activity in the soil, which improves nutrient cycling, availability and soil fertility (Kholssi et al., 2022). These properties have the potential to additionally promote deeper rooting in the Leptosol, which would contribute to the resistance of the vines to environmental stresses and at the same time increase the tolerance of the vines to abiotic stresses such as drought and heat. These combined effects underline the potential of *G. gracilis* as a natural solution to improve Leptosol quality and promote sustainable agriculture, especially in drought-prone environments.

Conclusions and future perspectives

Climate change threatens viticulture in Dalmatia by affecting soil conditions. Studies suggest that *G. gracilis* could improve Leptosol quality by enhancing soil structure, water retention, and microbial activity, increasing grapevine resilience to climate change. Future research is needed to assess *G. gracilis*' impact on grapevines and Leptosol, focusing on soil health, vine growth, and grape quality. These studies will help optimize application strategies and demonstrate *G. gracilis* as a sustainable soil amendment in viticulture. Integrating *Gracilaria* species into mariculture systems can boost productivity and sustainability, offering ecological and economic benefits.

References

- Ahmed, A., Tariq, A., & Habib, S. (2020). Interactive Biology of Auxins and Phenolics in Plant Environment. In *Plant Phenolics in Sustainable Agriculture* (pp. 117–133). Springer Singapore. https://doi.org/10.1007/978-981-15-4890-1_5
- Akbar, W. A., Rahim, H. U., & Rutigliano, F. A. (2024). Microbial- and seaweed-based biopolymers: Sources, extractions and implications for soil quality improvement and environmental sustainability - A review. *Journal of Environmental Management*, 359, 120964. <https://doi.org/10.1016/j.jenvman.2024.120964>

- Allam, A., Moussa, R., Najem, W., & Bocquillon, C. (2020). Hydrological cycle, Mediterranean basins hydrology. In *Water Resources in the Mediterranean Region* (pp. 1–21). Elsevier. <https://doi.org/10.1016/B978-0-12-818086-0.00001-7>
- Ammar, E. E., Aioub, A. A. A., Elesawy, A. E., Karkour, A. M., Mouhamed, M. S., Amer, A. A., & EL-Shershaby, N. A. (2022). Algae as Bio-fertilizers: Between current situation and future prospective. *Saudi Journal of Biological Sciences*, 29(5), 3083–3096. <https://doi.org/10.1016/j.sjbs.2022.03.020>
- APPRR Vinogradarski registar. (n.d.). <https://www.apprrr.hr/registri/>
- Babatsikou, E. , T. V. , P. E. , A. M. , B. G. , M. N. , Z. G. (2011). Developing a Biological Index of Soil Quality as an operational tool for the assessment of agricultural land management sustainability . In Th. Karyotis & D. Gabriels (Eds.), *Abstract Proceedings of the 6th International Congress of European Society for Soil Conservation – Innovative Strategies and Policies for Soil Conservation*.
- Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A. M., Cregger, M. A., Moorhead, L. C., & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, 6(8), 1–21. <https://doi.org/10.1890/ES15-00217.1>
- del Pozo, A., Brunel-Saldias, N., Engler, A., Ortega-Farias, S., Acevedo-Opazo, C., Lobos, G. A., Jara-Rojas, R., & Molina-Montenegro, M. A. (2019). Climate Change Impacts and Adaptation Strategies of Agriculture in Mediterranean-Climate Regions (MCRs). *Sustainability*, 11(10), 2769. <https://doi.org/10.3390/su11102769>
- Ebelhar, S.A.; Chesworth, W.; Paris, Q.; Spaargaren, O. (2008). Leptosols. In W. Chesworth (Ed.), *Encyclopedia of Soil Science. Encyclopedia of Earth Sciences Series*. (pp. 437–438). Springer International Publishing.
- FAO. (2019). *Soil erosion: the greatest challenge for sustainable soil management*. FAO.
- Francavilla, M., Franchi, M., Monteleone, M., & Caroppo, C. (2013). The Red Seaweed *Gracilaria gracilis* as a Multi Products Source. *Marine Drugs*, 11(10), 3754–3776. <https://doi.org/10.3390/md11103754>
- Gamito, S., Quental-Ferreira, H., Parejo, A., Aubin, J., Christensen, V., & Cunha, M. E. (2020). Integrated multi-trophic aquaculture systems: energy transfers and food web organization in coastal earthen ponds. *Aquaculture Environment Interactions*, 12, 457–470. <https://doi.org/10.3354/aei00375>
- Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., Zhi, L., Marquet, P. A., & Hijmans, R. J. (2013). Climate change, wine, and conservation. *Proceedings of the National Academy of Sciences*, 110(17), 6907–6912. <https://doi.org/10.1073/pnas.1210127110>
- Jang, C., Yang, B., Hong, W.-T., Ahn, J., & Jung, J. (2024). Soil improvement using agar gum polymer for seismic liquefaction mitigation. *Soil Dynamics and Earthquake Engineering*, 177, 108405. <https://doi.org/10.1016/j.soildyn.2023.108405>
- Jindo, K., Goron, T. L., Pizarro-Tobías, P., Sánchez-Monedero, M. Á., Audette, Y., Deolu-Ajayi, A. O., van der Werf, A., Goitom Teklu, M., Shenker, M., Pombo Sudré, C., Busato, J. G., Ochoa-Hueso, R., Nocentini, M., Rippen, J., Aroca, R., Mesa, S., Delgado, M. J., & Tortosa, G. (2022). Application of biostimulant products and biological control agents in sustainable viticulture: A review. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.932311>
- Kholssi, R., Lougraimzi, H., Grina, F., Lorentz, J. F., Silva, I., Castaño-Sánchez, O., & Marks, E. A. N. (2022). Green Agriculture: a Review of the Application of Micro- and Macroalgae and Their Impact on Crop Production on Soil Quality. *Journal of Soil Science and Plant Nutrition*, 22(4), 4627–4641. <https://doi.org/10.1007/s42729-022-00944-3>
- Morales Aparicio, M. M., Mateo Cid, L. E., Mendoza González, Á. C., & Ayala Niño, F. (2023). PROPUESTA DEL USO DE DOS ALGAS MARINAS EN UN SUELO ALCALINO CON UN CULTIVO DE ACELGA. In Palma López D.J.; Sánchez Hernández R. (Ed.), *Hacia un conocimiento global y multidisciplinario del recurso suelo* (pp. 252–257).
- Mouedden, R., Abdellaoui, S., El Madani, F., El Ouamari, N., Slimani, D., Kasmi, K., Taibi, M., Zahir, I., & Chaabane, K. (2024). *Gracilaria Gracilis* – A Review of Ecological Knowledge, Chemical Composition, Cultivation, and Applications. *Ecological Engineering & Environmental Technology*, 25(1), 276–287. <https://doi.org/10.12912/27197050/175506>

- Naulleau, A., Gary, C., Prévot, L., & Hossard, L. (2021). Evaluating Strategies for Adaptation to Climate Change in Grapevine Production—A Systematic Review. *Frontiers in Plant Science*, 11. <https://doi.org/10.3389/fpls.2020.607859>
- Neves, C., Morais, S., Vale, T., Soares, C., Grosso, C., Domingues, V. F., Vieira, M., & Delerue-Matos, C. (2023). Exploring the Bioactive Potential of *Gracilaria gracilis*: An Extraction Optimization Study Using Response Surface Methodology. *Foods* 2023, 13. <https://doi.org/10.3390/Foods2023-15026>
- Pacheco, D., Cotas, J., Rocha, C. P., Araújo, G. S., Figueirinha, A., Gonçalves, A. M. M., Bahcevandziev, K., & Pereira, L. (2021). Seaweeds' carbohydrate polymers as plant growth promoters. *Carbohydrate Polymer Technologies and Applications*, 2, 100097. <https://doi.org/10.1016/j.carpta.2021.100097>
- Rodrigo-Comino, J., Keesstra, S., & Cerdà, A. (2018). Soil Erosion as an Environmental Concern in Vineyards: The Case Study of Celler del Roure, Eastern Spain, by Means of Rainfall Simulation Experiments. *Beverages*, 4(2), 31. <https://doi.org/10.3390/beverages4020031>
- Rodrigues Dos Santos, A. S. (2023). *Deciphering putative biostimulant effects of a seaweed extract , from Gracilaria gracilis , in tomato plants*. University of Lisabon.
- Smart, D. R., Schwass, E., Lakso, A., & Morano, L. (2006). Grapevine Rooting Patterns: A Comprehensive Analysis and a Review. *American Journal of Enology and Viticulture*, 57(1), 89–104. <https://doi.org/10.5344/ajev.2006.57.1.89>
- Soil Bureau Network, E. (n.d.). *European soil : a global perspective Soil of the Mediterranean Region*.
- Tadić, M. P., Gajić-Čapka, M., Zaninović, K., & Cindrić, K. (2014). Drought Vulnerability in Croatia. In *Agriculturae Conspectus Scientifi cus* (Vol. 79, Issue 1). <http://www.dmcsee.eu/>
- Telo da Gama, J. (2023). The Role of Soils in Sustainability, Climate Change, and Ecosystem Services: Challenges and Opportunities. *Ecologies*, 4(3), 552–567. <https://doi.org/10.3390/ecologies4030036>
- Tsaniya, A. R., Dewi, E. N., & Anggo, A. D. (2021). Characteristics of liquid organic fertilizer from different composition types of seaweed between *Gracilaria* sp. And *Sargassum* sp. *Journal of Physics: Conference Series*, 1943(1). <https://doi.org/10.1088/1742-6596/1943/1/012071>
- van Leeuwen, C., Sgubin, G., Bois, B., Ollat, N., Swingedouw, D., Zito, S., & Gambetta, G. A. (2024). Climate change impacts and adaptations of wine production. *Nature Reviews Earth & Environment*, 5(4), 258–275. <https://doi.org/10.1038/s43017-024-00521-5>
- Yahyaoui, K., Traikia, M., Rihouey, C., Picton, L., Gardarin, C., Ksouri, W. M., & Laroche, C. (2024). Chemical characterization of polysaccharides from *Gracilaria gracilis* from Bizerte (Tunisia). *International Journal of Biological Macromolecules*, 266, 131127. <https://doi.org/10.1016/j.ijbiomac.2024.131127>
- Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. *MBio*, 6(2). <https://doi.org/10.1128/mBio.02527-14>

EXPERT PAPER

Capacity of Floodplains to Provide Ecosystem Services Based on Land Use Analysis

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Summary

Ecosystem services (ES) are the benefits that humans derive from nature, deeply intertwined with both human well-being and ecological health. Accurate mapping and visualization of ES, whether through monetary or non-monetary assessments, are crucial for understanding the supply-demand dynamics and assessing ecosystem conditions. Floodplains, as some of the most threatened ecosystems worldwide, provide vital and often unique ES. The paper introduces a method for mapping capacity of floodplain to provide ES, using the capacity matrix approach in combination with the CORINE LCL classification. The applicability, strengths, and limitations of this method are explored through a case study of the floodplain area of the Special Nature Reserve ‘Koviljsko-Petrovaradinski rit’, located within the Danube alluvium in North Serbia. The capacity of the area to provide the selected ecosystem service is absent in 11.51% of the area, while 53.28% has low capacity. Medium capacity is present in 1.24%, relevant capacity in 17%, and very high capacity in 16.97% of the area. No areas have high capacity.

Key words: Ecosystem services, CORINE LCL, Floodplains, Koviljsko-Petrovaradinski rit, Mapping

Introduction

Floodplains are geomorphological formations shaped by hydrological and biological processes, creating diverse natural conditions and habitat mosaics (Fischer et al., 2019; Tomscha et al., 2017). They are highly productive and provide numerous benefits to humans but are among the most endangered ecosystems due to climate change, land use changes, and human activities. Globally, floodplains have shrunk by approximately 50% (Tockner & Stanford, 2002), while in Europe, about 90% have been severely degraded (Tockner et al., 2009). In Serbia, only 10% of the original 338,000 hectares remain functional (Perosa et al., 2021). The concept of ecosystem services (ES) highlights the benefits nature provides to people, both directly and indirectly (Evans et al., 2022; La Notte, 2024; Meli et al., 2023). The concept and classification of ES were systematically introduced in the 2005 Millennium Ecosystem Assessment (MEA). This framework highlighted the importance of integrated management strategies for land, water, and other resources, advocating for conservation and sustainable use while acknowledging humans as a key component of ecosystems. Mapping ES is crucial for integrating them into sustainable decision-making (Burkhard and Maes, 2017; Daily and Matson, 2008; Maes et al., 2012), though challenges remain, such as data limitations, methodological uncertainties, and difficulties in capturing local-scale dynamics (de Sousa, 2018). In the Millennium Ecosystem Assessment (2005), it is highlighted that the most important direct driver of change in ecosystems is land-cover change, which includes ecosystem changes made by human interventions/management (Reyers et al., 2009). Land cover data, such as the CORINE Land Cover, provide essential inputs for ES evaluation. Proper mapping, whether in monetary or non-monetary terms, helps assess ES supply and

demand and ecosystem conditions (Burkhard and Maes, 2017). This study aims to demonstrate how to assess the floodplain capacity to provide ES, and to create thematic maps by using the methodology of Burkhard et al. (2009), focusing on the Special Nature Reserve "Koviljsko-Petrovaradinski Rit" (KPR) in Serbia. The research examines landscape aesthetic quality as a key ES defined by stakeholders within the IDES project (Srđević et al., 2021a).

Material and methods

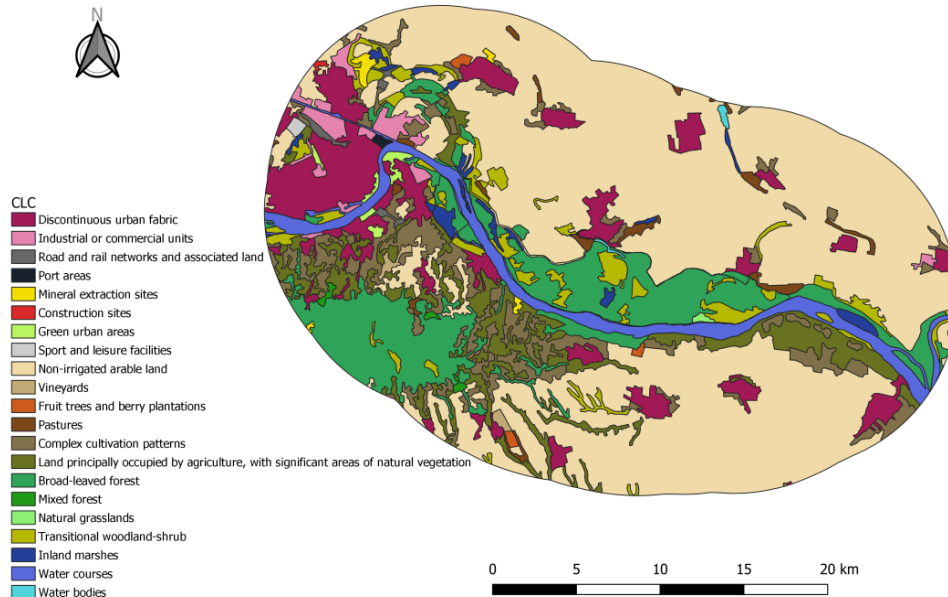
The floodplain of the SNR KPR along the Danube in northern Serbia is a vital area providing ES as flood protection, water purification, and climate resilience. Its forests and aquatic habitats support biodiversity and act as natural flood buffers. Beyond its ecological value, KPR holds cultural, historical, and recreational significance, promoting eco-tourism, education, and research. Traditional activities such as fishing and livestock grazing contribute to its sustainability. However, habitat loss, pollution, invasive species, and poor water management pose significant threats, highlighting the need for stronger conservation efforts (Ilić et al., 2024). The Copernicus Corine Land Cover Project (Gallardo & Cocero, 2023) provides a European land cover database with 44 classes, structured into a three-level nomenclature. This dataset is commonly used as a baseline for ES mapping in European case studies (Burkhard and Maes, 2017). Burkhard et al. (2009) introduced a methodology for mapping ES that assesses the capacity of land cover types to provide various ES. The approach is based on a matrix developed by experts, which cross-references 44 land cover types with 29 ES categories. Capacity is rated on a scale from 0 (no capacity) to 5 (very high capacity), with intermediate values indicating low (1), relevant (2), medium (3), and high (4) capacity, as described in Burkhard et al. (2009). Picture 1 illustrates part of this matrix, showcasing the capacity of different land cover types in the KPR study area to provide various ES. The last column highlights "Recreation & Aesthetic Values," used in this study to assess the floodplain's potential for the ES providing and mapping. While "landscape aesthetic quality" is not explicitly listed in Burkhard et al. (2009), it is encompassed within "Recreation & Aesthetic Values". To determine the land cover types, publicly available CORINE 2018 layers are used from the Internet address (<https://land.copernicus.eu/pan-european/corine-land-cover/clc2018>). Data analysis and processing have been done in the QGIS software, version 3.10.2.

	Ecological integrity	Biodiversity	Storage capacity	...	Provisioning services	Crops	Freshwater	...	Regulating services	Local climate regulation	Flood protection	...	Cultural services	Recreation & Aesthetic Values
Discontinuous urban fabric	7	1	1	...	3	1	0	...	0	0	0	...	...	0
Industrial or commercial units	2	1	0	...	0	0	0	...	0	0	0	...	...	0
Road and rail networks	4	2	0	...	0	0	0	...	0	0	0	...	...	0
Port areas	2	1	0	...	0	0	0	...	3	0	3	...	...	1
Mineral extraction sites	4	2	0	...	0	0	0	...	0	0	0	...	...	0
Construction sites	3	1	0	...	0	0	0	...	0	0	0	...	...	0
Green urban areas	18	3	2	...	2	0	0	...	11	2	0	...	...	3
Sport and leisure facilities	16	2	2	...	0	0	0	...	9	1	0	...	...	5
Non-irrigated arable land	22	2	4	...	21	5	0	...	5	2	1	...	...	1
Vineyards	14	2	2	...	5	4	0	...	3	1	0	...	...	5
Fruit trees and berries	21	3	3	...	13	5	0	...	19	2	2	...	...	5
Pastures	24	2	4	...	10	0	0	...	8	1	1	...	...	3
Complex cultivation patterns	20	3	3	...	9	4	0	...	5	2	1	...	...	2
Agriculture & natural vegetation	19	3	3	...	21	3	0	...	13	3	1	...	...	2
Broad-leaved forest	31	4	5	...	21	0	0	...	39	5	3	...	...	5
Mixed forest	32	5	5	...	21	0	0	...	39	5	3	...	...	5
Natural grassland	30	5	5	...	5	0	0	...	22	2	1	...	...	3
Transitional woodland shrub	21	4	2	...	5	0	0	...	3	1	0	...	...	2
Inland marshes	25	2	5	...	7	0	0	...	14	2	4	...	...	0
Water courses	18	4	1	...	12	0	5	...	10	1	2	...	...	5
Water bodies	23	4	4	...	12	0	5	...	7	2	1	...	...	5

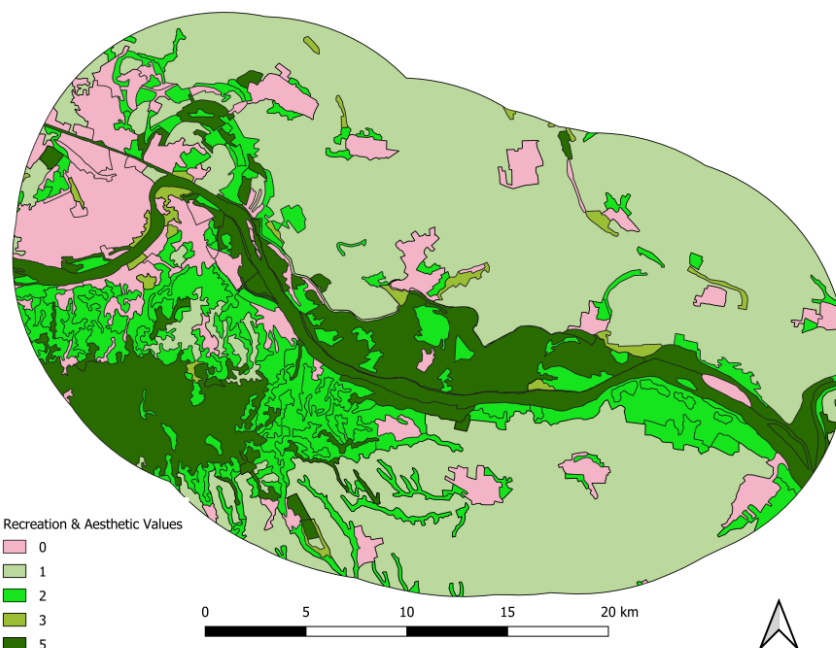
Picture 1. Matrix for the assessment of the different capacity of land cover types to provide ecosystem services (Adapted from Burkhard et al., 2009)

Results and discussion

Based on stakeholder engagement findings (Srđević et al., 2021b), a mapping of KPR's capacity to provide ecosystem services was conducted. Picture 2 shows the study area's land cover classes, including a 10 km buffer zone, with 21 distinct types, predominantly agricultural land, mainly arable, non-irrigated fields. Using IDES project results and stakeholder input, land cover classes were reclassified based on their capacity to provide the highest-ranked ES, Landscape Aesthetic Quality (Picture 3). While the matrix labels this ES as Recreation & Aesthetic Values, both terms reflect the same concept.



Picture 2. CORINE land cover classes in the KPR, including a 10 km-wide buffer zone



Picture 3. The KPR area's capacity to provide ecosystem service „Recreation & Aesthetic Values“

Of the total area, 11.51% has no capacity to provide Landscape Aesthetic Quality (value 0), mainly urban and industrial zones in the buffer area and terrestrial wetlands in the reserve. The majority (53.28%) has low capacity (value 1), mostly arable land with some river port areas. Relevant capacity (value 2) covers 17%, while medium capacity (value 3) is 1.24%. No areas have high capacity (value 4), and very high capacity (value 5) covers 16.97%, primarily water and forested areas. A key limitation in mapping ecosystem services using CORINE Land Cover is its lack of fine-scale data. Locally significant features, such as cultural sites, unique landscapes, and symbolic locations, are not captured. The capacity matrix method provides a broad assessment but relies on five broad land cover categories (artificial, agricultural, forest, wetland, and water bodies). CORINE's low spatial resolution hinders detailed ES evaluations. To improve accuracy, high-resolution, e.g. Sentinel satellite imagery could supplement data and refine local assessments. The CORINE CLC dataset indicates no significant changes in KPR's floodplain over the past two decades, which aligns with its protected status. However, changes in the buffer zone may impact the floodplain, requiring higher resolution analysis beyond CORINE's capabilities.

Conclusions

Floodplains are biodiversity hotspots and key providers of ecosystem services, but they face increasing degradation. Effective management is crucial to prevent loss and transformation. Given their broad impact—such as flood risk regulation—stakeholder involvement is essential in defining and assessing key ecosystem services. Due to the lack of detailed datasets, the capacity matrix approach, combined with publicly available CORINE data, proved useful but limited. Future research should refine this method using higher-resolution satellite imagery (e.g., Sentinel). Improved spatial ES maps would enhance decision-making, enable better floodplain protection, and promote sustainable development. At the local level, thematic maps can guide land-use planning, conservation, and risk assessment. For example, habitat suitability maps can identify priority areas for biodiversity protection. Adaptive management strategies, informed by these maps, can mitigate environmental risks and support sustainable agriculture, fisheries, and eco-tourism. Integrating ES maps into spatial planning and policymaking would improve environmental protection measures and raise public awareness. Participatory decision-making, based on these maps, could foster better floodplain management and sustainable land use. Finally, while ecosystem services are referenced in Serbian regulations, they remain underutilized in policy and planning. A comprehensive national strategy is needed to assess, value, and integrate ecosystem services into decision-making frameworks.

Acknowledgment

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References

- Burkhard, B., Kroll, F., Müller, F., Windhorst, W. (2009). Landscapes' capacities to provide ecosystem services—a concept for land-cover-based assessments. *Landscape Online*, 15, 1-22.
- Burkhard, B., Maes, J. (2017). *Mapping Ecosystem Services*. Pensoft Publishers, Sofia
- Daily, G.C., Matson, P.A. (2008). Ecosystem Services: From theory to implementation. *Proceedings of the National Academy of Sciences of the USA* 105(28), 9455-9456.
- de Sousa, L. P., Lillebø, A. I., Alves, F. L. (2018). Ecosystem Service Mapping: A Management-Oriented Approach to Support Environmental Planning Process. In: *Ecosystem Services and Global Ecology* (Hufnagel, L.), IntechOpen.

- Evans, D. L., Falagán, N., Hardman, C. A., Kourmpetli, S., Liu, L., Mead, B. R., Davies, J.A.C. (2022). Ecosystem service delivery by urban agriculture and green infrastructure –a systematic review. *Ecosystem Services*, 54, 101405.
- Fischer, C., Damm, C., Foeckler, F., Gelhaus, M., Gerstner, L., Harris, R., ... Scholz, M. (2019). The “habitat provision” index for assessing floodplain biodiversity and restoration potential as an ecosystem service - Method and application. *Frontiers in Ecology and Evolution*.
- Gallardo, M., & Cocero, D. (2023). Using the European CORINE land cover database: A 2011–2021 specific review. In: *Sustainable Development Goals in Europe*, 303-325. Springer, Cham.
- Ilić, M., Srđević, Z., Srđević, B., Stammel, B., Borgs, T., Benka, P., Grabić, J., Ždero, S. (2024). The nexus between pressures and ecosystem services in floodplains: New methods to integrate stakeholders' knowledge for water quality management in Serbia. *Ecosystem Services*, 68, 101643.
- La Notte, A. (2024). The importance of ecosystem services to support the governance of critical ecological assets. *Ecosystem Services*, 68, 101642.
- Maes, J., Egoh, B., Willemen, L., Liqueste, C., Vihervaara, P., Schägner, J. P., ... Bidoglio, G. (2012). Mapping ecosystem services for policy support and decision-making in the European Union. *Ecosystem services*, 1(1), 31-39.
- Meli, P., Vieli, L., Spirito, F., Reyes-Riveros, R., Gonzalez-Suhr, C., Altamirano, A. (2023). The importance of considering human well-being to understand social preferences of ecosystem services. *Journal for Nature Conservation*, 72, 126344.
- Millennium ecosystem assessment, MEA. (2005). *Ecosystems and human well-being* (Vol. 5, p. 563). Washington, DC: Island Press.
- Perosa, F., Fanger, S., Zingraff-Hamed, A., Disse, M. (2021). A meta-analysis of the value of ecosystem services of floodplains for the Danube River Basin. *Science of The Total Environment*, 777, 146062.
- Reyers, B., O'Farrell, P. J., Cowling, R. M., Egoh, B. N., Le Maitre, D. C., Vlok, J. H. (2009). Ecosystem services, land-cover change, and stakeholders: finding a sustainable foothold for a semiarid biodiversity hotspot. *Ecology and Society*, 14(1).
- Srđević Z., Benka P., Grabić J., Srđević B., Antić N., Ilić M., Ždero S. (2021a). How ecosystem services of the floodplains can contribute to the better water quality in the Danube region: IDES project. In: *Book of abstracts of the XII International Agriculture Symposium "AGROSYM 2021"*, 7-10 October 2021, Jahorina, Bosnia and Herzegovina.
- Srđević, Z., Ionescu B. S.C, Galmboš, L., Srđević, B., Grabić, J., Ždero, S., Ilić, M., Antić, N. (2021b). Stejkholderi u Specijalnom rezervatu prirode Koviljsko-petrovaradinski rit kod Novog Sada: Ko su i kako ih uključiti u procese identifikacije i integracije ekosistemskih usluga važnih za poboljšanje kvaliteta vode Dunava? *Vodoprivreda*, 53 (313-314), 241-248.
- Tockner, K., Stanford, J. A. (2002). Riverine flood plains: present state and future trends. *Environmental conservation*, 29(3), 308-330.
- Tockner, K., Uehlinger, U., Robinson, C. T. (2009). *Rivers of Europe*. Academic Press.
- Tomscha, S. A., Gergel, S. E., Tomlinson, M. J. (2017). The spatial organization of ecosystem services in river-floodplains. *Ecosphere*, 8(3), e01728.

ORIGINAL SCIENTIFIC PAPER

The effect of nitrogen fertilizers and variety on the yield and quality of winter wheat

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Summary

The aim of the study was to determine the influence of nitrogen fertilization and variety on yield, grain quality and other wheat parameters. Two varieties, Kraljica and Sofru, were used for sowing, and the field experiment was set up during the 2021/2022 growing season in three replications according to the RBC design. The basic fertilization with mineral fertilizer and two top dressings were the same for all treatments, while fertilization treatments 1 and 2 (FT1 and FT2) included an additional third foliar top dressing with Fertpol LN 30 in the usual and higher amounts. The results showed that there were certain differences in grain quality and yield between two varieties, but additional nitrogen fertilization did not have a statistically significant effect on the grain yield and quality compared to standard fertilization. Significant differences were found for varieties and the interaction of variety and fertilization treatments. The average wheat yield achieved in the experiment was 8.23 t ha⁻¹, the hectoliter weight was 77.61 kg hl⁻¹, while the average protein content was 11.07%.

Key words: nitrogen, winter wheat, weather conditions, yield, quality

Introduction

Wheat is still the most dominant cereal in the context of human nutrition. However, its production is under increasing pressure due to climate change, high inputs in production, low purchase price, complexity in production, etc. (Reynolds and Braun, 2022). In addition to weather conditions, all agricultural techniques are extremely important in the wheat production process, all interventions, from soil cultivation, variety selection, sowing, chemical protection to harvesting, affect the yield and quality of the grain. Wheat fertilization is an extremely important agrotechnical intervention that should not be omitted. In the Republic of Croatia, two to three fertilizations are most often carried out practice (basic fertilization and top dressing), and fertilizer is added according to the needs of the crop. In addition to the usual two top dressings, more and more producers are performing a third top dressing, which is intended to positively affect the quality of the grain while some authors are exploring the potential of other top dressing fertilizers and practice (Kastury et al., 2018; Gill et al., 2019). In recent years, the protein content has had a great influence on the purchase price of wheat, and the price has been determined according to the wheat classes. So, nitrogen fertilization and the correct choice of variety have a major impact on the protein content of wheat grains and are increasingly important in achieving high production. So, the aim of the research was to determine the influence of nitrogen fertilizers

and variety on the yield, agronomic and morphological properties of wheat during the 2021-2022 growing season.

Material and methods

Field trial description

The two factorial field trial was set up during the 2021/2022 wheat growing season on the agricultural land of the family farm Mirko Rumbočić in Mohovo, Vukovar-Srijem County (45°14'53"N 19°13'01"E). The two most common wheat varieties, Sofru (RWA seed company) and Kraljica (Agricultural Institute Osijek seed company), were sown in the trial on October 15 2021. The total experimental area was 600 m², with each variety sown on 300 m² in three replications according to the randomised complete block design (RCBD). In addition to the variety factor, the research also used a subfactor, which was fertilization with different amounts of nitrogen fertilizer. The basic fertilization was the same for the whole experiments. Before sowing, 100 kg ha⁻¹ of urea (46% N) and 350 kg ha⁻¹ of mineral fertilizer with a formulation of 15:15:15 (N:P:K) were added. The control treatment (CT) included two standard top dressings of wheat in the tillering (150 kg ha⁻¹ KAN) and steam elongation (100 kg ha⁻¹ KAN) phases, while fertilization treatments 1 and 2 (FT1 and FT2, respectively) included an additional third foliar top dressing with the preparation Fertpol LN 30 in the usual (16.6 l/ha) and double doses (33.2 l/ha). Foliar fertilizer Fertpol LN 30 contains 30% of nitrogen (N), of which 7.5% is nitrate, 7.5% is ammonium and 15% is amide form of N. Treatment was performed on May 19, 2022. Chemical protection measures included preventive herbicide treatment (Mustang, 0.5 l ha⁻¹ and Lancelot, 33 g ha⁻¹), fungicidal treatment (Prosaro 1 l ha⁻¹ and Priaxor 1 l ha⁻¹) and insecticide treatment (Karate zeon, 0.1 l ha⁻¹).

Samples preparation description

Wheat sampling was carried out manually on July 4, 2022 using scissors and a metal square with an area of 0.25 m². For each repetition, samples were taken four times to obtain a total area of 1 m². The samples were subsequently threshed and weighed using a small stationary thresher at the Centre for Seed and Seedlings, and the resulting grain mass was converted into t ha⁻¹. From these samples, grain moisture (%), 1000-grain mass (g), hectoliter mass (kg hl⁻¹), protein content (%), starch (%), wet gluten (%) and sedimentation value were determined. In addition to taking samples from 1 m², samples of thirty randomly selected plants were additionally taken to determine other properties (ear length in cm and number of grains per ear). All measurements were performed using special devices for counting and measuring the quality of wheat grain. Measurements were performed at the Faculty of Agrobiotechnical Sciences Osijek, and grain quality was determined in the Agrochemical laboratory of the Agricultural Institute Osijek using the InfratecTM 1241 Grain analyzer.

Weather analysis

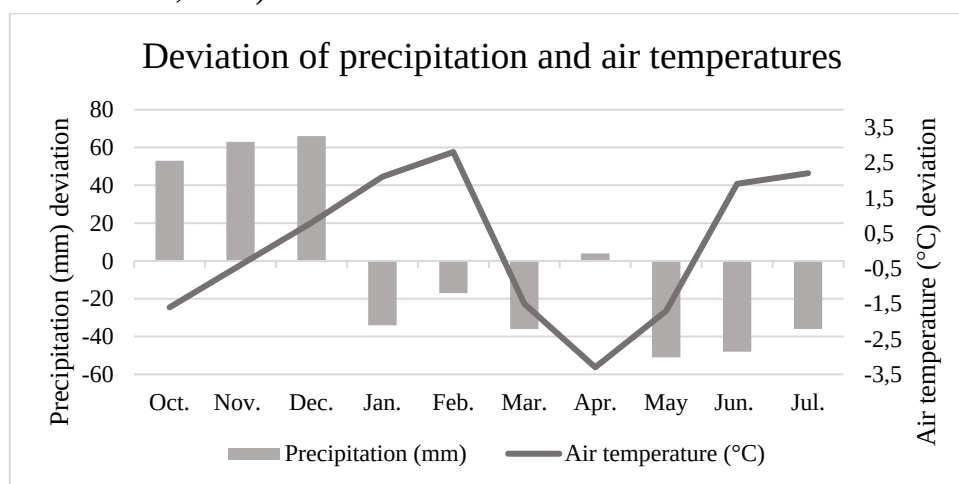
Official data from the State Hydrometeorological Institute of the Republic of Croatia with the Ilok meteorological station were used. Data on average monthly air temperatures (°C) and monthly precipitation (mm) during the wheat growing season 2021/2022 were used, as well as multi-year data for the period from 2001 to 2020.

Statistical analysis

The obtained results were processed in Excel and SAS Software 9.1.4. (SAS Institute Inc., 2003). Statistical analysis of data on the investigated traits was performed by individual analysis of variance using the F test. The significance of differences between the average values of the investigated factors and treatments was assessed by LSD.

Results and discussion

The amount of precipitation for the Ilok meteorological station during 2021/2022 was 36.1 mm lower than the multi-year average (517 mm and 553 mm), while the average temperature was very similar to the average (11.2°C and 11.0°C). However, certain discrepancies were found between precipitation amounts and temperatures in the studied period (Graph 1). Air temperatures during sowing were lower than optimal for wheat germination and sowing, and high precipitation and slow soil drying made sowing difficult. In general, precipitation during the winter months (October-December) was in surplus, while in all other months there was a high deficit in precipitation amount. A particularly large deficit was found in May and June, when wheat is in the most critical need for water because it goes through the stages of flowering, fertilization and grain filling. Air temperatures during the winter period (November-March) were above average values. In the spring period (March-May) they were below the limit values, while in the continuation of the vegetation period, when the wheat is sensitive to high temperatures, they were above average. In general, in the context of wheat cultivation, weather conditions were not optimal for high yields and good grain quality. In recent years, a major problem has been caused by increasingly noticeable climate changes that lead to extremely long dry periods, late spring frosts and low temperatures, as well as large temperature fluctuations during the wheat growing season. The result is frequent and large fluctuations in wheat yields in the Republic of Croatia, but also globally (Španić et al., 2023; Le Gouis et al., 2020).



Graph 1. Deviation of precipitation and air temperature during 2021/2022 growing season in comparison with multi-year data for the period from 2001 to 2020

Analysis of variance determined a significant influence of variety for 5 tested agronomic traits, as well as an interaction between variety and fertilization treatments for 4 agronomic traits, while fertilization treatments were not significant for any trait (Table 1). In this research relatively high grain yield (8.2 t ha⁻¹) was achieved compared to the average yield at the level of the Republic of Croatia (5.8 t ha⁻¹). The Sofru variety achieved grain yields that were about 14% higher than the Kraljica variety. Furthermore, it was found that foliar fertilization of wheat with nitrogen fertilizer did not have a significant impact on the average yield because the differences between the control treatment (8.08 t ha⁻¹) and the fertilization treatments (8.32 t ha⁻¹) were relatively low. Regarding the interaction of variety and fertilization treatments, it was determined that there was no statistically significant difference in the achieved yield, although the variation ranged from 7.50 t ha⁻¹ (Kraljica, FT1) to even 9.10 t ha⁻¹ (Sofru, FT1).

Also, a similar situation occurred with other important agronomic traits. For both, hectolitre weight and 1000 grain weight, statistical significance was shown in varieties and their interaction with fertilization treatment (Table 1). The absence of a third foliar top dressing is perhaps justified in this case because it is known that these properties depend mostly on the amount of precipitation and lower air temperatures in order to prolong the grain filling period (Martin et al., 2020). Given that the lack of precipitation in May and June was pronounced and air temperatures were above average, lower values of these two traits were achieved in this study (77.6 kg ha⁻¹ of hectoliter weight and 42.1 g of 1000 grain weight). Furthermore, the length of the ear in the study was 6.4 cm, and the number of grains per ear was 35.5, with significant differences between the varieties in favor of the Sofru variety (39.5 and 31.5).

Table 1. Statistical analysis for winter wheat yield, agronomic and morphological traits

Variety (A)	Fertilization treatment (B)							
	CT	FT1	FT2	Average	CT	FT1	FT2	Average
Yield (t ha ⁻¹)					Grain moisture (%)			
Kraljica	7.67	7.50	7.93	7.70^b	12.9 ^b	13.0 ^b	12.7 ^b	12.8^b
Sofru	8.50	9.10	8.70	8.77^a	13.5 ^a	13.5 ^a	13.0 ^b	13.3^a
Average	8.08	8.30	8.32	8.23	13.2	13.2	12.8	13.1
LSD _{0.05} (A) = 0.82; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = ns					LSD _{0.05} (A)=0.25;LSD _{0.05} (B)=ns; LSD _{0.05} (AxB)=0.33			
Hectolitre weight (kg hl ⁻¹)					1000 grain weight (g)			
Kraljica	78.2 ^{bc}	78.4 ^{ab}	79.8 ^a	78.8^a	38.7 ^b	39.2 ^b	39.6 ^b	39.2^b
Sofru	76.2 ^d	76.8 ^{cd}	76.2 ^d	76.4^b	44.4 ^a	46.0 ^a	44.5 ^a	45.0^a
Average	77.2	77.6	78.1	77.6	41.6	42.6	42.0	42.1
LSD _{0.05} (A) = 0.91; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = 1.43					LSD _{0.05} (A)=1.42; LSD _{0.05} (B)=ns;LSD _{0.05} (AxB)=2.63			
Grains number/ear					Ear lenght (cm)			
Kraljica	29.8 ^c	31.9 ^{bc}	32.6 ^b	31.5^b	6.4	6.6	6.5	6.5
Sofru	40.2 ^a	38.2 ^a	40.0 ^a	39.5^a	6.4	6.3	6.4	6.3
Average	35.0	35.0	36.3	35.5	6.4	6.5	6.5	6.4
LSD _{0.05} (A) = 1.58; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = 2.25					LSD _{0.05} (A) = ns; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = ns			

CT – control treatment; FT1 - fertilization treatment 1; FT2 – fertilization treatment 2; ns – not significant

After conducting research on wheat crops, it was determined that additional nitrogen fertilization did not have a significant impact on increasing protein content in the control and both fertilization treatments (Table 2). Given that the amount of precipitation during May and June was almost half as much as the multi-year average, this may be the reason for the lack of a greater impact of nitrogen fertilization (Graph 1). However, in the context of variety selection, it should be noted that the Kraljica variety had a 1.77% higher protein content than the Sofru variety. Silva et al. (2019) investigated the impact of top dressing on yield, as well as on the protein content in grain. According to their research, in low rainfall environments, nitrogen fertilization does not have a significant impact on yield and the amount of protein in grain, while in ideal conditions yields were increased. A similar situation occurred in our study. Out of a total of 4 quality characteristics of grain, the variety Kraljica showed a significant advantage in three quality characteristics (protein content, moist gluten and sedimentation).

Table 2. Statistical analysis for winter wheat quality traits

Variety (A)	Fertilization treatment (B)							
	CT	FT1	FT2	Average	CT	FT1	FT2	Average
Protein (%)					Starch (%)			
Kraljica	12.5 ^a	10.7 ^{ab}	12.6 ^a	11.9^a	69.6	71.7	69.8	70.4
Sofru	9.8 ^b	9.8 ^b	10.9 ^{ab}	10.2^b	71.2	71.7	70.2	71.0
Average	11.2	10.3	11.7	11.0	70.4	71.7	70.0	70.7
LSD _{0.05} (A) = 1.25; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = 2.1					LSD _{0.05} (A)=ns; LSD _{0.05} (B)=ns; LSD _{0.05} (AxB)=ns			
Wet gluten (%)					Sedimentation (ml)			
Kraljica	30.7 ^a	25.6 ^{ab}	31.0 ^a	29.1^a	41.2 ^a	31.8 ^{ab}	41.5 ^a	38.2^a
Sofru	22.1 ^b	22.2 ^b	24.9 ^{ab}	23.1^b	25.6 ^b	25.4 ^b	27.7 ^b	26.3^b
Average	26.4	23.9	28.0	26.1	33.4	28.6	34.6	32.2
LSD _{0.05} (A) = 3.70; LSD _{0.05} (B) = ns; LSD _{0.05} (AxB) = 6.25					LSD _{0.05} (A)=6.46; LSD _{0.05} (B)=ns; LSD _{0.05} (AxB)=11.2			

CT – control treatment; FT1 - fertilization treatment 1; FT2 – fertilization treatment 2; ns – not significant

Conclusions

Although weather conditions during the growing season were not optimal, the wheat yield was more than satisfactory. In this study, the role of winter wheat variety and the interaction of variety and fertilization treatment had a positive effect. Unfortunately, foliar application of nitrogen fertilizer had no effect on yield, agronomic and morphological characteristics, or grain quality. Variety Sofru achieved better agronomic traits (yield, 1000 grain weight and grains number per ear) while Kraljica achieved better grain quality traits (protein content, wet gluten and sedimentation value). Therefore, it is recommended to use both varieties in production to achieve both high yields and good quality.

References

- Gill S. C., Sharma R. K., Tripathi S. C., Chhokar R. S., Meena R. P., Jha, A. (2019). Nitrogen top dressing just before irrigation improves wheat growth, productivity, nitrogen use efficiency and profitability. *Journal of Cereal Research*, 11(1).
- Kastury F., Rahimi Eichi V., Enju A., Okamoto M., Heuer S., Melino V. (2018). Exploring the potential for top-dressing bread wheat with ammonium chloride to minimize grain yield losses under drought. *Soil Science and Plant Nutrition*. 64 (5): 642–652.
- Le Gouis J., Oury F.X., Charmet, G. (2020). How changes in climate and agricultural practices influenced wheat production in Western Europe. *Journal of Cereal Science*. 93, 102960.
- Martin T. N., Minussi J. E., Stecca J. D. L., Burg G. M., Pinto M. B. (2020). Meteorological conditions and their associations with the productive performance of wheat. *Australian Journal of Crop Science*. 14(1): 28–35.
- Reynolds M.P., Braun H.J. (2022). *Wheat Improvement - Food Security in a Changing Climate*. Springer Nature Switzerland. 657. Available from: <https://link.springer.com/book/10.1007/978-3-030-90673-3>
- Silva R. R., Zucareli C., Fonseca C. D. B., Riede C. R., Benin G., Gazola D. (2019). Timing and growing conditions of nitrogen topdressing influence the grain yield and protein content of four wheat cultivars. *Bragantia*. 78: 361-370.
- Španić V., Jukic G., Zoric M., Varnica, I. (2023). Some agronomic properties of winter wheat genotypes grown at different locations in Croatia. *Agriculture*, 14 (1): 4.

ORIGINAL SCIENTIFIC PAPER

Soil respiration of soils treated with different seaweed products and byproducts from the seaweed industry

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Summary

Often seaweed industry, producing fertilizers from seaweed, can result in byproducts or in harvested unwanted seaweed species. In our study, we compare soil respiration of two different soil types (light soil and heavier soil) amended with three different seaweed material (*Fucus ssp.* seaweed, *Ascophyllum sp.* byproduct and, *Ascophyllum sp.* seaweed) in two different applied concentrations (2% and 4%) as an indicator of the effect of the material on soil health and quality. The soil respiration was highest when *Ascophyllum sp.* byproduct was added on both soil types in both concentrations. *Fucus sp.*, seaweed that commonly is not used in fertilizer production did not show a negative impact on respiration and therefore on the microbial community compared to *Ascophyllum sp.* and as such it could have the potential to be used as fertilizer or soil conditioner. Further tests involving plant growth would give a more comprehensive insight into using the investigated materials.

Key words: *Ascophyllum sp.*, *Fucus sp.*, nutrients, soil conditioner

Introduction

Brown seaweeds dominate the seaweed industry due to their outstanding nutrient composition and the presence of beneficial compounds that promote plant growth and resilience. Kelp, *Ascophyllum nodosum*, is among the most popular brown seaweed used in the seaweed fertilizers and animal feed industry due to its high content of nutrients, alginates, and growth-promoting hormones. It is particularly valued for its high concentration of potassium, trace minerals, and natural plant hormones like auxins, cytokinins, and gibberellins. While harvesting *Ascophyllum sp.*, often a certain percentage of *Fucus sp.* is harvested as well. The seaweed from the *Fucus* family is not used in fertilizer or animal feed production due to its somewhat elevated levels of toxic trace elements. This results in a certain amount of unused leftovers from the harvest. Furthermore, seaweed processing of *Ascophyllum sp.* in some cases results in process byproducts. The purpose of our paper is to examine the impact of *Fucus sp.* and seaweed industry byproducts on soil, in comparison to the *Ascophyllum*-based product used as a soil fertilizer.

Material and methods

Seaweed properties

Three types of seaweed material were used in the analysis (two *Ascophyllum sp.* and one *Fucus sp.*). The seaweed material from *Ascophyllum sp.* was: a) granulated fertilizer available on the market, and b) a byproduct from the production of liquid fertilizer derived from *Ascophyllum sp.*. The third seaweed analyzed was a *Fucus sp.*, which is a seaweed family that is not commonly used in fertilizer production. The information on seaweed pH was obtained from the manufacturer (the seaweed company providing us with the seaweed material). The seaweed content of TOC and Tot N was analysed on Skalar C/N analyzer, the

concentration of nutrients (P and K) and toxic elements (As and Cd) (ISO 2009), were analyzed at the Faculty of Agrobiotechnical Sciences Osijek.

Soil properties

Two soils from Croatia used in the soil respiration analysis were two different types, one was from the Tvrdavica site which has light soil, and the other was from the Antunovac site which has more heavy soil. Soil properties determined at the Faculty of Agrobiotechnical Sciences Osijek, were: pH (ISO 1994), available P and K (Egner et al. 1960), TOC, and total N (C/N Skalar analyzer). Soils used in the analysis had similar pH but they differed in nutrient content, specifically phosphorous and potassium (Table 1.).

Table 1. Soil properties

	BD (gcm⁻³)	pH (KCl)	TN (%)	TOC (%)	AL-K₂O mg100g⁻¹	AL-P₂O₅ mg100g⁻¹
Tvrdavica	1,55	7,12	0,12	1,04	19,8	122,1
Antunovac	1,35	6,72	0,21	1,95	30,0	27,7

Soil C respiration

Soil respiration was determined by the titration method (Bakhshandeh et al. 2019). The experimental design consisted of 2 soils (lighter and heavier) x 3 seaweed products x 2 concentrations (2% and 4%). A weighted amount of 25 g of soil, or a mixture of soil and seaweed, was placed in a 1000 mL glass jar. A separate container with 10 mL of 0.2 M NaOH was positioned next to the soil sample to absorb released CO₂. On the titration day, BaCl₂ was added to the NaOH solution, and titration was carried out using 0.1 M HCl with thymolphthalein as an indicator. A 100 g quartz sand sample served as a blank control. Titrations were performed at specific intervals (days 1, 2, 4, 7, 10, 14, 17, and 21), and the amount (mg) of released CO₂ was calculated using the formula:

$$\text{mg CO}_2 = (a - b) \times F$$

where “a” is the volume of acid used for titrating the blank sample, “b” is the volume of acid used for titrating the test sample, and “F” is a factor based on the molarity of HCl.

The analysis of the seaweed material indicated some differences in seaweed properties as Asco. byproduct had much higher levels of toxic Cd but also much higher levels of nutrients P and K, but lowest levels of N (Table 1.) and by that the widest C/N ratio. Fucus which is usually avoided due to high levels of toxic elements had somewhat elevated levels of Cd, but much lower compared to reported levels in other studies (Balina et al. 2016). Furthermore, the concentration of Cd in Fucus as well as in Asco. byproduct in our study do not exceed the limits prescribed by the regulation on the protection of agricultural land from pollution (NN 9/2014). The levels of As were similar in all three investigated materials. So from the toxicity point of view (Cd and As), the investigated seaweed materials do not pose a threat to soil pollution.

Table 2. – Seaweed material properties

	n	TC (%)	TN (%)	Na mgkg⁻¹	As µgkg⁻¹	Cd µgkg⁻¹	K mgkg⁻¹	P mgkg⁻¹
Asco. byproduct	3	35,2	0,36	13357	20193	2239	55050	4691
Asco.	3	34,1	1,03	20673	26250	184,7	26930	1217
Fucus	3	34,9	0,71	17383	23253	315,4	27173	825

Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) were performed using Minitab® Statistical Software version 17. Descriptive statistics were performed for soil and seaweed properties. Analysis of variance and Tukey pairwise comparison was performed for values of C respiration to determine significant differences among treatments (different soils, different seaweed products with different concentrations applied).

Results and discussion

Previous studies found that long-term use of seaweed fertilizer improves crop yield, soil properties, and soil bacterial diversity. It also increases the abundance of bacteria with nitrogen-fixing capabilities, enhancing organic matter and providing essential nutrients for plant growth. The continuous use of seaweed fertilizer has no side effects and promotes sustainable green agriculture (Qiqin et al., 2023). In our study, we tested byproducts from the seaweed industry as well as *Fucus* seaweed species that are not commonly used in the seaweed industry in comparison with commonly used seaweed on two different soils. As expected control soil without any added seaweed material had the lowest C respiration rate. Samples with added seaweed material had significantly higher C respiration because the soil microorganisms had more energy for their growth and therefore they respire more CO₂. The highest C respiration was observed when the *Ascophyllum* byproduct (byproduct from fertilizer production from *Ascophyllum* species) was mixed into the soil. For both concentrations 2% and 4% (Table 3). *Fucus* species, which is not commonly used in fertilizer production, had C respiration rates similar to *Ascophyllum* fertilizer, suggesting no differences in soil microorganism activity due to the incorporation of *Fucus* or *Ascophyllum* species into the soil. Two different concentrations (2% and 4%) of the applied seaweed follow the same pattern, although when 4% of *Ascophyllum* is applied on lighter soil with lower organic matter content (Tvrđavica site) the C respiration increases significantly compared to Antunovac sample or soil samples amended with *Fucus*. In addition, soil C respiration of control soils showed higher C respiration and therefore higher microorganism activity in Antunovac soil (Table 3.) which had more organic matter compared to the control soil from Tvrđavica (Table 1.).

Table 3. Soil respiration of two soils (AN and TV) amended with 2% and 4% of seaweed material (Asco. Byproduct, Asco. and *Fucus*) after 21 days

		2%	4%
	n	CO ₂ mgg ⁻¹ DM	CO ₂ mgg ⁻¹ DM
AN Asco. byproduct	3	1.8978 ^a	2.801 ^a
TV Asco. byproduct	3	1.6637 ^b	2.314 ^b
TV Asco.	3	1.3501 ^c	2.189 ^b
AN <i>Fucus</i>	3	1.3477 ^c	1.664 ^c
AN Asco.	3	1.1965 ^{cd}	1.635 ^c
TV <i>Fucus</i>	3	1.1265 ^d	1.669 ^c
ctrl soil AN	3	0.7245 ^e	0.7245 ^d
ctrl soil TV	3	0.4724 ^f	0.4724 ^d

AN – site Antunovac (clay soil); TV – site Tvrđavica (sandy soil)

The overall increase in respiration did not double when twice as much seaweed was added. It increased, depending on the seaweed material, by 62% to 72% for soil from Tvrđavica and by 68% to 81% for soil from Antunovac. This suggests a higher activity of microorganisms in heavier soil from Antunovac compared to lighter soil from Tvrđavica.

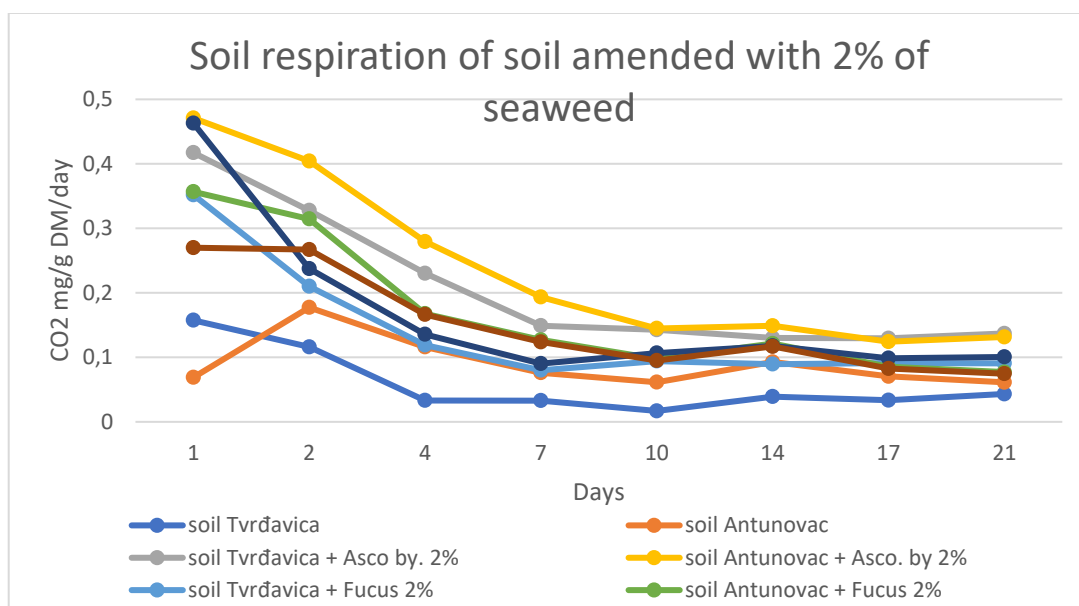


Figure 1. Soil respiration of two soils (Antunovac and Tvrdavica) amended with 2% of seaweed material

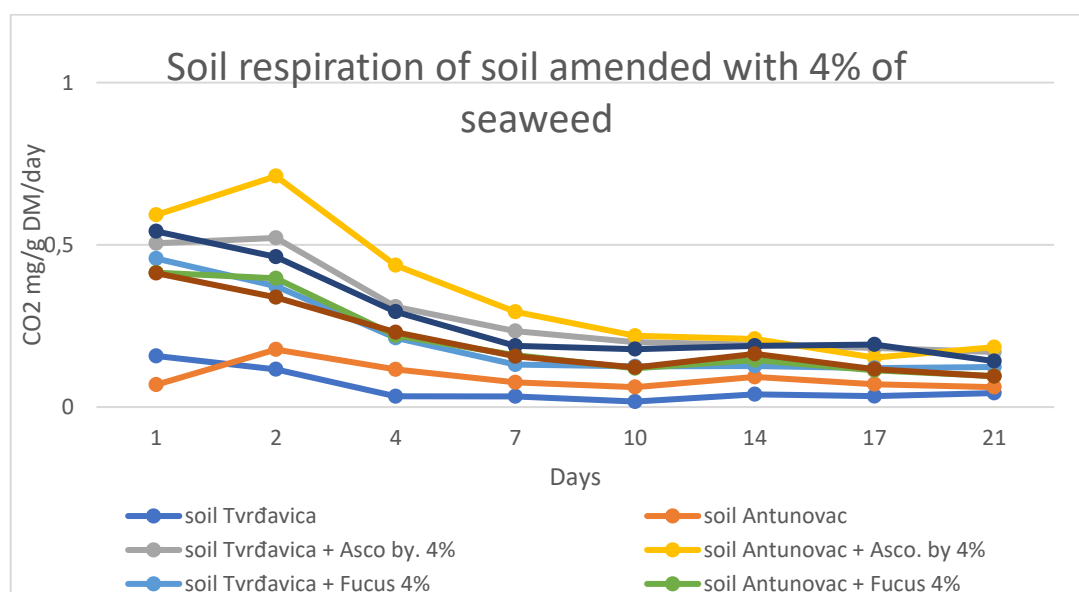


Figure 2. Soil respiration of two soils (Antunovac and Tvrdavica) amended with 4% of seaweed material

When 2% of seaweed is added the burst of respiration is observed in the first four days while the significantly increased respiration lasts for 7 days when more seaweed is added (4%). As expected when more organic material is added the period of elevated soil respiration is extended as the microorganisms have a larger energy pool and it takes them more time to break down labile organic matter. When the organic material (seaweed) is utilized the respiration comes to equilibrium and there are no more significant differences between the respiration in the control soil and the respiration of soils amended with seaweed (Table 4.).

Table 4. Soil respiration per day for both soils combined amended with 2% and 4% of seaweed material for the period of 21 day

day	n	CO ₂ mgg ⁻¹ DM (2%)	CO ₂ mgg ⁻¹ DM (4%)
1	24	0.319 ^a	0.394 ^a
2	24	0.257 ^b	0.387 ^a
4	24	0.156 ^c	0.232 ^b
7	24	0.109 ^d	0.159 ^c
14	24	0.107 ^d	0.145 ^{cd}
10	24	0.095 ^d	0.130 ^{cd}
21	24	0.089 ^d	0.114 ^d
17	24	0.089 ^d	0.123 ^{cd}

Conclusions

Adding *Ascomyces* byproduct resulted in the highest soil C respiration, indicating mobilization or the highest activity of microorganisms when *Asco.* byproduct was added. *Fucus* species did not differ from the *Asco.* in respiration nor in seaweed properties, suggesting that this material, at least the one used in our study, could be used as a soil conditioner. Further tests involving plants should be conducted to get a better insight into using the investigated materials.

Acknowledgement

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References

- Balina, K., Romagnoli, F., Blumberga, D. (2016). Chemical Composition and Potential Use of *Fucus Vesiculosus* from Gulf of Riga. *Energy Procedia*, Volume 95, 43-49,
- Bakhshandeh, E., Francaviglia, R., Renzi, G. (2019). A cost and time-effective method to evaluate soil microbial respiration for soil quality assessment. *Applied Soil Ecology*, 140, 121-125.
- Egner H., Riehm H., Domingo, W. R. (1960). Investigations on the chemical soil analysis as a basis for assessing the soil nutrient status II. Chemical extraction methods for phosphorus and potassium determination. *Kungliga Lantbrukshögskolans Annaler* 26: 199-215.
- ISO. (1994). Soil quality – Determination of pH. [10390:1994]. Geneva, Switzerland: International Organization for Standardization.
- ISO (2009). Soil quality — Determination of selected elements by inductively coupled plasma mass spectrometry (ICP-MS). [23161:2009]. Geneva, Switzerland: International Organization for Standardization.
- NN 9/2014 – Pravilnik o zaštiti poljoprivrednog zemljišta od onečišćenja
- Qiqin, L., Huaguang, Z., Minxiu, S., Qian, L., Haijun, F., Haimin, C., Rui, Y. (2023). Improvement of Soil Structure and Bacterial Composition by Long-Term Application of Seaweed Fertilizer. *J Soil Sci Plant Nutr* 23, 5122–5132 .

ORIGINAL SCIENTIFIC PAPER

Comparison of phosphorus solubilization efficiency of some fluorescent bacteria

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Summary

The aim was to select a suitable growing medium for determining the efficiency of phosphorus (P) solubilization and to compare the P solubilizing efficiency between isolated bacterial strains of the genus *Pseudomonas* spp. from the soils of Osijek-Baranja and Vukovar-Srijem counties and controlstrains. Pikovskaya's agar medium showed better bacterial growth performances, greater challenges for bacterial strains in solubilizing P and better differences in measured efficiency. Highest efficiency was achieved by the bacterial strain labeled "U-10-BR" and was 9.97% higher compared to the value achieved by *Pseudomonas putida*, 57.10% higher compared to the value achieved by *Pseudomonas fluorescens* and 183.93% higher compared to the value achieved by *Pseudomonas rhizosphaerae*. The results indicate the possibility of activating P reserves in the soil by using of phosphorus-solubilizing microorganisms.

Key words: *Pseudomonas* spp., plant-available P, bacterial growing media, microorganisms

Introduction

Phosphorus (P) is the second most necessary macroelement for plant growth and development, right after nitrogen, and is considered a critical factor in many plant production systems because its availability in soils, in plant-available forms, is limited (Xiao et al., 2011). Phosphorus is found in the soil, plants and nature in the pentavalent form and is part of very important organic compounds such as nucleoproteins, enzymes, phospholipids, etc. Plants absorb phosphorus exclusively in the anionic form, of which H_2PO_4^- and HPO_4^{2-} are the most abundant, which further depends on the pH reaction of the soil itself (Mahidi et al., 2011). Unlike nitrogen and sulfur, P is incorporated into organic matter without reduction. Vukadinović and Lončarić (1998) stated that the phosphorus cycle consists of the decomposition of phosphorus compounds in the soil, their uptake by plants and the re-formation of soil minerals, and that the amount of phosphorus in plants is on average 0.3-0.5%. There are several ways in which the availability of phosphorus can be increased, such as liming, ameliorative phosphorus fertilization and the input of larger amounts of organic matter like manure. Sometimes, those measures are economically and agroecologically unjustified. Most agricultural soils have huge reserves of phosphorus, the greater part of which, about 95 to 99%, is in inaccessible forms and thus is actually useless for plants (Pradhan and Sukla, 2005). These facts have prompted research into the possibility of increasing the solubility of phosphorus from its reserves (Kahn et al., 2007), and recently, one of the ways to increase the availability of P in the soil has been the use of phosphorus-solubilizing microorganisms (PSM) that will affect phosphorus reserves in soils where its amount is negligible. Thus, it is believed that phosphorus-solubilizing bacteria (PSB), used as inoculants, stimulate phosphorus uptake by crops. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* belong to the most powerful phosphorus-solubilizing bacteria

(Rodriguez and Fraga, 1999). Some are capable of dissolving and mineralizing insoluble soil phosphorus that is inaccessible to plants. In the natural environment, numerous soil and rhizosphere microorganisms are effective in releasing phosphorus from total soil phosphorus through solubilization and mineralization (Bhattacharyya and Jha, 2012). Therefore, the aim of the research can be divided into two parts. The first part involved the selection of a suitable growing medium for determining the efficiency of phosphorus solubilization by our tested microorganisms. The second part involved determining the efficiency of isolated bacterial strains of the genus *Pseudomonas* spp. from the soils of Osijek-Baranja and Vukovar-Srijem counties in relation to control strains (*Pseudomonas putida* ATCC12633, *Pseudomonas rhizosphaerae* DSM16299 and *Pseudomonas fluorescens* ATCC13525).

Material and methods

Pure cultures of bacteria from the genus *Pseudomonas* spp. (*P. putida* ATCC12633, *P. rhizosphaerae* DSM16299 and *P. fluorescens* ATCC13525) of known origin, as control strains, and 9 autochthonous bacteria of the genus *Pseudomonas* spp. isolated from soils of Osijek-Baranja and Vukovar-Srijem counties (Tenja, Osijek, Ernestinovo, Đakovo, Slakovci, Jarmina, Gaboš, Habjanovci) were used during the research. Isolation was carried out from freshly collected soil samples, by the method of dilution and purification to pure cultures according to the protocol of Steinbüchel et al. (2013). The bacteria were grown on solid and liquid King's B medium. Two growing media were prepared, differing in the concentration of phosphorus in the form of tricalcium phosphate. The ready-made Pikovskaya's agar (PIK) medium, which contains 5 g L⁻¹ phosphorus in the form of tricalcium phosphate, and the modified PIK medium for testing the efficiency of phosphorus dissolution (FOP) which contains 2 g L⁻¹ phosphorus in the same form. After sterilizing the media and pouring them into Petri dishes, the control strains were inoculated to test the suitability of the media for further testing. Inoculation was performed by pipetting 20 µl of bacterial sample onto the medium approximately in the central part of the Petri dish. The media are milky white in color, and after an incubation period (5 days at a temperature of 30 °C), a clear zone, formed from the solubilization of P in medium, appears (Figure 1). The efficiency of individual bacterial strains for phosphorus solubilization was determined by measuring the diameter of the clear zone (Figure 1), and obtained value (mm) was used to compare the efficiency of phosphorus solubilization between reference strains as control and isolated ones.

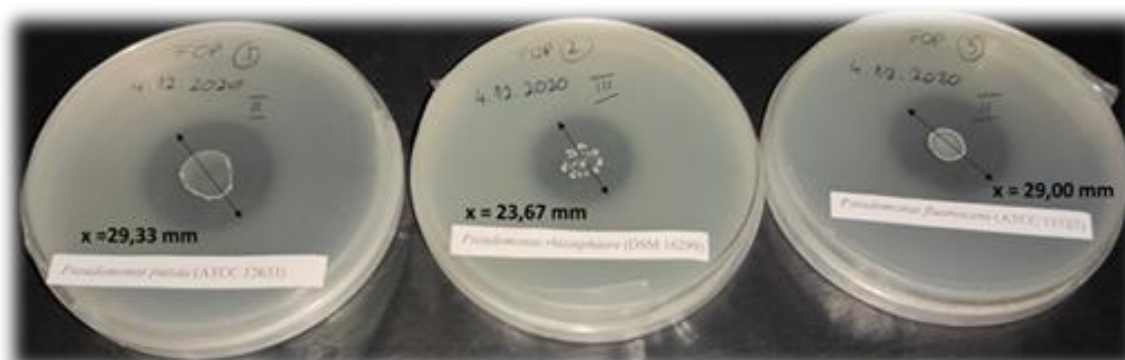


Figure 1. Clear zones due to P solubilization from the medium by *Pseudomonas* strains
Data analysis included descriptive statistics parameters, and all data were processed in SAS Enterprise Guide 7.1. Statistically significant differences between the observed parameters were determined by the F test (LSD=0.05).

Results and discussion

On Pikovskaya's growing medium *P. putida* showed the highest phosphorus solubilization efficiency, which was 26.67 mm, and this value was 43.70% higher than the average value. Statistically significant differences were found between the efficiency of all control strains (Table 1). On the FOP medium higher values for the efficiency were obtained, probably due to lower concentration of P source in the media. *P. putida* also achieved the highest phosphorus solubilization efficiency on this medium, which was 29.33 mm. Also, the bacterium *P. fluorescens* indicates a very high phosphorus solubilization efficiency, and the achieved value was 29.0 mm. Statistically significant differences were found between the strains *P. putida* and *P. rhizosphaerae*, as well as between *P. fluorescens* and *P. rhizosphaerae* (Table 1).

Table 1. Efficiency of phosphorus solubilization on Pikovskaya's Agar (PIK) and modified PIK medium (FOP) using control strains (*P. putida* ATCC12633, *P. rhizosphaerae* DSM16299 and *P. fluorescens* ATCC13525)

Growing media	Bacterial strain	Efficiency of phosphorus solubilization (mm)
Pikovskaya's Agar (PIK)	<i>P. putida</i>	26.67 A
	<i>P. fluorescens</i>	18.67 B
	<i>P. rhizosphaerae</i>	10.33 C
	LSD (0.05)	7.738
Modified PIK medium (FOP)	<i>P. putida</i>	29.33 A
	<i>P. fluorescens</i>	29.00 A
	<i>P. rhizosphaerae</i>	23.67 B
	LSD (0.05)	2.978

Capital letters indicate significant differences among strains in P solubilization efficiency

Based on the results obtained, the PIK medium, which contains a higher concentration of phosphorus in the form of tricalcium phosphate, showed better bacterial growth performances greater challenges for bacterial strains in solubilizing phosphorus and better differences in measured efficiency. Therefore, this media was used for further research. The results obtained, namely the fact that clear zones were formed under bacterial cultures, correspond to the statements of numerous authors (Fliessbach et al., 2009; Kumar and Singh, 2001; Rodriguez and Fraga, 1999; Troxler et al., 1997) that bacteria of the genus *Pseudomonas*, with their exudates that are products of their metabolism, can dissolve phosphorus in the soil and convert them into plant-available forms. The average value of phosphorus solubilization efficiency on Pikovskaya's Agar (PIK) medium was 21.72 mm (Table 2). The highest value (29.33 mm) was achieved by the bacterial strain labeled "U-10-BR", while the lowest value was achieved by *P. rhizosphaerae*, with a value of 10.33 mm. The mentioned value achieved by strain U-10-BR was 9.97% higher compared to the value achieved by *P. putida* (ATCC12633), 57.10% higher compared to the value achieved by *P. fluorescens* (ATCC13525) and 183.93% higher compared to the value achieved by *P. rhizosphaerae* (DSM16299). Statistically significant differences were found between the efficiency of the bacterial strains labeled U-10-BR and the bacterial strains labeled U-2-O, U-17-J-P, U-17-S-P, U-7-E, U-20-H-J, U-19-K-SU, *P. fluorescens* and *P. rhizosphaerae* (Table 2).

Table 2. Comparison of P solubilization efficiency on Pikovskaya's Agar (PIK) medium between control strains and isolated strains

Growing media	Bacterial strain	Efficiency of phosphorus solubilization (mm)
Pikovskaya's Agar (PIK)	<i>P. putida</i>	26.67 ab
	<i>P. fluorescens</i>	18.67 cd
	<i>P. rhizosphaerae</i>	10.33 f
	U-10-BR	29.33 a
	U-1-ZP	26.00 ab
	U-4-T	25.33 ab
	U-2-O	24.00 b
	U-17-J-P	23.33 b
	U-14-S-P	22.67 bc
	U-7-E	22.67 bc
	U-20-H-J	17.33 de
	U-19-K-SU	14.33 ef
	Average	21.72
	Minimum	10.33
	Maximum	29.33
	LSD (0.05)	4.250

Lowercase letters indicate significant differences among strains in P solubilization efficiency

Most isolated bacterial strains, exactly 6 of them U-1-ZP, U-4-T, U-2-O, U-17-J-P, U-17-S-P and U-7-E achieved a phosphorus solubilization efficiency value that was at the same level as *P. putida*, which means that no statistically significant differences were found between them. This leads to the fact, that the isolated bacterial strains from Osijek-Baranja and Vukovar-Srijem county soils have the possibility to solubilize P and increase P mineralization. Park et al. (2008) already mentioned the possibility of increased phosphorus mineralization by bacteria from the genus *Pseudomonas* spp., indicate the potential possibility of reducing mineral phosphorus fertilizers. Also, numerous authors stated that soils are rich in indigenous populations of rhizobacteria that have optimally adapted to environmental conditions and that they they represent an indispensable participant in the cultivation of crops using sustainable agricultural methods (Deubelet et al., 2000; Di Simine et al., 1998; Fliessbach et al., 2009; Kumar and Singh, 2001).

Conclusions

The results indicate a very significant role of microorganisms in the nutrient cycling, especially when it comes to plant-available P, which is among the most significant limiting factors in agricultural production. Furthermore, the possibility of activating P reserves in the soil by using phosphorus-solubilizing microorganisms would lead to a reduction in mineral P fertilizers. Additional research is needed to get the isolated strains tested in field trials and use them in agricultural crop production.

References

- Bhattacharyya, P.N., Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*. 28: 1327–1350.
- Deubelet, A., Gransee, A., Merbach, W. (2000): Transformation of organic rhizodepositions by rhizosphere bacteria and its influence on the availability of tertiary calcium phosphate. *Journal of Plant Nutrition Soil Science*. 163: 387–392.
- Di Simine, C.D., Sayer, J.A., Gadd, G.M. (1998): Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. *Biology and Fertility of Soils*. 28: 87– 94.

- Fliessbach, A., Winkler, M., Lutz, M.P., Oberholzer, H.R., Mader, P. (2009): Soil amendment with *Pseudomonas fluorescens* CHA0: lasting effects on soil biological properties in soils low in microbial biomass and activity. *Microbial Ecology*. 57: 611–623.
- Khan, M.S., Zaidi, A., Wani, P. (2007). Role of phosphate solubilizing microorganisms in sustainable agriculture. *Agronomy for Sustainable Development*. 27: 29-43.
- Kumar, V., Singh, K.P. (2001.): Enriching vermicompost by nitrogen fixing and phosphate solubilizing bacteria. *Bioresource Technology*. 76: 173–175.
- Mahidi, S.S, Hassan, G.I., Hussain, A., Faisul-ur-Rasool (2011). Phosphorus availability issue: Its fixation and role of phosphate solubilizing bacteria in phosphate solubilization – Case study. *Res. J. Agri. Sci.* 2: 174-179.
- Park, K.H., Park, G.T., Kim, S.M., Lee, C.Y., Son, H.J. (2008): Conditions for soluble phosphate production by environment-friendly biofertilizer resources *Pseudomonas fluorescens*. *Journal of Environmental Science*. 17: 1033–1037.
- Pradhan, N., Sukla, L.B. (2005). Solubilization of inorganic phosphate by fungi isolated from agriculture soil. *Afr. J. Biotechnol.* 5: 850-854.
- Rodriguez, H., Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* 17: 319-339.
- Troxler, J., Zala, M., Moënne-Loccoz, Y., Keel, C., Défago, G. (1997): Predominance of non-culturable cells of the biocontrol strain *Pseudomonas fluorescens* CHA0 in the surface horizon of large outdoor lysimeters. *Applied and Environmental Microbiology*. 63: 3776–3782.
- Vukadinović, V., Lončarić, Z. (1998). *Ishrana bilja*. Poljoprivredni fakultet u Osijeku. Osijek
- Xiao, C.Q., Chi R.A., Li, X.H., Xia, M., Xia, Z.W. (2011). Biosolubilization of rock phosphate by three stress-tolerant fungal strains. *Appl. Biochem. Biotechnol.* 165: 719-727.

PRELIMINARY COMMUNICATION

Soil Microorganism: Key Drivers of Soil Fertility and Plant Health

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Summary

This paper highlights the importance of the biological component of soil and provides guidelines for achieving an optimal abundance of soil microorganisms through proper soil management. Microorganisms are an integral part of the soil's living phase, contributing to the decomposition of organic matter and driving biogeochemical cycles, including carbon, nitrogen, sulfur, phosphorus, and other elements. Additionally, they produce compounds that enhance nutrient availability, such as siderophores that facilitate iron uptake. Microorganisms also increase plant resistance to biotic and abiotic stresses, induce defense mechanisms against phytopathogens, and promote plant growth through the biosynthesis of growth regulators. Moreover, they play a key role in the degradation of xenobiotics, helping to reduce environmental pollutants. Their extracellular polymeric substances contribute to the formation of stable soil aggregates, improving soil aeration and water infiltration. As a fundamental biological component of soil fertility and crop productivity, microorganisms are essential for maintaining biological balance and ensuring overall ecosystem health.

Key words: soil health, nutrient cycling, PGPM, biopesticides, sustainable agriculture

Introduction

Soil is an ideal habitat for microscopic and macroscopic organisms, and its composition depends on physical, chemical and biological properties as well as environmental conditions. Microbial biomass in soil accounts for 1–5% of total organic matter, with fungi accounting for up to 90% of this biomass (Hopkins and Dungait, 2010). The highest concentration of microorganisms is found in the surface layers of the soil, where organic matter is most abundant. Soil microorganisms play a crucial role in maintaining the balance and health of the soil. In addition to decomposing organic matter, they are also involved in various biochemical processes and contribute to nutrient cycling and humus formation, which increases soil fertility. Understanding the microbial processes in soil and the interactions between abiotic and biotic factors is essential for maintaining soil fertility through sustainable agricultural practices.

Microorganisms in the element cycling and in soil aggregation

Microorganisms enhance the availability of nutrients by participating in the biogeochemical cycles of carbon, nitrogen, phosphorus, potassium, sulfur, iron and other microelements. Carbon cycling is essential for maintaining the natural carbon balance and has a significant impact on climate change and ecosystem health. Heterotrophic microorganisms contribute to carbon released into the atmosphere through organic material decomposition and respiration. Carbon is fixed in the soil by the assimilation of CO₂ through photosynthesis by photoautotrophs, phototrophic and chemoautotrophic bacteria. In anaerobic soil conditions, during the decomposition of organic matter, methanogenic bacteria produce methane, which volatilizes into the atmosphere and influences the negative effects of climate change. Aerobic and anaerobic microorganisms participate in the decomposition of organic matter

in the process of ammonification. Various species of the genus *Nitrosomonas* and *Nitrobacter* oxidize ammonia to nitrite in the nitrification process, while nitrites formed during denitrification are reduced to elemental nitrogen by denitrifying bacteria. Free-living soil bacteria of the genus *Azotobacter*, *Clostridium*, *Beijerinckia*, *Azospirillum* and other diazotrophs, fix elemental nitrogen and reduce it to ammonia. Symbiotic nitrogen fixers such as bacteria of the genus *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, *Frankia* and cyanobacteria *Anabaena* and *Nostoc*, which can also live freely, form a mutualistic community with higher plants and fix significant amounts of nitrogen. Microorganisms, especially bacteria and fungi, play a key role in phosphorus mineralization, the process in which organic phosphorus substances are broken down into inorganic phosphate forms through the catalytic action of phosphatase. Phosphorus is present in the soil in large quantities as poorly soluble inorganic phosphates such as $\text{Ca}_3(\text{PO}_4)_2$ or FePO_4 , which are unavailable to plants. Phosphorus solubilization enables bacteria of the genus *Pseudomonas*, *Bacillus* and fungi *Penicillium*, *Aspergillus*, *Fusarium* and mycorrhizae to break down these compounds into plant-available forms with the help of organic acids.

Various species of the genus *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Acidithiobacillus* and others participate in the potassium cycle and its availability to plants. Through processes such as mineral decomposition, mineralization of organic matter, production of organic acids and formation of complexes with potassium, microorganisms help release potassium from unavailable forms in the soil making it accessible for plant absorption. These microbiological processes are essential for soil health and crop productivity, providing plants with the potassium necessary for growth and development. Microorganisms play a key role in many stages of the sulfur cycle, facilitating the transformation of sulfur between different chemical forms. Microorganisms in soil and aquatic ecosystems can break down organic sulfur compounds, releasing sulfur in the H_2S form. Chemo- and photolithotrophs such as green sulfur and purple sulfur bacteria use H_2S as a source of electrons in the process of anaerobic photosynthesis. The oxidation of elemental sulfur to sulfate is carried out by photosynthetic and chemolithoautotrophic microorganisms, while the reduction of sulfate by sulfate-reducing bacteria of the genus *Desulfovibrio* and *Desulfomonas* results in the formation of hydrogen sulfide. Microorganisms are involved in the iron cycle in nature, which, although an essential element for many biological processes, is not always in an easily accessible form. Microorganisms influence the oxidation-reduction state of iron, primarily by producing siderophores that bind Fe^{3+} and reduce it to Fe^{2+} . In this way, siderophores help maintain the balance of iron in nature, making it available in forms that would otherwise be difficult for plants to use. Additionally, microorganisms influence the accessibility of other micronutrients such as zinc, copper, manganese, molybdenum, boron and cobalt. Bacteria play a significant role in forming stable soil aggregates, which help maintain soil quality, structure, and productivity. The stability of these aggregates improves aeration, water permeability, root growth and nutrient retention. Extracellular polymeric substances, especially polysaccharides are crucial for the stability of micro and macro aggregates, as they help retain water within the aggregates, reducing erosion. This creates better conditions for plants, making them more resilient to external factors such as rain, wind, or human activity. Furthermore, these substances reduce soil compaction by maintaining structural stability and increasing soil porosity, which improves air and water flow. The network of fungal hyphae also connects soil particles and forms stable macroaggregates that are more resistant to erosion and breakage.

Plant growth promoting microbes

PGPM (Plant growth promoting microbes) represent various categories of microorganisms that positively influence plant growth through different mechanisms. These microbes

improve plant health, enhance productivity, increase stress resistance and improve soil nutrient efficiency. The most common microorganisms in this category include bacteria from the genus *Azospirillum*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Enterobacter* and others, as well as fungi such as mycorrhiza and species from the genus *Trichoderma*. These microbes often exist in symbiosis with plants or reside in the rhizosphere where they directly influence plant growth. In addition to their role in nitrogen fixation, phosphorus solubilization and soil aggregation, these microorganisms produce plant hormones, such as auxins, cytokinins, and gibberellins, which stimulate plant growth (de Andarde et al., 2023). These hormones promotes root development, promote the formation of new shots and improve nutrient absorption. Microorganisms from the genus *Bacillus* and *Pseudomonas* synthesize secondary antimicrobial metabolites, including iturin, surfactin 2,4-diacetyl phloroglucinol (DAPG), and phenazines – compounds that inhibit pathogenic microorganisms, induce systemic resistance (ISR) in plants and improve plant disease resistance and can be used both preventively and curatively in the biological control of plant diseases (Dimkić et al., 2021). Furthermore, microorganisms help plants cope with environmental stress such as drought, by producing antioxidants that mitigate oxidative stress, enhancing water uptake, and lowering ethylene levels via ACC deaminase production. The use of PGPM in agriculture is becoming increasingly popular due to its environmental and economic benefits. However, a deeper understanding of the interactions between microbes, plants and soil is necessary to determine the conditions under which these microorganisms function most effectively (Moore et al., 2022).

Environmentally friendly and effective pesticides

The intensive use of agrochemicals, including plant protection products, to prevent and control plant diseases and to achieve high yields is common in agricultural production. However, their use can lead to several negative impacts, such as persistence, bioaccumulation, leaching into water resources, toxicity to non-target organisms, the development of resistance in pests and potential risks to human health. To minimize these adverse effects, it is crucial to research and develop safe, sustainable alternatives. One strategy includes optimizing the chemical structure of new plant protection products, such as heterocyclic compounds like imidazole. Heterocyclic compounds have potential advantages as next-generation pesticides due to their specific biological activity, faster environmental degradation, lower toxicity, greater selectivity, reduced risk of resistance development, and lower potential for bioaccumulation. They represent a safer alternative to traditional broad-spectrum chemical pesticides, by minimizing negative environmental and health impacts (Rastija et al., 2021). The design of new molecules with desirable properties involves optimizing their chemical structures to enhance biological activity and selectivity using the Computer-Aided Molecular Design (CAMD) approach. This includes methods such as Quantitative Structure-Activity Relationship (QSAR) analysis, which examines the relationship between the chemical structure and the biological activity.

How to increase the abundance of soil microorganisms?

The optimal abundance of beneficial microorganisms in the soil can be achieved through sustainable soil management, which improves soil fertility and structure while increasing plant resilience. Some important guidelines for maintaining healthy, microbiologically rich soil include supporting soil interactions by encouraging symbiotic relationships between plants and microorganisms, such as mycorrhiza and PGPM, which are essential for soil health. Plants that interact harmoniously with microorganisms create favorable conditions for their growth and reproduction. Regular application of organic material such as compost, and manure, which serves as a food source for microorganisms, stimulates microbial activity,

maintaining their abundance and diversity. It is also important to maintain optimal soil moisture to support microbial life. Reducing the use of chemical pesticides and fertilizers, which can negatively impact beneficial microorganisms, is crucial; instead, integrated approaches to pest control and nutrient optimization should be pursued. Crop rotation and diversification help sustain soil health while minimizing excessive tillage to preserve microbial community dynamics. Sowing cover crops and using crop residues helps to conserve moisture, reduce nutrient leaching and prevent erosion and organic matter loss. Additionally, applying microbial inoculants such as biofertilizers and biopesticides containing beneficial microorganisms enhances microbial biodiversity. Implementing these guidelines will help maintain the balance of microbial communities in the soil, ensuring long-term fertility and sustainability in agricultural production.

Conclusions

Soil microorganisms are essential for maintaining soil fertility and promoting plant health by enhancing plant nutrient uptake and improving crop yields, while microbial diversity ensures ecosystem stability. However, the excessive use of chemical fertilizers and pesticides in agricultural practices can disrupt microbial communities, leading to soil degradation. Sustainable agricultural practices help preserve microbial diversity and boost soil productivity. Additionally, climate change and environmental factors significantly affect microbial composition, influencing their ability to sustain soil fertility. Understanding soil microbiomes has led to the biofertilizers and biopesticides development, reducing reliance on synthetic chemicals and promoting sustainable agriculture. Therefore, the conservation and restoration of microbial communities are vital for long-term soil health, agricultural productivity and global food security. Future research on soil microbiomes will offer innovative solutions for mitigating environmental challenges and ensuring a sustainable agricultural future.

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References

- de Andrade L.A., Santos C.H.B., Frezarin E.T., Sales L.R., Rigobelo E.C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms* 21;11(4):1088.
- Dimkić I., Janakiev T., Petrović M., Degrassi D., Fira Dj. (2021). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms - A review. *Physiological and Molecular Plant Pathology* 117: 101754.
- Hopkins D.W., Dungait J.A.J. (2010). Soil Microbiology and Nutrient Cycling. In: *Soil Microbiology and Sustainable Crop Production*, Dixon G., Tilston E. (ed), 59-80. Dordrecht, Springer.
- Moore J.A.M., Abraham P.E., Michener J.K., Muchero W., Cregger M.A. (2022). Ecosystem consequences of introducing plant growth promoting rhizobacteria to managed systems and potential legacy effects. *New Phytologist* 234: 1914-1918.
- Rastija V., Vrandečić K., Čosić, J., Majić I., Šarić G.K., Agić, D., Karnaš M, Lončarić, M., Molnar, M. (2021). Biological Activities Related to Plant Protection and Environmental Effects of Coumarin Derivatives: QSAR and Molecular Docking Studies. *International Journal of Molecular Science* 22: 7283.

ORIGINAL SCIENTIFIC PAPER

Assessment of Heavy Metal Contamination in Garden Soils of the Petrinja Town

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Summary

To assess soil quality in the Petrinja town, soil samples were taken from ten private urban gardens to determine soil reaction (pH), organic matter content, and heavy metals concentration (Cd, Hg, Pb, Mo, As, Ni, Co, Cu, Cr, Zn). The results showed elevated levels of zinc and lead above the legal limits at two locations. The pH values were close to neutral, while the organic matter content ranged from low to moderate. A strong positive correlation between the organic matter content and the concentrations of Cd, Co, Cr, Ni and Zn was observed and very weak to weak correlation with soil reaction. For a more accurate assessment of heavy metal concentrations in Petrinja, further studies are recommended, especially in the areas along the Kupa River that have been cleared of landmines and are being converted for agricultural use.

Keywords: private gardens, heavy metals, soil quality, Petrinja

Introduction

Among the various forms of soil degradation, contamination with heavy metals is of increasing importance and attracts great public attention (Adriano, 2001). This contamination refers to heavy metal concentrations in soil that cause observable or measurable disturbances to soil functions (Alloway, 2012), particularly its fertility and suitability for supporting natural or cultivated plant growth (Blum, 2005). Chemically, heavy metals are defined as elements with a density of more than 5 g/cm³ and an atomic number of more than 20 (Kisić, 2012). Biologically, a more nuanced classification takes into account the complexing properties. Alloway (2012) categorizes heavy metals into essential elements for some organisms (V, Cr, Mn, Fe, Co, Zn, Mo), elements necessary for plant growth (Mn, Fe, Cu, Zn, Mo, and possibly Ni), and phytotoxic elements (Cd, Hg, Pb). Some heavy metals therefore serve as micronutrients, but can also be phytotoxic, although the range between beneficial and harmful concentrations is small. Of the 65 metallic elements in the periodic table, seven of the ten most abundant in the lithosphere are heavy metals (Kabata-Pendias & Pendias, 2001). Romić and Romić (2003) associate elevated heavy metal levels with industrial or agricultural activities or link them to the geogenic origin of soils. Ajmone-Marsan and Biasioli (2010) compiled data from 153 studies on heavy metals in urban soils in various cities worldwide, and found elevated levels of lead, copper, zinc, and nickel in almost all cases. Cicchella et al. (2020) found that elements such as Al, Ca, Fe, K, Mg, Na, P, S, and Ti are naturally distributed without significant human influence, while elements such as Ag, As, Au, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Sb, Sn, Tl, V, and Zn have high concentrations, especially in densely populated areas, at industrial sites and along roads. Manta et al. (2002) attribute elevated levels of Pb, Zn, Cu, Sb, and Hg to anthropogenic pollution, while elevated concentrations of Mn, Ni, Co, Cr, V, and Cd are associated with the source material. Pecina et al. (2021) assessed urban soil quality and its

impact on human health in 101 Chinese cities, and analyzed over 17,000 soil samples. Their evaluation showed that 11% of the cities had highly contaminated soils. In 15 cities, risk levels for young children exceeded standard tolerances, and carcinogenic risk assessments indicated potential hazards in cities with a population of nearly 30 million. Cr and As were identified as the most dangerous elements, with cadmium posing the greatest risk to urban green spaces. The authors argue for an urgent change in urbanization policy and the development of functional urban green spaces adapted to the high levels of pollution (Pecina et al., 2021). To determine the current soil quality, regular soil analysis and continuous monitoring are essential, including soil sampling, laboratory testing, and interpretation of the results. Soil reaction and organic matter content are crucial factors for the assessment of heavy metal concentrations. This study aimed to assess the heavy metals (Cd, Hg, Pb, Mo, As, Ni, Co, Cu, Cr, Zn) contamination of garden soils of the Petrinja town and the relationship between metals and organic matter content and pH value.

Materials and methods

The study included ten private garden locations (plus two control samples) in the narrow area of the Petrinja town. The control samples included a meadow (sample 2-1), which was located in the vicinity of the private gardens, and had not been used for agricultural production or fertilized with organic or mineral fertilizers (Figure 2). The second control sample (3-1) was taken in an uncultivated urban area without any vegetation. The locations of the private family gardens were selected on the basis of land use criteria — namely, that conventional vegetable cultivation had been practiced there continuously since 1995. The most commonly grown crops included peppers, tomatoes, cucumbers, potatoes, pumpkins, beans, and various types of lettuce. The size of the gardens ranged from around 50 to 200 square meters. The soil samples were taken on May 21, 2019. The exact sampling locations are shown in Figure 1. Depending on the size of the garden, two or three composite samples were taken from each site at a depth of 20 cm. Approximately one kilogram of soil was taken from each site (Figure 2). In the laboratory, the samples were homogenized and air-dried at room temperature and then prepared for analysis according to HRN ISO 11464:2009. The following parameters were determined in all soil samples: soil reaction (pH) using a modified ratio of 1:2.5 w/v according to HRN ISO 10390:2005, organic matter content using a modified method according to HRN ISO 14235:2004 (Zgorelec et al., 2011) and concentrations of selected heavy metals. The analysis of pH and soil organic matter content was carried out at the Faculty of Agriculture, University of Zagreb, Department of General Agronomy. The analysis of heavy metal concentrations (Cd, Hg, Pb, Mo, As, Ni, Co, Cu, Cr and Zn) was carried out at the Quality Control Institute in Sisak. Soil digestion was performed using aqua regia (0.5 g sample/10 ml solution; HCl:HNO₃ = 3:1 v/v) in accordance with HRN ISO 11466:2004. Detection and quantification of metals were performed using the ICP-OES technique (ICPE 9000 Shimadzu) following the HRN EN ISO 11885:2010 method. The contamination assessment was carried out on the basis of the Regulation on the Protection of Agricultural Land from Pollution (Official Gazette 71/19), which specifies the pollutants and their maximum allowable concentrations (MAC) in soils, while relationship between metals and organic matter content and soil pH was determined by calculating Pearson's correlation coefficients using SAS 9.1.3. statistical program.



Figure 1. Locations of private gardens in the Petrinja town where soil sampling was conducted



Figure 2. Soil sampling in a private garden (left) and in a meadow control location (right)

Results and discussion

Soil reaction and organic matter content in the analyzed samples are presented in Figures 3 and 4. The highest measured soil pH value among the analyzed samples from the Petrinja town was 7.72 (samples 1 and 8), while the lowest was 5.70 (control sample 3-1). The average pH value was 7.17. On average, garden soils in the Petrinja town exhibit a neutral to very slightly alkaline reaction. The organic matter content in the analyzed soils was generally up to 3%, with the exception of location 7, where a value of 5.4% was recorded (Figure 4). Surprisingly, the garden soils in Petrinja are, on average, low in organic matter. Given that low organic matter content was observed at most locations, fertilization with well-composted farmyard manure or other organic soil amendments is recommended to improve soil quality and enhance its suitability for vegetable production (Kisić, 2018).

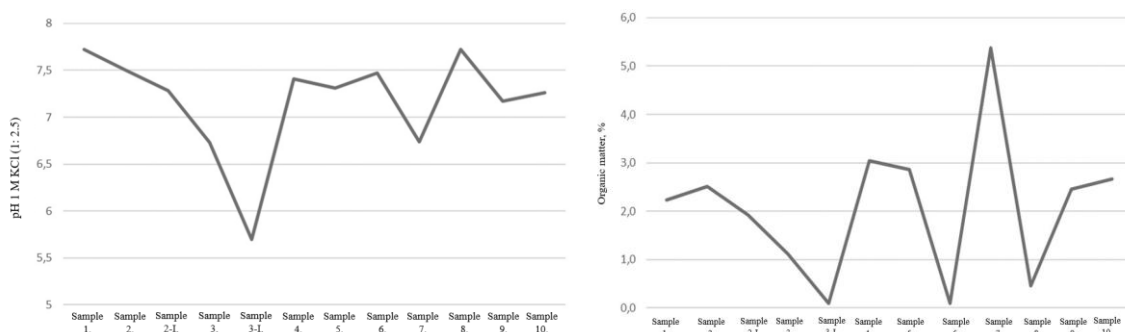


Figure 3 and 4. Soil reaction in the research area; Organic matter content in the researched soils

Tables 1 and 2 present the concentrations of metals in the analyzed soil samples. In total, concentrations of ten elements were compared with MAC values (30 mg As kg⁻¹, 2 mg Cd kg⁻¹, 60 mg Co kg⁻¹, 120 mg Cr kg⁻¹, 120 mg Cu kg⁻¹, 75 mg Ni kg⁻¹, 150 mg Pb kg⁻¹, 200 mg Zn kg⁻¹, 15 mg Mo kg⁻¹ and 1.5 mg Hg kg⁻¹) according to Regulation on the Protection of Agricultural Land from Pollution Concentrations of As, Cd, Co, Cr, Cu, Hg, Mo, and Ni were within the permissible limits. Exceptions include Zn at location 2-1 (control) and location 4, where concentrations exceed MAC values for children's playgrounds and agricultural soils (Mesić et al., 2008). Pb concentrations at location 4 also exceed the permissible values. In all analyzed garden soil samples from Petrinja, mercury and molybdenum concentrations were below the detection limit.

Table 1. Concentration of metals according to analyzed locations, mg/kg of soil

	LOCATION					
	1	2	2-1-control	3	3-1-control	4
As	6.49	6.28	4.94	9.18	4.86	6.90
Cd	0.34	0.45	0.55	0.22	0.17	1.11
Co	12.2	8.81	10.1	9.97	9.45	8.69
Cr	37.5	33.8	45.9	35.3	27.7	29.3
Cu	42.5	33.7	54.3	25.7	15.5	72.6
Ni	24.0	20.2	24.7	16.5	15.5	19.2
Pb	25.1	27.2	49.2	29.4	30.2	210
Zn	136	131	237	134	86.4	261
Mo	< 0.0004					
Hg	< 0.004					

Table 2. Concentration of metals according to analyzed locations, mg/kg of soil

	LOCATION					
	5	6	7	8	9	10
As	5.31	12.3	10.1	6.90	4.42	8.31
Cd	0.58	0.87	1.13	0.29	0.31	0.71
Co	8.89	13.3	29.4	8.27	13.1	11.9
Cr	25.9	31.5	66.7	27.3	22.9	39.0
Cu	55.9	48.9	38.2	21.6	29.1	116
Ni	16.6	28.4	47.6	16.7	17.3	38.3
Pb	51.2	30.3	49.0	28.4	30.3	88.7
Zn	188	116	160	90.4	131	152
Mo	< 0.0004					
Hg	< 0.004					

Previous studies conducted in the Sisak-Moslavina County, in particular in the towns of Glina (Bašić et al., 1994) and Sisak (Šorša and Halamić, 2014), were used to compare the concentrations of the investigated metals. Almost identical concentrations of certain heavy metals were determined during the investigations in the Glina area. Higher levels of lead and zinc were found in the Petrinja area compared to Glina. Compared to the Sisak town —

where increased concentrations of arsenic, copper, zinc, cadmium and lead were found in certain places — the soil quality in Petrinja is significantly more favorable. This increase in contamination in Sisak is related to the previously developed industrial activities in the town. The highest concentrations of certain heavy metals were found in the zones of former industrial complexes. This indicates that industrial activities (such as the oil refinery – hydrocarbon processing; the thermal power plant – fossil fuel combustion; the steel mill – ore smelting and metal processing; and the chemical industry – pesticide production) were the main sources of soil pollution in Sisak. Since there were no large industrial plants in the Petrinja area, the soils in this region are of better quality, i.e. less polluted.

The correlation analysis, shown in Table 3, was carried out using the Roemer-Orphal scale. The results show a strong positive correlation between the organic matter content and the concentrations of Cd ($r = 0.60$), Co ($r = 0.65$), Cr ($r = 0.63$), Ni ($r = 0.61$) and Zn ($r = 0.50$). On the contrary, soil pH showed a very weak to weak correlation with metal content. A very strong correlation was found between Ni and Cr ($r = 0.84$), as well as Co and Ni ($r = 0.82$). Pb and Zn also showed a strong mutual correlation ($r = 0.73$).

Table 3. Correlation relationship of the investigated parameters

	pH	Organic matter	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
pH	1									
Organic matter	0.11	1.00								
As	0.10	0.02	1.00							
Cd	0.20	0.60	0.51	1.00						
Co	-0.19	0.65	0.47	0.53	1.00					
Cr	-0.12	0.63	0.40	0.50	0.82	1.00				
Cu	0.32	0.34	0.17	0.54	-0.03	0.14	1.00			
Ni	0.01	0.61	0.57	0.66	0.82	0.84	0.46	1.00		
Pb	0.13	0.33	0.00	0.63	-0.11	-0.04	0.58	0.07	1.00	
Zn	0.25	0.50	-0.16	0.58	-0.02	0.22	0.52	0.11	0.73	1.00

Conclusion

The heavy metal concentrations determined in the selected gardens in the Petrinja town indicate that the current use of these soils has not led to increased heavy metal concentrations, except to garden at location ‘‘4’’, where content of zinc (261 mg/kg) and lead (210 mg/kg) exceed the MAC values according to Croatia national regulation. Moreover, industrial facilities in the Petrinja town and nearby Sisak have not caused elevated heavy metal concentrations in the soils of Petrinja's urban gardens. Further research, involving a larger number of samples and investigated locations, would certainly help clarify some of the uncertainties revealed in this preliminary study.

References

- Adriano D. C., (2001). Environmental Contamination and Regulation. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals, p. 861.
- Ajmone-Marsan F., Biasioli M. (2010). Trace elements in soils of urban areas. *Water, Air, & Soil Pollution*, 213, 121-143.
- Alloway B. J. (Ed.). (2012). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (Vol. 22). Springer Science & Business Media, p. 368
- Bašić F., Mesić M., Butorac A. (1994). Teške kovine u tlima općine Glina. *Agronomski glasnik*. 1-2.
- Blum W.E.H. (2005). Functions of soil for society and the environment. *Reviews in Environmental science and Biotechnology*, 4/3: 75-79.
- Cicchella D., Zuzolo D., Albanese S., Fedele L., Di Tota I., Guagliardi, I., Lima A. (2020). Urban soil contamination in Salerno (Italy): Concentrations and patterns of major, minor, trace and ultra-trace elements in soils. *Journal of Geochemical Exploration*, 213, 106519.

- Halamić J., Miko S. (2009). Geokemijski atlas Hrvatske. Hrvatski geološki institut Zagreb, str. 87.
- ISO (2004a). Soil quality – Determination of pH (modified; w/v = 1 : 2,5). [HRN ISO 10390:2005]. Geneva, Switzerland: International Organization for Standardization.
- ISO (2004b). Soil quality – Determination of organic carbon by sulfochromic oxidation (modified; titrimetric determination, Tjurin method, bichromate method). [HRN ISO 14235:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO (2004c). Soil quality – Extraction of trace elements soluble in aqua regia. [HRN ISO 11466:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO (2009). Soil quality – Pretreatment of samples for physico-chemical analysis. [HRN ISO 11464:2009]. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010). Water quality – Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES). [HRN EN ISO 11885:2010]. Geneva, Switzerland: International Organization for Standardization.
- Kabata-Pendias A., Pendias H. (2001). Trace elements in soils and plants. Third Edition, CRC Press Book, p. 413.
- Kisić I. (2018). Gradska poljoprivreda. Agronomski fakultet Sveučilišta u Zagrebu, str. 311.
- Kisić I. (2012). Sanacija onečišćenog tla. Agronomski fakultet Sveučilišta u Zagrebu, str. 276.
- Manta D. S., Angelone M., Bellanca A., Neri R., Sprovieri M. (2002). Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. *Science of the total environment*, 300(1-3), 229-243.
- Mesić H. i sur. (2008) Program trajnog motrenja tala Hrvatske. Agencija za zaštitu okoliša, str. 131.
- Pecina V., Brtnický M., Baltazár T., Juříčka D., Kynický J., Galiová M. V. (2021). Human health and ecological risk assessment of trace elements in urban soils of 101 cities in China: A meta-analysis. *Chemosphere*, 267, 129215.
- Romic M., Romic D. (2003). Heavy metals distribution in agricultural topsoils in urban area. *Environmental geology*, 43, p. 795-805.
- Šorša A., Halamić J. (2014). Geokemijski atlas Siska. Sisak – Zagreb, str. 200.
- Zgorelec Ž., Mesić M., Vuković I., Jurišić A., Komesarović B., Klaić D., Sajko K. (2011). Comparison of Methods for Soil Sampling and Carbon Content Determination. *Agriculturae Conspectus Scientificus*, 76(1), 15-18.

EXPERT PAPER

Research progress on biogas production from lignocellulose pretreated with ultrasound based on bibliometric analysis

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Summary

This study aims to explore the role of biogas production in achieving Sustainable Development Goals (SDGs), with a focus on ultrasound-assisted pretreatment of lignocellulosic biomass. A bibliometric analysis using Web of Science data (2010–2023) highlights a rise in research activity after the SDGs' introduction in 2015. Ultrasound, microwave, and steam explosion emerged as the most studied pretreatment methods. The geographic analysis revealed Spain, China, and India as leading contributors. Results emphasize biogas's contribution to environmental, economic, and social sustainability, while noting untapped potential for commercializing ultrasound pretreatment technologies.

Keywords: anaerobic digestion, physical pretreatment, sustainable development goals, waste utilization

Introduction

Rapid urbanization and industrialization have led to increased energy consumption and environmental harm. Addressing energy shortages requires a transition to renewable energy, energy efficiency, and alignment with the Sustainable Development Goals (SDGs) established in 2015 (Archana et al., 2024). SDGs aim to end poverty, improve health and education, and address climate change while promoting economic growth (UN, <https://sdgs.un.org/goals>). The global energy goal is covered in SDG 7 (Affordable and Clean Energy) but this topic is also related to several other SDGs. Countries cannot achieve development without affordable and reliable energy supply, and universal access to modern energy services therefore one of the targets is growing the share of renewable energy in global energy production and consumption (Gielen et al., 2019). One of such solutions is biogas production from lignocellulose biomass. Biogas, which is renewable and eco-friendly energy alternative, directly supports several SDGs and aligns with the broader goals of a circular economy and sustainable development. Scientific research, especially interdisciplinary research worldwide, is essential for contributing to SDGs because the scientific community helps translate global goals into practical national and local agendas (Meschede, 2020). Lignocellulose, derived from agricultural and forestry waste, holds great potential for biogas production and aligns with the sustainable bioeconomy approach. However, its complex structure, with 10 to 30% lignin, requires pretreatment for effective anaerobic digestion (Ruett et al., 2024). No pretreatment method has been found suitable for all lignocellulosic types. Different methods act diversely on lignocellulose due to its heterogeneous chemical structure and complexity. Effective pretreatment should minimize investment and energy costs, enhance feedstock degradation, reduce lignin recalcitrance, and increase biogas yield. Methods are typically classified into mechanical, physical, chemical, biological, and combination of pretreatments (Kamperidou and Terzopoulou, 2021). Physical pretreatment methods (which also includes ultrasound) alter lignocellulose's

physical properties (Wang et al., 2023) without using chemicals or microorganisms (Camargo et al., 2023).

Ultrasound pretreatment of lignocellulose has gained attention as an effective, eco-friendly alternative to chemical methods for improving biomass conversion. High-intensity ultrasound generates mechanical stress and cavitation effects, disrupting cell walls, increasing surface area, and enhancing polysaccharide accessibility for enzymatic hydrolysis (Ong and Wu, 2020). This paper presents a bibliometric analysis of research on biogas production and its role in achieving SDGs, focusing on studies involving ultrasound-assisted pretreatment. It also compares the number of published papers on this topic and maps the geographic distribution of ultrasound pretreatment research to identify key regions contributing to this field.

Material and methods

The literature review was conducted using the Web of Science Core Collection (WoSCC) in December 2024. A bibliometric analysis was performed based on topic searches, retrieving records with specified terms in fields like title, abstract, author keywords, and Keywords Plus. Boolean operators defined relationships between terms, focusing on biogas production and SDGs. SDG indexing in WoSCC was done systematically, combining bibliometric analysis and manual curation. Each SDG was linked to a specific Micro Citation Topic, derived from citation connections in the database. Regular reviews and re-clustering ensured accuracy and relevance in mapping research on sustainable development. The bibliographic analysis of physical pretreatment methods in biogas production focused on topic searches combining "biogas" with "ultrasound/ultrasonic pretreatment", "microwave pretreatment", "steam explosion pretreatment", "pyrolysis pretreatment", "autoclave pretreatment", "extrusion pretreatment" and "oven pretreatment". These methods were chosen for their prominence in research. The analysis also examined the number of papers published on each method over 14 years (2010–2023). The analysis examined the geographic distribution of ultrasound pretreatment studies in biogas research, focusing on the top 10 countries with the most publications on ultrasound pretreatment of lignocellulose for biogas production. Using author affiliations and citation analysis from WoSCC, the research identified the geographic origins of publications through detailed metadata, with manual curation to ensure accuracy and consistency.

Results and discussion

Figure 1 shows the relationship between biogas production and SDGs, highlighting how waste management and energy production can advance multiple goals. It presents the number of studies per dominant SDG in biogas research from a WoSCC search on "biogas". At least 7 SDGs were listed as those that can be advanced by applying anaerobic digestion: Responsible consumption and production (SDG 12) with 20 735 papers, Affordable and clean energy (SDG 7) with 5 174 papers, Climate action (SDG 13) with 4 134 papers, Clean water and sanitation (SDG 6) with 3 771 papers, Sustainable cities and communities (SDG 11) with 1 577 papers, Zero hunger (SDG 2) with 1 537 papers, and Good health and well-being (SDG 3) with 904 papers.



Figure 1. The total number of studies per dominant SDGs in biogas research indexed in WoSCC for the period from 2010 to 2023

Biogas production significantly contributes to several SDGs by addressing environmental issues such as waste management, pollution, and resource depletion. It helps reduce air and water pollution (SDG 12) and mitigates health risks (SDG 3), while offering a renewable, clean energy source that supports SDG 7 (Frankowski and Czekala, 2023). By reducing greenhouse gas emissions and replacing chemical fertilizers, biogas production also contributes to SDG 13 (Dhungana et al., 2022). Moreover, biogas plants can treat wastewater, improving water quality (SDG 6) and minimizing waste in landfills, promoting sustainable cities (SDG 11) (Piadeh et al., 2024). Additionally, biogas supports SDG 2 by enhancing smallholder productivity, improving yields, and increasing farmers' income through the use of digestate (Triple Line Consulting, 2020). The bibliographic analysis of physical lignocellulose pretreatments in biogas production highlighted common methods like ultrasound, microwave, and steam explosion, while extrusion and conventional thermal treatments (hot air oven and autoclave) were less represented. A notable increase in publications occurred between 2016 and 2018, coinciding with the introduction of the UN's SDGs in 2015. Extrusion, mainly used in other industries, is gaining attention in biogas but has seen limited progress (Konan et al., 2022). Conventional heating methods are more common in biogas research on sludges and manures.

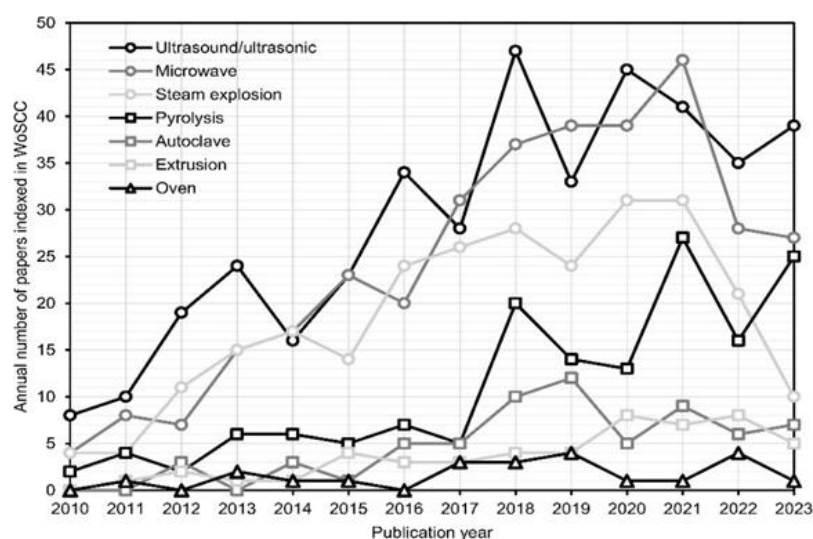


Figure 2. The annual number of biogas studies indexed in WoSCC according to major physical pretreatment methods for the period from 2010 to 2023

The literature review revealed that ultrasound pretreatment research is conducted worldwide. Further analysis explored the geographic distribution of ultrasound pretreatment studies in biogas research (Figure 3) and the number of papers published in the top 10 countries (Table 1). Spain leads with 77 papers, followed by China (73), India (58), Italy (48), and Poland (37). The UK, Germany, Australia, France, and South Korea each have around 20 papers.

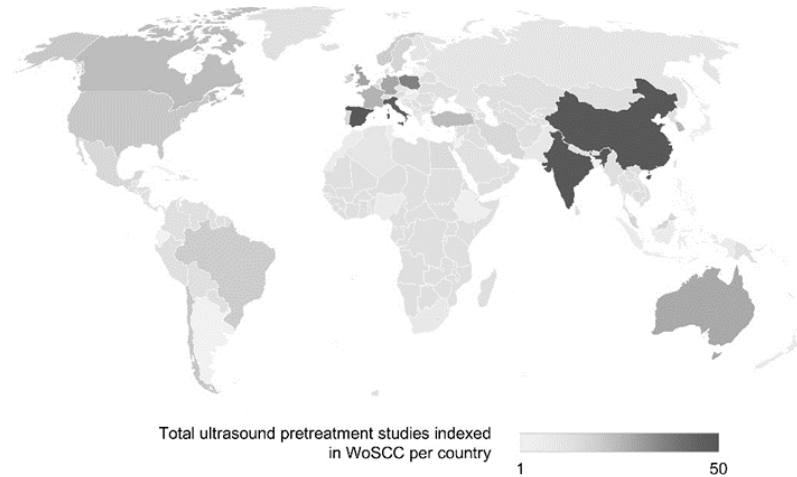


Figure 3. The geographic distribution of ultrasound pretreatment studies in biogas research according to WoSCC.

Table 1. Number of studies on ultrasound pretreatment of substrates for biogas production in the leading countries (period from 2010 to 2023).

Country	Number of published papers
Spain	77
China	73
India	58
Italy	48
Poland	37
United Kingdom	26
Germany	25
Australia	24
France	21
South Korea	19

Conclusions

Biogas production plays a key role in achieving multiple SDGs by offering sustainable solutions for waste management, renewable energy, climate change, and agricultural productivity. A surge in published papers between 2016 and 2018 aligns with the SDGs' introduction in 2015, indicating growing interest in biogas research linked to global sustainability. Biogas supports a comprehensive approach to sustainable development, addressing environmental, economic, and social aspects.

A bibliometric analysis of physical pretreatment methods for lignocellulosic biomass in biogas production shows that ultrasound, microwave, and steam explosion are the most studied. While these methods are used in research, there's still significant potential for their commercialization.

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References

- Archana K., Viskram A.S., Senthil Kumar P., Manikandan S., Saravanan A., Natrayan L. (2024). A review on recent technological breakthroughs in anaerobic digestion of organic biowaste for biogas generation: Challenges towards sustainable development goals. *Fuel* 358:130298.
- Camargo F.P., Rabelo C.A.B.S., Duarte I.C.S., Silva E.L., Varesche M.B.A. (2023). Biogas from lignocellulosic feedstock: A review on the main pretreatments, inocula and operational variables involved in anaerobic reactor efficiency. *International Journal of Hydrogen Energy*, European Fuel Cells and Hydrogen Piero Lunghi Conference 2021 48, 20613–20632.
- Dhungana B., Lohani S.P., Marsolek M. (2022). Anaerobic Co-Digestion of Food Waste with Livestock Manure at Ambient Temperature: A Biogas Based Circular Economy and Sustainable Development Goals. *Sustainability* 14:3307.
- Frankowski J., Czekala W. (2023). Agricultural Plant Residues as Potential Co-Substrates for Biogas Production. *Energies* 16:4396.
- Gielen D., Boshell F., Saygin D., Bazilian M.D., Wagner N., Gorini R. (2019). The role of renewable energy in the global energy transformation. *Energy Strategy Reviews* 24:38–50.
- Kamperidou V., Terzopoulou P. (2021). Anaerobic Digestion of Lignocellulosic Waste Materials. *Sustainability* 13:12810.
- Konan D., Koffi E., Ndao A., Peterson E.C., Rodrigue D., Adjallé K. (2022). An Overview of Extrusion as a Pretreatment Method of Lignocellulosic Biomass. *Energies* 15:3002.
- Meschede C. (2020). The Sustainable Development Goals in Scientific Literature: A Bibliometric Overview at the Meta-Level. *Sustainability* 12:4461.
- Obaideen K., Shehata N., Sayed E.T., Abdelkareem M.A., Mahmoud M.S., Olabi A.G. (2022). The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. *Energy Nexus* 7:100112.
- Ong V.Z., Wu T.Y. (2020). An application of ultrasonication in lignocellulosic biomass valorisation into bio-energy and bio-based products. *Renewable and Sustainable Energy Reviews* 132:109924.
- Piadeh F., Offie I., Behzadian K., Rizzuto J.P., Bywater A., Córdoba-Pachón J.-R., Walker M. (2024). A critical review for the impact of anaerobic digestion on the sustainable development goals. *Journal of Environmental Management* 349:119458.
- Ruett J., Abdelshafy A., Walther G. (2024). Using miscanthus and biochar as sustainable substrates in horticulture: An economic and carbon footprint assessment of their primary and cascading value chains. *Sustainable Production and Consumption* 49:163–178.
- Triple Line Consulting [WWW Document], 2020. URL <https://tripleline.com/search/eyJyZXN1bHRfcGFnZSI6InNlYXJjaFwvaW5kZXgiLCJrZXI3b3JkcyI6InNkZyBiaW9nYXMiMifQ> (accessed 11.29.24).
- United Nations, n.d. Take Action for the Sustainable Development Goals. U. N. Sustainable Development URL <https://www.un.org/sustainabledevelopment/sustainable-development-goals/> (accessed 11.19.24).
- Wang J., Ma D., Lou Y., Ma J., Xing D. (2023). Optimization of biogas production from straw wastes by different pretreatments: Progress, challenges, and prospects. *Science of the Total Environment* 905:166992.

ORIGINAL SCIENTIFIC PAPER

The influence of the orientation and type of hedgerows on agricultural land shading

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Summary

Hedgerows are recognised as linear structures of woody and shrubby vegetation occurring in different agricultural ecosystems. The general public primarily identifies conservation and environmental values, which have been extensively researched with numerous studies available. When farmers manage hedgerows within their cultivated areas, they often face challenges as hedgerows can obstruct their farming activities. Farmers emphasize that shading crops primarily reduces the cultivated area, increases competition for soil moisture and nutrients between agricultural crops and hedgerows. Based on responses gathered through a questionnaire distributed to 1,197 farmers, where a significant proportion expressed concerns about reduced field productivity due to the presence of hedgerows, we further analysed the actual extent of shading on agricultural land. Our aim was to determine how the shading of agricultural land by hedgerows is affected by both the type and orientation of the hedgerows. For calculating the percentage of shading, we use ArborShadow R4 software for two different types of hedgerows: a tree hedgerow with a height of 20 meters and a shrub hedgerow with a height of 5 meters. The input data included latitude and longitude, hedgerow orientation, tree composition, time, and date. The results indicate a strong correlation between the percentage of shading on agricultural land and both the type and orientation of hedgerows. Shrub hedgerows consistently shade a smaller percentage of adjacent agricultural areas compared to taller tree hedgerows, regardless of orientation. Notably, the lowest shading levels were observed when the shrub hedgerow was oriented in the east–west direction.

Key words: hedgerow, shading, agricultural area, agriculture

Introduction

One of the oldest and traditional forms of agroforestry systems is hedgerows bordering arable fields, grasslands, and other land-use types. Their structural characteristics and species diversity vary considerably depending on their origin - planted, spontaneous, or remnant - as well as on adjacent farming practices and the intensity and tradition of their management (Forman, 1984). Hedgerows significantly affect the microclimatic conditions of adjacent agricultural land, with their orientation and structural type playing a crucial role in determining the extent and pattern of shading throughout the growing season (Pollard, 1974; Earnshaw, 2004). In the past, they served to mark property boundaries between individual plots, to separate pastures from arable land, and to provide firewood. Over time, the number of hedgerows began to decline due to the expansion of arable land and the construction of road infrastructure (Burel, 1996; Montgomery, 2020; Holden, 2019). In Slovenia, hedgerows are the most recognized agricultural landscapes, and the official records include 4522 hedgerows totaling 458.5 km (Borec and Lešnik, 2024; Lampič and Kastelic, 2021). In recent years, their value has been increasingly acknowledged - not only in terms of biodiversity conservation and cultural heritage - but also for their agronomic functions,

which are regaining importance. Hedgerows noticeably change the microclimatic conditions of the nearby agricultural land. Lower soil temperatures in shaded agricultural areas can inhibit nitrification and limit root water uptake (Knauer and Leskošek, 1991). Hedgerows reduce incoming solar radiation, moderate wind erosion, intercept precipitation, and modify the temperature and humidity profile of nearby soils (Wales, 1972; Dierschke, 1974; Pollard et al., 1974). Temperature differences of approximately 0.5°C have been recorded between the sunny and shaded sides of a hedgerow (Pollard et al., 1974), with relative humidity higher on shaded soils. Wind speeds are reduced near the base of hedgerows and increase towards their tops (Caborn, 1976). Consequently, soil evaporation is reduced in shaded zones, although transpiration may rise due to wind exposure (Ballard, 1979). Recent observations in Slovenia corroborate that soil temperature and moisture levels are directly influenced by hedgerow shading, with measurable impacts on gravimetric soil water and soil temperature (Borec et al., 2023). Despite their recognized ecological and landscape functions, the maintenance, removal, or transformation of hedgerows depends largely on farmers. As daily users of the land, farmers often perceive hedgerows as an obstacle to efficient cultivation. Notably, the potentially negative agronomic impacts of hedgerows - such as shading, competition for soil moisture and nutrients, and increased weed pressure - remain under-researched, with limited literature addressing this perspective (Lešnik, 2024). Shading by hedgerows can reduce the effective cultivation area and lead to reduced yields, especially in intensively managed fields. The presence of hedgerows may also favour the spread of invasive plant species and necessitate greater use of herbicides, contributing to increased input costs and reduced profitability (Oreszczyn and Lane, 2000). Based on farmers' feedback indicating that shading from hedgerows negatively affects cultivation and reduces yields, this study aims to quantify and compare the shading effects of different hedgerow types (tree vs. shrub) and orientations, using modelling tools to assess the extent of shaded agricultural areas. Understanding these interactions is crucial for balancing the ecological functions of hedgerows with the practical needs of agricultural production, especially in regions where land use intensity and biodiversity goals must be reconciled. Hedgerows in the landscape occur at various heights, with diverse vegetation structures and orientations. For the purpose of this study, we analysed typical hedgerows—a 6 m high shrub-type and a 20 m high tree-type, in both north-south and east-west orientations.

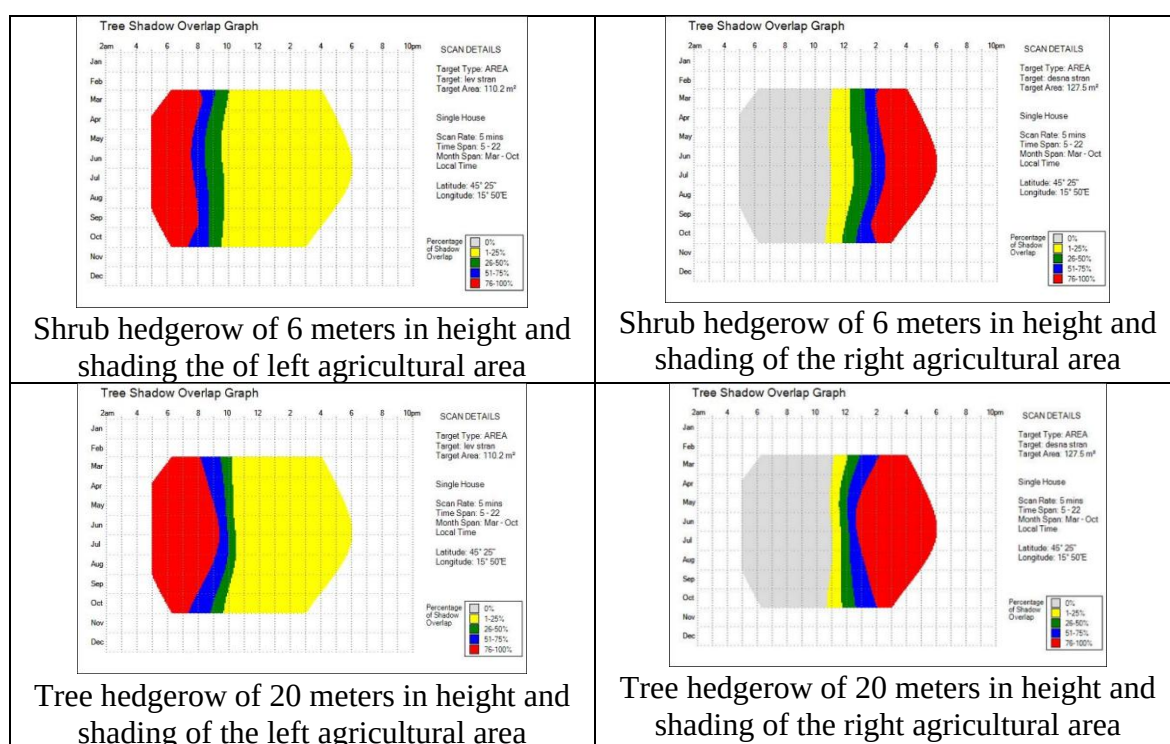
Material and methods

Survey input data are supported by a questionnaire designed to assess farmers' awareness of the impact of landscape elements. It included 11 semi-open and closed-ended questions related to farm characteristics, land use, and attitudes toward the presence and potential establishment of landscape features. Altogether, a total of 1,197 responses were carried out between February and April 2022, using the online tool 1KA. Farms were located in the area of the Chamber of Agriculture and Forestry of Slovenia – Ptuj Regional Unit. In the context of this article, we focused on a question in which farmers were asked to express their level of agreement (1 – do not agree, 2 – partially agree, 3 – fully agree) with the following statements: hedgerows presence reduces crop yield and hedgerow presence improves crop quality relating to shading effect on agricultural land. The results of farmers' perceptions were further supported with a spatial shading analysis using ArborShadow R4 software (2016). The model simulated the extent of shading caused by hedgerows based on geographic coordinates in Ptuj (Slovenia), tree height (6 and 20 meters), orientation (north–south and east–west), and time of day between 4:00 and 18:00 during the growing season of 2022. Shading intensity was expressed as a percentage and categorized into four classes: red (76–100%), blue (51–75%), green (26–50%), and orange (1–25%).

Results and discussion

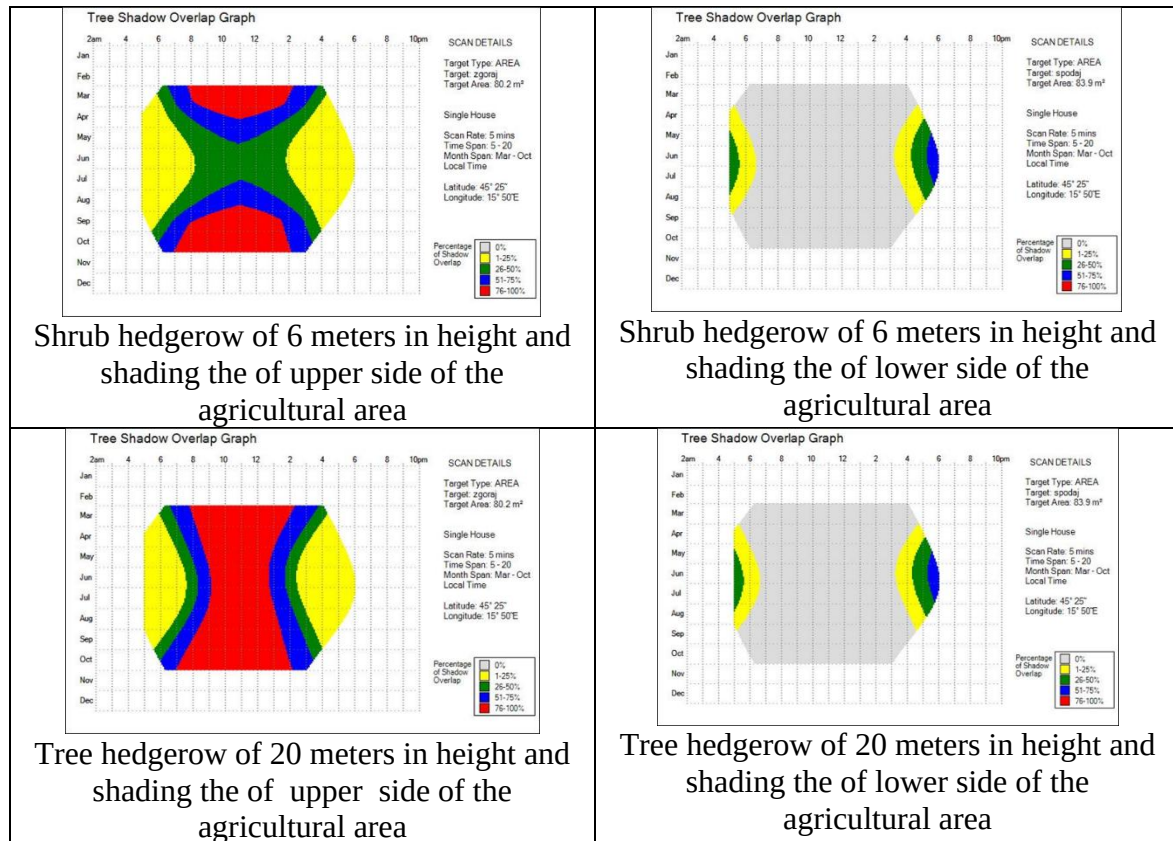
The results of the survey (1,197 farmers) show that the majority believe the presence of hedgerows reduces crop yields due to the shading of agricultural land. A total of 29.5% (353 farmers) completely agree with this statement, while 53.6% (642 farmers) partially agree, and 16.9% (202 farmers) do not agree. Opinions on the quality of agricultural production are more divided. While 39.9% (526 farmers) do not agree that hedgerows improve crop quality, 39.2% (469 farmers) partially agree, and 16.9% (202 farmers) completely agree with this statement.

Graph 1 illustrates the patterns caused by shrub (6 m) and tree (20 m) hedgerows oriented in the north–south direction. Because of this orientation, shading shifts laterally throughout the day, affecting the left side of the field in the morning and the right side in the afternoon. Shrub hedgerows mostly cause low to moderate shading (1–50%), while tree hedgerows create extensive high-intensity shading (up to 100%) that can significantly reduce light availability for adjacent crops.



Graph 1. Hedgerow shading with north–south orientation

Graph 2 shows the shading patterns by shrub (6 m) and tree (20 m) hedgerows oriented in the east–west direction. In this orientation, the shading is concentrated directly north and south of the hedgerow, meaning that the upper and lower sections of the adjacent agricultural land are affected more persistently throughout the day. Shrub hedgerows cause low to moderate shading (1–50%), while tall tree hedgerows result in more extensive and symmetrical shading, with central areas reaching up to 100% overlap, which may limit crop growth in those zones.



Graph 2. Hedgerow shading with east-west orientation

The quantitative data confirm the visual simulations shown in Graphs 1 and 2. On average, tree hedgerows cause substantially more shading than shrub hedgerows, regardless of orientation. With a north-south orientation, shrub hedgerows result in an average shading of 34.5%, while tree hedgerows increase this to 53.0%. In the east-west orientation, overall shading levels are lower, with shrub hedgerows contributing 17.09% and tree hedgerows 33.3%. These results highlight the dominant influence of hedgerow height on shading extent and also confirm that north-south oriented hedgerows generally cast broader and longer-lasting shadows, especially in the early morning and late afternoon. This has practical implications for field planning and crop selection near taller, vertically oriented hedgerows.

Table 1. Percentage of shading (%) according to hedgerow orientation and type

Hedgerow type	Hedgerow orientation north-south	Hedgerow orientation east-west
Shrub hedgerow	34.5%	17.09%
Tree hedgerow	53.0%	33.3%

Conclusions

The status of hedgerows, their impact on agricultural land, and their conservation largely depend on farmers, who interact with them daily while cultivating their land. Farmers cultivating land adjacent to hedgerows often report yield losses in shaded zones due to reduced light availability and increased competition for resources. These areas are perceived as less productive, especially in intensive farming systems where every square metre of arable land counts. This study confirms that both the type and orientation of hedgerows significantly influence the extent and intensity of shading on adjacent agricultural land. Tall tree hedgerows cause notably more shading than shrub hedgerows, regardless of orientation,

while north–south oriented hedgerows produce more extensive and temporally dynamic shading patterns compared to east–west oriented ones. These findings align with farmer perceptions, as shading is frequently cited as a factor that limits cultivable area and reduces crop productivity. In light of these results, effective field planning and hedgerow management are essential to balance the ecological benefits of hedgerows with their agronomic impacts, particularly in areas where agricultural operations such as land consolidation, drainage improvement, and irrigation development are or will be implemented.

References

- Ballard J.T. (1979). Fluxes of water and energy through the Pine Barrens ecosystems. Pine Barrens: ecosystem and landscape. *Academic press*, New York: 133–146.
- Borec A., Lešnik T. (2024). *Common agricultural policy incentives for hedgerow management: a Slovenian case study*. International Scientific Agriculture Symposium "AGROSYM". 1417–1423.
- Borec A., Lešnik T., Šumenjak T.K., Muršec M. (2023). The Effects of Hedgerows Shading on Soil Temperature and Gravimetric Soil Water Content. *Agricultura Scientia*. 20(2): 1-9.
- Burel F. (1996). Hedgerows and their role in agricultural landscapes. *Critical reviews in plant sciences*. 15(2): 169-190.
- Caborn J.M. (1976). Changements du paysage et protection contre le vent. Les Bocages: histoire, ecologie, economie. University of Rennes, Rennes, France: 109–114.
- Dierschke H. (1974). Saumgesellschaften im Vegetations und Standortsgefalle an Waldrändern. Verlag Erich Goltz, Gottingen, FRG: 246.
- Earnshaw S. (2004). Hedgerows for California agriculture. *Community Alliance for Family Farmers: Davis, CA, USA*.
- Forman R.T., Baudry J. (1984). Hedgerows and hedgerow networks in landscape ecology. *Environmental management*. 8: 495–510.
- Holden J., Grayson R.P., Berdeni D., Bird S., Chapman P.J., Edmondson J.L., ... Leake J. R. (2019). The role of hedgerows in soil functioning within agricultural landscapes. *Agriculture, ecosystems & environment*. 273: 1-12.
- Knauer N., Leskošek M. (1991). *Žive meje in obrobni pasovi vodotokov kot sestavni del agrarnih ekosistemov in njihov kmetijsko-ekološki pomen v kulturni krajini*.
- Lampič B., Kastelic A. (2021). Identification and recording of hedgerows: Testing different methods in a pilot area of the Ljubljana marshes. *Dela*. 56: 29-51.
- Lešnik T. (2024). Vloga mejic v kmetijskem prostoru z vidika zmanjšanja površin, izgube pridelka in spremembe talnih razmer : magistrsko delo (str. 1 spletni vir (1 datoteka pdf (vii, 8–93, 1))) [Magistrsko delo, T. Lešnik]. Digitalna knjižnica Univerze v Mariboru. Available from: <https://dk.um.si/IzpisGradiva.php?lang=slv&id=87200>
- Montgomery I., Caruso T., Reid N. (2020). Hedgerows as ecosystems: service delivery, management, and restoration. *Annual Review of Ecology, Evolution, and Systematics*. 51(1): 81-102.
- Oreszczyn S., Lan, A. (2000). The meaning of hedgerows in the English landscape: Different stakeholder perspectives and the implications for future hedge management. *Journal of Environmental Management*. 60(1): 101-118.
- Pollard E., Hooper M.D., Moore N.W. (1974). *Hedges*. W. Collins Sons, London. 256.
- Wales B.A. (1972). *Vegetation analysis of northern and southern edges in a mature oak-hickory forest*. *Ecol. Monogr*: 451-471.

ORIGINAL SCIENTIFIC PAPER

Analysis of agri-environmental payments for permanent grassland on farms with and without livestock

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The aim of the paper is to analyze whether there are internal reserves of permanent grasslands among existing farms in Slovenia, which are managed by farms without livestock and are included in nature conservation measures. Such farms generally do not even need to adjust their agricultural practices to implement the measures. In contrast, farms with livestock, due to their dependence on their own grasslands for feed production, face significantly greater requirements when participating in the measures. We used a comparative analysis to assess the extent of permanent grassland under nature conservation measures on farms with and without livestock. The results indicate the untapped potential of grasslands on farms without livestock, which could be better used to increase domestic self-sufficiency in feed.

Key words: permanent grassland, agri-environmental measures, agricultural policy

Introduction

Permanent grasslands have an important multi-functional role, as in addition to their production role they also contribute to the conservation of nature, biodiversity and climate stability. Key ecosystem services of permanent grasslands include carbon sequestration, protection of water resources and provision of habitat for numerous plant and animal species (Hammes et al., 2016). In addition, grasslands represent the main source of feed for herbivorous animals, which is of great importance from the point of view of food security. In Slovenia, permanent grasslands and pastures cover almost 60% of all agricultural land in use, which exceeds the European average (Eurostat, 2025). As a result, cattle breeding (for both meat and milk production) is the most important production sector in Slovenia (Zeleno poročilo za leto 2023, 2024). Due to the numerous ecosystem functions of permanent grasslands and their role in providing self-produced forage for herbivorous animals, the management of these areas is one of the important issues of sustainable agriculture - especially in the context of climate change and limited natural resources. Food producers are aware of the nature conservation importance of meadows, which is also reflected in their inclusion in various agri-environmental-climate measures aimed at preserving nature and the environment in meadow areas (Hammes et al., 2016). These measures usually include restrictions such as specific mowing times, reduced or prohibited fertilization, and a ban on grazing. In exchange for respecting these restrictions, farmers receive financial compensation. Despite the compensation, however, involving into such measures can be challenging, as there may be a conflict between biodiversity conservation objectives and the animals' needs for sufficient high-quality feed (Žnidaršič et al., 2019). Late mowing reduces the quality of the forage (Verbič et al., 2003; Verbič in Žnidaršič, 2018), and a smaller number of mowings per year reduces both; the total amount of forage produced and its energy value (Verbič et al., 2003; Verbič in Verbič, 2019). As a result, inclusion in nature conservation measures may mean less and lower quality feed, which increases the cost of purchasing feed and the environmental footprint due to transport, and with greater dependence on purchasing feed, various risks in the livestock farming process increase. Such circumstances can be

particularly problematic for specialized cattle farms, while the decision to include in the measures is easier for farms where livestock farming is not the main source of income. In addition to the limitations associated with implementing nature conservation practices, a significant challenge for some farms is the lack of their own meadows, which further complicates the provision of sufficient quantities of quality domestic feed. This is a so-called new concept that is increasingly becoming part of discussions in agricultural policy - feed import dependence or the need for greater self-sufficiency in domestic feed for animals. The aim of this paper is to investigate whether there are internal reserves in the form of permanent grasslands among existing farms in Slovenia, which are owned by farms without livestock and are included in nature conservation measures. These farms do not require major adjustments to agricultural practices or none at all to implement the measures, so their inclusion in the measures is very simple. On the other hand, farms with livestock are largely dependent on their own grasslands and their production capacity, so inclusion in the measures requires major adjustments to agricultural practices. Through a comparative analysis, we wanted to assess the scope of eligible areas for payment of nature conservation measures on grassland. We compared farms with livestock and without livestock and checked to what extent the potential of farms with permanent grasslands and no livestock could be used to increase domestic feed self-sufficiency.

Material and methods

For the purposes of the research, we asked the Ministry of Agriculture of Slovenia, which prepared and provided us with the necessary data on the implementation of measures on grassland. The analysis is based on data from application forms of the farms for agri-environment-climate measures (AECM) in two programming periods of the Common Agricultural Policy. For the programming period 2014–2022, we used data for grassland measures in 2020: Permanent grassland I, Permanent grassland II, Preservation of special grassland habitats, Preservation of grassland habitats of butterflies, Preservation of humid extensive meadows for bird conservation, Preservation of litter meadows, Steep slopes mowing and Humpy meadows mowing. For the current programming period (CAP Strategic Plan 2023–2027), we collected data for grassland measures for 2023: Preservation of special grassland habitats, Preservation of grassland habitats of butterflies, Preservation of litter meadows, Preservation of humid meadows, Maintenance of extensive Karst pastures, Steep slopes mowing, Humpy meadows mowing, Preservation of humid extensive meadows for bird conservation, and additionally data for a grassland measure Natura 2000 payments. Data for individual farms included the extent of utilised agricultural area (UAA) and permanent grassland, as well as data of livestock units (LU) and the amounts of payments for individual grassland measures. The analysis of the inclusion of farms in grassland measures additionally included an overview of the inclusion of farms without livestock, taking into account agricultural holdings with 0 to 1 LU per farm, as 1 livestock represents a negligible number of animals and is more likely a hobby or livestock is present for reasons other than a source of income.

Results and discussion

Of the 241.000 ha of permanent grassland in Slovenia (Zeleno poročilo za leto 2023, 2024), approximately 24.400 ha were included in grassland measures in 2020, cultivated by 1.759 farms (Table 1). The average size of farm was 17.4 ha of UAA, of which 14 ha of permanent grassland, on which an average of 11.4 LU were grazed. Farms received an average of 93 EUR per ha of permanent grassland for inclusion in grassland measures. The greatest interest was in inclusion in the measure Preservation of special grassland habitats, where more than half

(13.650 ha) of the total grassland areas in the measures were included. Just under a quarter of the areas were included in the measure Permanent grassland II.

Of the 1.759 farms, 332 did not raise livestock. They cultivated 1.979 ha and received an average of 197 EUR per ha of permanent grassland for this. Farms without livestock were smaller, on average just under 11 ha. Of the 3.402 ha under grassland measures, the largest area (2.109 ha) is included in the measure Preservation of special grassland habitats, followed by areas (also in combination with other measures) in the measure Preservation of humid extensive meadows for bird conservation with 667 ha.

Table 1. Involvement of farms in agri-environmental measures on permanent grassland in year 2020

	2020	
	Farms	Farms without livestock
Number of farms	1.759	332
Permanent grassland area (net; ha)	24.561	1.979
Permanent grassland under nature conservation measures (%)		8,1
Average UAA per farm (ha)	17,4	10,5
Average permanent grassland area per farm (ha)	14,0	7,4
Average payment per ha of permanent grassland (EUR/ha)	93,1	196,6
Average LU	11,4	0,1
Agri-environmental measures on permanent grassland (gross; ha), of which*:	24.397	3.402
Humpty meadows mowing	2	0
Preservation of humid extensive meadows for bird conservation	1.680	667
Steep slopes mowing	325	35
Preservation of special grassland habitats	13.650	2.109
Preservation of litter meadows	11	7
Permanent grassland I	1.921	4
Permanent grassland II	5.637	67
Preservation of grassland habitats of butterflies	1.171	514

* Due to the possibility of simultaneously implementing two or more measures on the same grassland, the areas when added together may be larger than the net areas.

With the new programming period (Strategic plan CAP 2023–2027), the number of farms that were included in at least one of the grassland measures in 2023 increased to around 2.400 in 2023, cultivating a total of 34.000 ha of permanent grassland (Table 2). The average area of UAA per farm also increased by 1.5 ha, while the average area of permanent grassland per farm was at a similar level to 2020 (14.2 ha). Although the number of farms and the area of total grassland areas increased by a two-thirds, the area included in at least one grassland measure was half as large as in 2020, namely 12.900 ha. Similar to 2020, in 2023, by far the largest area was included in the Preservation of special grassland habitats measure, followed by inclusion in the Preservation of humid extensive meadows for bird conservation and Maintenance of extensive Karst pastures measures. There was less interest in the Preservation of humid meadows, Natura 2000 payments and Preservation of grassland habitats of butterflies measures, as less than 1.300 ha were included in total.

Table 2. Involvement of farms in agri-environmental measures on permanent grassland in year 2023

	2023	
	Farms	Farms without livestock
Number of farms	2.393	728
Permanent grassland area (net; ha)	33.971	3.958
Permanent grassland under nature conservation measures (%)		11,7
Average UAA per farm (ha)	18,9	8,6
Average permanent grassland area per farm (ha)	14,2	5,4
Average payment per ha of permanent grassland (EUR/ha)	155,6	228,1
Average LU	12,3	0,1
Agri-environmental measures on permanent grassland (gross; ha), of which*:	14.075	2.679
Humpy meadows mowing	38,3	1,4
Preservation of humid extensive meadows for bird conservation	1.506	456
Steep slopes mowing	361	51
Maintanance of extensive Karst pastures	1.208	0
Preservation of special grassland habitats	10.069	1.903
Preservation of litter meadows	5	3
Preservation of humid meadows	100	17
Natura 2000 payments	244	94
Preservation of grassland habitats of butterflies	544	154

* Due to the possibility of simultaneously implementing two or more measures on the same grassland, the areas when added together may be larger than the net areas.

Significant changes are evident in 2023 on the farms without livestock. The number of those farms more than doubled, and the total area of grassland also doubled. This means that the average area of permanent grassland per farm decreased by almost 2 ha. The fact that small farms have become more intensively involved in grassland measures is also shown by the average area of UAA. This decreased from 10.5 ha of UAA in 2020 to an average of 8.6 ha of UAA in 2023. In 2023, farms without livestock included around 2.700 ha of grassland area in grassland measures, which is 720 ha less than in 2020. Even at farms without livestock, in the structure of all grassland measures, the greatest interest is in the measures Preservation of special grassland habitats and Preservation of humid extensive meadows for bird conservation, while the share of inclusion in the remaining measures is around 12% of the areas included in agri-environmental measures.

Conclusions

The aim of the article was to highlight the importance of permanent grasslands, which cover 34% of agricultural land in the EU (Eurostat, 2025), as a key source of many ecosystem services. Grass also remains the most affordable source of quality feed for efficient meat and milk production in ruminants (Van Den Pol et al., 2018). Based on these points, we raised the issue of equality between farmers with and without livestock in receiving public support.

We find that nature conservation measures require only minimal or almost no adaptations from managers of grasslands without livestock, while farmers with livestock often face significantly

higher demands. Although farms without livestock also contribute to achieving environmental goals, the question of their role in ensuring food security remains open. In contrast, livestock farmers, who directly contribute to the food supply, often suffer a reduction in the quantity and quality of feed, with the same payments, which means higher costs and the need for additional feed sources. In this context, we highlight an additional problem - feed imports and the fact that animals are often fed with feed produced on arable land that could otherwise be used to produce food for people (Di Paola et al., 2017). If livestock farmers had access to a larger amount of grassland, the need to import feed and feed from arable land could be reduced. The reserve potential for such expansion may lie precisely with managers of permanent grassland without livestock. The results presented in the article reveals internal reserves in existing grassland areas: in 2023, 12% of all permanent grassland under nature conservation measures was managed by farms without livestock. Based on these findings, we propose that agricultural policy consider separate payments for nature conservation measures on grassland; separately for farms with livestock and those without livestock. Among the more ambitious proposals, we highlight the possibility of redirecting the management of permanent grassland to those farmers who raise herbivorous animals and are inextricably linked to the production of fodder on grassland.

References

- Di Paola, Rulli M.C., Santini M. (2017). Human food vs. animal feed debate. A thorough analysis of environmental footprints. *Land Use Policy*, 67.
- Eurostat. (2025). Agricultural statistics. <https://ec.europa.eu/eurostat/data/database>
- Hamess V., Eggers M., Isseslstein J., Kayser M. (2016). The attitude of grassland farmers towards nature conservation and agri-environmental measures – A survey-based analyses. *Land Use Policy*. Vo. 59. 528-535 <https://www.sciencedirect.com/science/article/pii/S0264837715302179>
- Van Den Pol A., Becker T., Botana Fernandez A., Hennessy T., Peratoner G. (2018). Social and economic impacts of grass based ruminant production. 27th European Grassland Federation General Meeting Cork, European Grassland Federation EGF, Zürich.
- Verbič J., Verbič J., Babnik D., Čop J. (2003). Vpliv časa košnje na pridelek in hranilno vrednost travnikov Ljubljanskega barja, 12. Posvetovanja o prehrani domačih živali »Zdravčevo-Erjavčevi dnevi«. 84-100.
- Zeleno poročilo za leto 2023. (2024). Travnikar T., Bedrač M., Bele S., Bleiweis A., Brečko J., Kožar M., Moljk B., Telič V., Zagorc B.. Poročilo o stanju kmetijstva, živilstva, gozdarstva in ribištva v letu 2023. Ljubljana. Slovenija. Kmetijski Inštitut Slovenije. 294.
- Žnidaršič T., Verbič J., Dakskobler I., Vreš B., Šilc U. (2019). Vpliv načina gospodarjenja na vrstno bogatih travnikih na pridelke, krmno vrednost, količino hranil v tleh in botanično pestrost. *Proceedings of the 28th International Scientific Symposium on Nutrition of Farm Animals. Zdravec-Erjavac Days 2019, Murska Sobota. Slovenija. Kmetijsko-gozdarski zavod Murska Sobota.* 249-255.

ORIGINAL SCIENTIFIC PAPER

Status of microelements and macroelements in apple leaves during hydroponic cultivation after exclusion of some nutrients

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Summary

The aim of this research was to examine the impact of the exclusion of certain elements in the hydroponic solution on the uptake and concentration of biogenic elements in apple (*Malus × domestica*) leaves cv. 'Braeburn'. Plants were grown using Hoagland's solution with 4 fertigation treatments: complete solution (control), nitrogen excluded, iron excluded, and magnesium excluded. Leaf samples were taken in triplicate. Results showed that apple leaf N content ranged 1.13–2.63% DM, magnesium 0.25–0.50% DM, and iron 56.6–157.6 mg/kg DM. The exclusion of nitrogen and iron affected the leaf content of almost all elements, while the exclusion of magnesium affected phosphorus and copper content in apple leaf.

Key words: biogenic elements, cv. Braeburn, fertigation, hydroponics, deficiency symptoms

Introduction

The apple (*Malus × domestica*) represents one of the most economically significant fruit crops globally and serves as a benchmark for evaluating the profitability of other fruit tree species (Krpina et al., 2004). In Croatia, apple production reached 47,792 tons in 2022, marking it as the most abundantly produced fruit in that year (DZS, 2024). Despite a slight decline in production volumes in recent years, apples remain the most extensively cultivated fruit species in the country. Consequently, maintaining the optimal nutritional status of apple trees is essential to prevent yield reduction and ensure sustainable production. Understanding the impact of specific nutrient deficiencies on the uptake and dynamics of other essential elements is crucial for improving fertilization strategies. In recent years, reduced nitrogen application has gained attention as a sustainable agrotechnical practice due to its potential positive effects on crop quality and environmental sustainability (Wen et al., 2024; Xing et al., 2024; Wang et al., 2022). However, this practice should not be misinterpreted as a justification for complete omission of nitrogen or other essential nutrients under the guise of environmental stewardship. Instead, fertilization practices should aim to achieve an optimal balance which can achieve the dual goals of enhancing soil nitrogen supply capacity and minimizing environmental risks while improving orchard productivity (Chen et al., 2024). The assessment of the mineral composition of apple leaves plays a critical role in evidence-based orchard management and is an indispensable tool in modern fruit production systems. Leaf tissue analysis provides a reliable means of evaluating the nutritional status of plants. Furthermore, an understanding of the interactions among essential nutrients—particularly how the presence or absence of one element influences the uptake and distribution of others is vital for formulating precise and effective fertilization regimes. Table 1 presents the concentrations of macroelements in apple leaves as reported in various literature sources. Gjamovski et al. (2017) examined the cultivar 'Granny Smith' grafted onto different rootstocks and identified rootstock M9 as the most

efficient in terms of mineral uptake from the soil. Leaf samples from trees grafted onto M9 exhibited the highest concentrations of iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). Moreover, this rootstock demonstrated superior capacity for mineral translocation within the plant system.

Abdalla et al. (1982) investigated the impact of rootstocks with varying levels of vegetative vigor on the leaf mineral composition of the cultivar 'Golden Delicious' in a ten-year-old orchard. Similarly, Ashby et al. (1968) assessed the effect of the growth inhibitor "Alar" on the macroelement content in the leaves of the 'Jonathan' apple cultivar. Table 2 provides data on microelement concentrations in apple leaves derived from several studies. In a study on the mineral composition of apple leaves in response to fungicide application, Young (1982) reported the concentrations of microelements in both washed and unwashed leaf samples. While washing had no significant impact on macroelement concentrations, elevated levels of microelements were detected in unwashed samples, suggesting potential surface contamination. The values presented in Table 2 refer specifically to washed leaf samples to ensure consistency and accuracy in nutrient quantification. Kucukyumuk et al. (2011) reported microelement concentrations in leaves of apple trees grafted onto the M9 rootstock, as part of a study evaluating the influence of rootstock selection on mineral nutrition. Additionally, Abdalla et al. (1982) provided data on leaf mineral composition from a long-term experiment with 'Golden Delicious' trees grafted onto rootstocks differing in growth vigor.

Table 1. Macroelements content in apple leaves according to different authors

Source	% in dry weight				
	N	P	K	Ca	Mg
Gjamovski et al., 2017	2.40	0.11	0.65	1.11	0.25
Abdalla et al., 1982	-	-	1.57	1.22	0.25
Ashby et al., 1968	2.06	0.18	1.38	2.00	-
Kucukyumuk et al., 2011	2.0	0.13	1.18	0.81	0.26

Table 2. Microelements content in apple leaves according to different authors

Source	mg/kg in dry weight			
	Fe	Mn	Cu	Zn
Milošević et al., 2008	157	35.1	4.37	27.8
Young, 1982	69	157	7.00	101
Kucukyumuk et al., (2011	116	46.0	9.70	25.8
Abdalla et al., 1982	-	80	-	-

The aim of this research is to examine the impact of the exclusion of certain elements in the hydroponic solution on the uptake and concentration of biogenic elements in apple (*Malus × domestica*) leaves cv. 'Braeburn'.

Material and methods

An experimental hydroponic apple orchard (*Malus × domestica*) was established in spring 2022 at the experimental site of the University of Zagreb Faculty of Agriculture. The cultivar 'Braeburn', grafted onto M9 dwarfing rootstock, was used. Two-year-old seedlings were planted in 30 L pots filled with inert perlite substrate. The study involved growing of apples using complete Hoagland's solution with 4 fertigation treatments: complete solution (control), nitrogen (N) excluded, Fe excluded, magnesium (Mg) excluded. Each treatment included 40 plants, totalling 160 plants. Each pot received 2 L of nutrient solution daily. Leaf samples were collected on September 13, 2022, with four replicates per treatment. Samples were dried at 105 °C, ground, homogenized, and digested using concentrated HNO₃ and HClO₄ in a

microwave system. Phosphorus (P) was determined by spectrophotometry; potassium (K) by flame photometry; calcium (Ca), Mg, Fe, Zn, Mn, and Cu by atomic absorption spectrometry (AOAC, 2023). Total N was determined using the modified Kjeldahl method (HRN ISO 11261:2004). Statistical analysis was conducted using one-way ANOVA in SAS software (version 9.1, SAS Institute Inc.), and differences between means were assessed using Tukey's HSD post-hoc test .

Results and discussion

Table 3 presents the concentrations of selected macroelements in apple leaves under different fertigation treatments. Nitrogen leaf concentrations were elevated in the treatments where Fe and Mg were excluded, compared to the control treatment represented by complete Hoagland's solution. The highest N concentration (2.63% N) was recorded in the Fe-excluded treatment and was statistically significant compared to all other treatments. Nitrogen concentrations in the Fe and Mg-excluded treatments were statistically similar. In contrast, the N-excluded treatment exhibited the lowest nitrogen concentration across all treatments, a difference that was statistically significant. The highest P concentration (0.45% P) was observed in the N-excluded treatment and was statistically higher than in all other treatments. The phosphorus concentration in the Mg-excluded treatment and control treatment (0.19% P) represented the lowest statistically significant value recorded. The Fe-excluded treatment had a P concentration slightly higher, indicating a moderate but statistically significant increase. Potassium concentrations were highest in the Fe-excluded treatment (2.45% K) and were significantly greater than those in other treatments. The lowest K concentrations were observed in the N and Mg-excluded treatments. Calcium concentrations reached their significantly highest value in the N-excluded treatment (1.50% Ca), compared to all other treatments. The Mg-excluded treatment exhibited Ca concentrations with no statistically significant difference to the control treatment. The lowest Ca concentration was recorded in the Fe-excluded treatment and was statistically distinct from all others. Magnesium concentrations were also highest in the N-excluded treatment (0.50% Mg). The treatments with excluded Fe and Mg did not show a statistically significant difference in Mg content. The lowest Mg concentration was found in the Fe-excluded treatment and was statistically significant compared to the other treatments. In table 4, the concentrations of selected microelements in apple leaves under different fertigation treatments are presented. The highest statistically significant Fe concentration was recorded in the N-excluded treatment, amounting to 158 mg Fe/kg. The lowest Fe concentration was observed in the Fe-excluded treatment. There were no significant differences between control and Mg-excluded treatments. The highest Zn concentration, 66.8 mg Zn/kg, was observed in the Fe-excluded sample and was significantly higher compared to all other treatments. The control treatment and the N-excluded treatment showed the lowest Zn concentration, ranging from 42.5-46.3 mg Zn/kg. The maximum Mn concentration, 66.6 mg Mn/kg, was observed in the Fe-excluded treatment and was statistically higher than in all other treatments. The magnesium-excluded and control treatments were statistically similar regarding Mn concentration. The lowest Mn concentration, 42.8 mg Mn/kg, was recorded in the N-excluded treatment. The highest Cu concentrations, were found in the Mg-excluded treatment as well as in control one and they were statistically higher than in the N and Fe-excluded treatments.

Table 3. Macroelements content in apple leaves in different fertigation treatments

Fertigation treatments	% in dry weight				
Hoagland's solution	N	P	K	Ca	Mg
Complete solution	2.34 b	0.19 c	1.64 b	1.19 b	0.32 b
Excluded N	1.13 c	0.45 a	1.32 c	1.50 a	0.50 a
Excluded Fe	2.63 a	0.25 b	2.45 a	0.87 c	0.25 c
Excluded Mg	2.62 a	0.19 c	1.39 c	1.27 b	0.27 c

Different letters in the same column represent significantly different values according to Tukey's test, $p \leq 0.05$.

Table 4. Microelements content in apple leaves in different fertigation treatments

Fertigation treatments	mg/kg in dry weight			
Hoagland's solution	Fe	Zn	Mn	Cu
Complete solution	125 b	46.3 c	54.0 b	3.41 a
Excluded N	158 a	42.5 c	42.8 c	2.29 b
Excluded Fe	57.0 c	66.8 a	66.6 a	2.44 b
Excluded Mg	115 b	51.0 b	52.6 b	3.45 a

Different letters in the same column represent significantly different values according to Tukey's test, $p \leq 0.05$.

As expected, the lowest N content was recorded in the N-excluded treatment. However, the measurable amount of N still present in the plant tissue despite N exclusion can be attributed to the mobilization of N reserves from the woody tissues of the plant or the foliar absorption of dissolved nitrogen oxides from rainfall. Among the other macronutrients, only K also exhibited its lowest concentration in the N-excluded sample. Among the micronutrients Mn, and Cu showed their lowest values in the same treatment when are compared with the control. Conversely, the highest concentrations of P, Ca and Mg were observed in the N-excluded sample. With respect to micronutrients, Fe reached its maximum concentration in this treatment. In the Fe-excluded sample, the highest values were recorded for N and K, as well as for Zn and Mn among the micronutrients. As anticipated, Fe content was lowest in the Fe-excluded treatment. Additionally, Ca and Mg recorded their lowest concentrations in this same treatment. When compared to the average biogenic element concentrations in apple leaves reported by other authors, the mean values of macronutrients in this study were significantly higher, whereas the micronutrient concentrations were considerably lower. These discrepancies may be explained by differences in cultivar and rootstock selection. Rootstock type can influence nutrient translocation efficiency; in this study, the use of the low-vigor M9 rootstock, known for its poor, shallow, and fragile root production, as well as reduced translocation capacity, may have acted as a limiting factor in nutrient distribution within the plant (Akbari et. al, 2015). Furthermore, exposure to foliar-applied plant protection agrochemicals during the experiment may also affect the mineral composition of leaves, particularly the concentration of micronutrients, as these substances can alter nutrient dynamics when applied directly to the foliage. These findings underscore the pivotal N role in regulating elemental interactions within the plant system (Petek et al., 2017). The nitrogen accumulation and assimilation into plant compounds may be either inhibited or facilitated by the presence of other nutrients. Given the inherently complex nature of nutrient interactions (Mengel and Kirkby, 1987), deficiencies or excesses of individual elements can initiate a cascade of physiological responses. Thus, changes in nutrient concentrations may not always result directly from antagonistic effects (Bergmann, 1992), but rather from indirect influences exerted by other nutrients that modify the bioavailability, uptake, or physiological function of the antagonistic element (Marschner, 2012).

Conclusions

The concentrations of all examined elements exhibited their minimum and maximum values in treatments where N, Mg or Fe were excluded. Notably, in the control treatment, none of the examined elements reached their highest concentrations. In the N-excluded treatment, the lowest concentrations were observed for K, Mn, Cu and, as anticipated, N itself. Conversely, in the same treatment the highest concentrations of P, Mg, Ca and Fe were determined. Furthermore, the highest N, K, Zn, and Fe concentrations were noted in the Fe-excluded treatment. Statistical analysis confirmed that the most extreme element concentration values, both highest and lowest, across the entire experiment were found in the N-excluded treatments. When compared to the control treatment, almost all elements examined displayed either its maximum or minimum concentration in the absence of N, indicating that N availability has a significant effect on the uptake and accumulation of other elements in plant tissues.

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References

- Abdall O., Khatamian H., Miles N. (1982). Effect of Rootstocks and Interstems on Composition of 'Delicious' Apple Leaves. *J. Amer. Soc. Hort. Sci.* 107. 730-733. 10.21273/JASHS.107.5.730.
- Akbari M., Maejima T., Otagaki S., Shiratake K., Matsumoto S. (2015). Efficient Rooting System for Apple (M.9) Rootstock Using Rice Seed Coat and Smocked Rice Seed Coat. *International Journal of Agronomy*, 107906, <http://dx.doi.org/10.1155/2015/107906>
- AOAC (2023). *Official Method of Analysis of AOAC International*, Gaithersburg, Maryland, USA.
- Ashby D.L., Looney N.E. (1968). Mineral content of fruit and leaves of spartan apple trees treated with succinic acid 2,2-dimethyl hydrazide. *Canadian Journal of Plant Science*. 48(4): 422-423. <https://doi.org/10.4141/cjps68-081>
- Bergmann W. (1992). *Nutritional disorders of plants*. Gustav Fischer Verlag Jena, Stuttgart, New York.
- Chen S., Zhang S., Li H., Hu T., Sun G., Cui X., Liu J. (2024) Optimizing irrigation and nitrogen management improves soil soluble nitrogen pools and reduces nitrate residues in a drip-fertigated apple orchard on the Loess Plateau. *Agricultural Water Management*, 295:108770
- DZS, Državni zavod za statistiku https://podaci.dzs.hr/media/v0n1cfyb/si-1712_poljoprivredna-proizvodnja-u-2022.pdf, (Pristupljeno 20.08.2024.)
- Gjamovski V., Cvetkovic J., Stafilov T., Bačeva Andonovska K. (2017). Influence of rootstocks on mineral composition of apple cultivar granny smith. *Bulgarian Journal of Agricultural Science*. 23:4.
- HRN ISO 11261: (2004). (Kakvoća tla - Određivanje ukupnog dušika - Prilagođena Kjeldahlova metoda (ISO 11261:1995)).
- Krpina I. (2004.). *Voćarstvo*. Nakladni zavod Globus. Zagreb.
- Kucukyumuk Z., Erdal I. (2011). Rootstock and cultivar effect on mineral nutrition, seasonal nutrient variation and correlations among leaf, flower and fruit nutrient concentrations in apple trees. *Bulgarian Journal of Agricultural Science*. 17. 633-641.
- Marschner P. (2012). *Marschner's Mineral Nutrition of Higher Plants*. Academic press, Amsterdam.
- Mengel K., Kirkby E. A. (1987). *Principles of Plant Nutrition*. International Potash Institute, Bern.
- Milošević T., Glišić I., Milošević N., Glišić I. (2008). Microelement composition of apple leaves as affected by agrozel presence in the soil. *Cereal Research Communications*. 36. 2087-2090.
- Petek M., Toth N., Pecina M., Karažija T., Lazarević B., Herak Čustić M. (2017) Mineral ratios in beetroot under different fertilization. 3rd International symposium for agriculture and food – ISAF 2017, Proceedings, 76-85
- Wang F., Ge S., Lyu M., Liu J., Li M., Jiang Y., Xu X., Xing Y., Cao H., Zhu Z., Jiang Y. (2022). DMPP reduces nitrogen fertilizer application rate, improves fruit quality, and reduces environmental cost of intensive apple production in China. *Sci Total Environ*. 802:149813, doi: 10.1016/j.scitotenv.2021.149813.

- Wen S., Cui N., Li M., Gong D., Xing L., Wu Z., Fan J., Zhang Y., Wang Z. (2024). Optimizing irrigation and nitrogen fertilizer management to improve apple yield, quality, water productivity and nitrogen use efficiency: A global meta-analysis. *Scientia horticulturae*, 332, id. id.113221, DOI 10.1016/j.scienta.2024.113221
- Xing Y., Feng Z.-Q., Zhang X., Cao H.-X., Liu C.-L., Qin H.-H., Jiang H., Zhu Z.-L., Ge S.-F., Jiang Y.-M. (2024). Nitrogen reduces calcium availability by promoting oxalate biosynthesis in apple leaves, *Horticulture Research*, 11:uhae208, <https://doi.org/10.1093/hr/uhae208> Article
- Young E. (1982). Spray materials and washing solution affect apple foliar analyses. *HortScience*, 17(1), 61-63.

ORIGINAL SCIENTIFIC PAPER

Comparison of digestate quality from three biogas plants near KriževciSonja Rajić-Bistrović ter Veer ¹, Silvija Zeman ²¹ *Faculty of Agrobiotechnical Sciences Križevci, M. Demerca 1, Križevci, Croatia (sbistrovic@vguk.hr)*² *Međimurje Polytechnic in Čakovec, J. Jelačić 22 A, Čakovec, Croatia***Summary**

In our era of sustainable agriculture, one of the global priorities is to replace fossil fuels with renewable energy sources, properly manage organic waste and reduce greenhouse gas emissions. Proper use of digestate would complete the entire cycle of energy production (electricity and/or heat) from organic waste from farms and energy crops. In this context, AD biogas plants (anaerobic digestion) offer an ideal solution and a major step towards the goals of the European Green Deal (EGD) to achieve climate neutrality by 2050. According to EBA (2023) forecasts of the European Green Deal by 2050., 93% of conventional mineral and organic nitrogen-based fertilizers could be replaced by 'RENU products', whose list contains digestate as a substance with great potential, since it contains nitrogen partially obtained from livestock manure, and after processing it can be used in areas with nitrogen pollution of waters, as defined in the EU legislation 'Nitrates Directive (91/676/EEC)'. Although digestate is rich in valuable nutrients, especially nitrogen (N), phosphorus (P) and potassium (K), the main problem is the wide range of chemical composition that can be expected from digestate. The aim and purpose of this work is to compare the components of solid and liquid digestates from three biogas plant locations with literature data. This study shows that digestates from all three locations (Gradec, Gregurovec and Sveti Petar Orehovec) are of its chemical composition, mainly in terms of the content of the main macroelements N, P, and K, a high-value substance that can be used as a quality fertilizer or soil improver.

Keywords: digestate, macroelements NPK, anaerobic digestion, fertilizer**Introduction**

Today, Europe is the largest producer of biogas, i.e. biomethane, in the world. Globally, over 1000 Mt of biomethane could be produced in 2040. with average costs falling by 15% compared to costs in 2018. Anaerobic digestion is a valuable biochemical process for the production of biogas, i.e. green energy - RES from organic waste of plant (biomass) but also of animal origin (wastewater and livestock manure) (Koszela and Lorencowicz, 2015).

In addition to biomethane, the AD process produces a nutritionally valuable by-product called digestate. The total annual amount of digestate in Germany in 2012. was around 65.5 million m³. This amount contains: 390.15Mg nitrogen (N); 74.075Mg phosphorus (P) and 331.47Mg potassium (K) (Möller and Müller, 2012). Also according to Arthurson (Arthurson, 2009) the entire amount of digestate produced in Sweden since 2006. was used in agriculture. Data from 2021 according to Doyeni et al. (Doyeni et al. 2021) show that the annual amount of anaerobic digestate produced in the entire EU is 180 million tons. This huge potential in the field of fertilizer or soil conditioner production has only been used by about 20% (Arthurson, 2009). The key element in the digestate is nitrogen (N), which is found in both inorganic and organic form. Already in the AD digester, and later in the soil, nitrogen changes into the mineralized form NH₄⁺. The ammonium ion makes organic complex compounds . Part of the ammonium ions are used for the growth of beneficial anaerobic bacteria in the digester. There are studies

on the addition of nematodes which have the ability to reduce the percentage of mineralized forms of nitrogen with the effect to increase the carbon to nitrogen ratio C/N in the digestate, or to increase nitrogen mineralization with a decrease in the C/N ratio (Ferris et al., 1998). These ratios are regulated depending on the type of AD plant, the type of thermophilic or mesophilic processes, the C/N ratio, but also the composition of the input raw material. Therefore, the composition of the digestate is variable, for example, adding straw would increase the total carbon (C) content, but also the bio-immobilization of nitrogen in the digestate would increase. At the same time, the level of nitrate content would decrease (Barlóg et al., 1997). Thermal treatment of raw manure and manure in the AD thermophilic digester can be beneficial due to the destruction of pathogens and weed seeds present, as this reduces germination. The advantages of digestate are the increased content of as much as 25% of mineralized, easily accessible forms of nitrogen in relation to the raw organic manure used as raw material for the AD process (Arthurson, 2009). It is therefore important to monitor whether $N_{min}/N_{tot} > 90\%$ (mineralized nitrogen content to total nitrogen), which is one of the criteria for processing the digestate into the 'RENURE' chemical Haber–Bosch fertilizer, which should harmonize the ecological properties of the environment. AD digestate contains nitrogen, which is completely or partially derived from livestock excreta (Reuland et al., 2021).

It is also important to know the value of 'nitrogen use efficiency' (NUE), defined as the ratio of total nitrogen (TN) to nitrogen that is usable by the crop. The higher the 'NUE' value, the lower the risk of soil leaching and harmful nitrogen greenhouse gas emissions (Reuland et al., 2021). In highly developed countries, long-term fertilization could lead to oversaturation of the soil with nutrients and, consequently, the release of excess nitrogen in the form of toxic ammonia (NH_3) or nitrogen(I)-oxide (N_2O), which would further burden the environment with greenhouse gas emissions (Barlog et al., 2020). Digestates are richer in NH_4^+ nitrogen compounds compared to mineral and organic fertilizers. Plants can easily use it from soil that has been treated with digestate and therefore this is an important argument for their application (Möller and Müller, 2012). Although nitrogen is a key component of digestate, there is growing interest in the use of digestate as a soil improver with phosphorus. Phosphorus plays a key role in plant growth, increasing yield and its quality. Raising the pH of the fertilizer shifts the chemical equilibrium towards the formation of phosphate ($HPO_4^{2-} \rightarrow PO_4^{3-}$). This reduces the solubility, or leaching of phosphorus from the soil because the fractions of dissolved, mineralized phosphorus, during the AD process, associate with suspended solids (Arthurson, 2009). In non-calcareous soils, inorganic phosphorus in soil solution (P_i) has a strong tendency to bind to metal oxides and hydroxides, mainly iron (Fe) and aluminum (Al) (Lee et al., 2020). In soils with a high concentration of calcium (Ca) (or magnesium), minerals of magnesium or calcium-phosphate composition precipitate. (e.g. $Ca_3(PO_4)_2$). According to Barlog (Barlóg et al., 1997) application of digestate increased the content of plant-available phosphorus in the soil. For potassium (K) to be optimally utilized, its chemical form is important, as is the ratio with phosphorus P/K, which determines whether the soil will be sufficiently supplied with potassium or whether it will need to be additionally supplied with mineral fertilization when digestate is continuously used as fertilizer (Czekala, 2022).

Material and methods

Digestate samples were sampled and prepared according to the standard (ISO 11464:2004) for analysis in the authorized Agrochemical Laboratory of the Križevci University of Applied Sciences (VUK). This means that samples of fresh solid and liquid digestate were brought in plastic sample bags, and then thoroughly dried in a thermostat and desiccator, crushed and sieved. The following chemical analyzes were determined in the prepared average samples: pH values (HRN ISO 10390:2005), determination of organic carbon by sufchromic oxidation of humus (ISO 14235 1998), analysis of total N% by the modified Kjeldahl method (ISO 11261;

1995), determination of plant-available phosphorus P_2O_5 and potassium K_2O (preparation of samples by Egner's the Al method), after which readings were made of the amount of phosphorus (P) on a spectrophotometer (Shimadzu UV-1280 UV-VIS spectrophotometer) and of potassium (K) on a flame photometer (Sherwood M410 Flame photometer).

Results and discussion

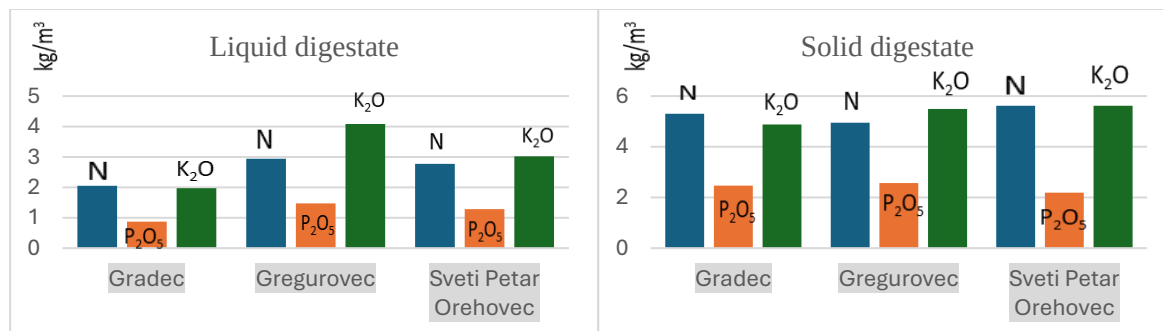
Tabular and graphical presentation of the data are presented through the key parameters of digestate quality as fertilizer: the content of total nitrogen (N), phosphorus (P) and potassium (K) for three different AD biogas plants in Gradec, Gregurovac and Sveti Petar Orehovec. Some other properties of the digestate at the mentioned locations were also analyzed. Thus, it was determined that the pH values ranged from 8.11 to 9.23 for solid digestate and slightly more acidic from 7.45 to 8.11 for liquid digestate. The content of dry matter (DM) varied from 18.32% to 28.24% in solid digestate, and in liquid digestate from 2.78% to 8.44% DM. C_{tot} values of total carbon for solid digestate (SD) were from 44.17 to 51.20%, and for liquid digestate (LD) of all samples from 32.43 to 37.22%.

Table 1. Total mineral content in fresh digestate samples from three locations

Location of Biogas plant	Solid digestate			Liquid digestate		
	N kgm ⁻³	P ₂ O ₅ kgm ⁻³	K ₂ O kgm ⁻³	N kgm ⁻³	P ₂ O ₅ kgm ⁻³	K ₂ O kgm ⁻³
Gradec						
I	4.97	2.50	4.50	2.40	1.10	1.80
II	5.83	2.52	5.39	1.51	0.61	1.67
III	5.11	2.39	4.76	2.23	0.90	2.44
Average	5.30	2.47	4.88	2.05	0.87	1.97
Gregurovec						
I	4.40	2.39	4.71	2.02	1.30	3.80
II	5.02	2.46	5.64	2.62	1.28	3.22
III	5.43	2.85	6.12	4.17	1.82	5.21
Average	4.95	2.57	5.49	2.94	1.47	4.08
Sveti Petar Orehovec						
I	4.47	2.30	4.91	2.58	1.20	2.90
II	5.19	1.92	5.08	2.87	1.48	3.26
III	7.29	2.36	6.86	2.85	1.15	2.91
Average	5.62	2.19	5.62	2.77	1.28	3.02

The composition of digestate is highly variable. For example for: dry matter (DM) from 15 to 437g kg⁻¹; for N_{tot} from 1.2 to 15 g kg⁻¹; while the C/N ratio varies from 2.0 to 24.8. All values obtained from samples from all locations of this work range within this data. All T-test results for N, P₂O₅ and K₂O at all locations are not statistically significant because all p-values are less than 0.05 (p < 0.05). The T-test thus suggests that there are no significant differences in nutrient concentrations between different locations. The mean values of the quantitative analysis of the amount of total nitrogen in the solid digestate (SD) ranged from 4.95 to 5.62 kg m⁻³, the mean values of the amount of total phosphorus ranged (SD) from 2.19 to 2.57 kg m⁻³, while the mean values for potassium ranged (SD) from 4.88 to 5.62 kg m⁻³. If we compare the amounts of these three nutrients (N, P and K) according to the selected biogas plants, it can be concluded that the lowest average nitrogen content in fresh solid digestate was in Gregurovec, and the highest in

Orehovec, the lowest phosphorus content in Orehovec, and the highest in Gregurovec, and the lowest potassium content in Gradec, and the highest in Orehovec. According to the mean values of the quantitative analysis of total nitrogen in the liquid digestate, the nitrogen content ranged from 2.05 to 2.94 kg m⁻³, phosphorus from 0.87 to 1.47 kg m⁻³ and potassium from 1.97 to 4.08 kg m⁻³.



Graph 1. Comparison of the amounts of major nutrients (N, P, K) in solid and liquid samples of digestate from three biogas plants Gradec, Gregurovec, Sveti Petar Orehovec

Total nitrogen content affects crop quality and yield, but also affects the environment, especially in the process of nitrification and transformation in the soil during the growth of agricultural crops (Czekala, 2022). Literature data show that with short-term, e.g. one-year use of digestate, the SOC and TN content in the soil will not increase as quickly as with the application of mineral NPK fertilizer (Barłóg et al., 1997). It is only after several years of applying fertilization with digestate that a very positive effect on the properties of the soil is observed, which is harmonized and enriched with nutrients C, N, P, K and others. Digestate increases the content of organic matter in the soil and increases soil pH. Mineralization of N and P in combination with a significant increase in the pH of digestate as a fertilizer can improve the formation and crystallization of the complex compound survite. With this procedure, the amounts of forms of N and P available to the plant can be regulated in order to reduce the load on oversaturated soils (Arthurson, 2009). During anaerobic digestion, the more labile organic materials and, to a certain extent, non-reactive organic molecules (lignin and non-hydrolyzable lipids) are degraded. These changes in organic materials result in a higher degree of biological stability. Poor management of livestock manure and slurry can significantly increase the amount of methane (CH₄) and ammonia (NH₃) emissions, and to a lesser extent nitrous oxide (N₂O) and (CO₂) released into the environment. This acidifies the soil, i.e. reduces pH, and can also lead to leaching of nutrients, mainly nitrogen (N) and phosphorus (P) from the soil, because the pH of the fertilizer greatly affects the solubility of phosphorus and micronutrients (Reuland et al., 2021) and (Lee et al., 2020). When digestate samples are compared, it can be seen that fresh solid digestate contains more nitrogen, phosphorus and potassium (kg/m³) compared to the content of these nutrients in fresh liquid digestate.

Conclusion

Analyses show that digestate from all three locations can be used as a fertilizer that balances nitrogen or phosphorus overloaded soil, but also enriches soil poor in major nutrients. Even after just one application of AD digestate, this fertilizer harmonizes the soil for a longer period of time. The use of AD digestate improves the dynamics and content of nutrients in the soil and their bioavailability to plants. AD digestate's contribution to maintaining the living microbial mass in the soil is indispensable, thereby increasing the enzymatic activity of the soil. Since AD digestate has a more balanced nutrient profile than conventional fertilizers, their lower input into the soil during fertilization is required. The digestate has a very positive effect on the

formation of Norg - organic forms of nitrogen compounds that enter the soil in the long term and prevent soil erosion. Digestate, a by-product of the thermophilic and mesophilic processes in AD, suppresses crop diseases and reduces the consumption of plant protection products. Consequently, there is a reduction in soil pollution, but also in emissions of greenhouse gases NH_3 , CO_2 , N_2O . Thus AD digestate contributes to better soil quality where it is applied as fertilizer. There is a need for further monitoring of the biochemical processes of the main nutrients introduced into the soil by AD digestate as fertilizer. Since AD digestate is an excellent candidate for the 'RENURE' product list, there is a need for further studies of N and P mineralization at the molecular level as well as monitoring of coefficients such as NUE (nitrogen use efficiency) and NFRV(N-fertiliser replacement values) for a particular group of solid or liquid AD digestate.

References

- Koszela M., Lorencowicz E. (2015). Agricultural use of biogas digestate as a replacement fertilizers. *Agriculture and Agricultural Science Procedia* 7 (2015) 119 – 124. Available from: <http://www.sciencedirect.com/>
- Ferris H., Venette R.C. van der Meulen H.R., Lau S.S. (1998). Nitrogen mineralization by bacterial feeding nematodes: verification and measurement. *Plant and Soil* 203 (1998) 159–171, Davis, California, USA: 1998 Kluwer Academic Publisher, Netherland
- Barlóg P., Hlisnikovský L., Kunzová E. (1997). Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization . *Agronomy* 10 (2020) 379. Poznan, Poland, Available from: <http://www.mdpi.com/journal/agronomy/>
- Reuland G., Sigurnjak I., Dekker H., Michels E., Meers E. (2021). The Potential of Digestates and the Liquid Fraction of Digestate as Chemical Fertilizer Substitutes under the RENURE Criteria. *Agronomy* 11 (2021) 1374. Brussels, Belgium: Licensee MDPI, Basel, Switzerland. Available from : <http://www.mdpi.com/journal/agronomy/>
- Lee M.E., Steiman M.W., St. Angelo S.K. (2020). Biogas digestate as a renewable fertilizer : effects of digestate application on crops growth and nutrients composition. *Renewable Agriculture and Food Systems* 36, 173-181. Published online by Cambridge University Press. Available from: <https://doi.org/10.1017/S1742170520000186>
- Arthurson V. (2009). Closing the Global Energy and Nutrient Cycles through Application of Biogas Residue to Agricultural Land – Potential Benefits and Drawbacks . *Energies* 2 (2009) 226-242. Uppsala, Sweden. Available from: www.mdpi.com/journal/energies
- Möller K. and Müller T., (2012). Effects of anaerobic digestion on digestate nutrients availability and crop growth: A review. *Eng. Life Sci.* 12 (2012) No. 3, 242–257. Hohenheim, Stuttgart, Germany: 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. Available from: www.els-journal.com
- Doyeni M.O., Stulpinaite U., Baksinskaite A., Suproniene S. and Tilvikiene V. (2021). The Effectiveness of Digestate Use for Fertilization in an Agricultural Cropping System. *Plants* 10 (2021) 1734. Kedainiai, Lithuania. Licensee MDPI, Basel, Switzerland. Available from: <https://doi.org/10.3390/plants10081734>
- Czekala W. (2022). Digest as a source of Nutrients: Nitrogen and its Fractions. *Water* 14 (2022). 4067. Poznań, Poland . 2022 by the author. Licensee MDPI, Basel, Switzerland. Available from: <https://www.mdpi.com/journal/water>

ORIGINAL SCIENTIFIC PAPER

Allelopathic influence of common milkweed (*Asclepias syriaca* L.) water extracts on lettuce

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Summary

Common milkweed (*Asclepias syriaca* L.) is an invasive perennial plant species belonging to the Asclepiadaceae family. The aim of the study was to examine the allelopathic effects of water extracts from *A. syriaca* dry biomass in three concentrations (1%, 2.5%, 5%) on the germination and growth of lettuce (*Lactuca sativa* L.). Results showed that *A. syriaca* extract in the lowest concentration significantly promoted shoot length and fresh weight of lettuce. However, the increase in concentration resulted in negative effect with the highest extract concentration causing inhibition of germination, root and shoot length, and fresh weight of seedlings by 7.4%, 69.5%, 36.4% and 43.7%, respectively. Further investigations in order to quantify the content and composition of allelochemicals present in *A. syriaca* are needed.

Key words: allelopathy, weeds, germination, inhibition, invasive species

Introduction

Herbicides have long been a key tool in efficient weed management, but their ongoing use has led to the occurrence of weed resistant populations, and concerns about their environmental and health impacts. Furthermore, the discontinuation of many active ingredients, combined with the growing shift toward organic farming, has accelerated the adoption of more sustainable and eco-friendly weed control methods in recent years (Hasan et al., 2021). Allelopathy is a biological phenomenon defined as the direct or indirect, positive or negative effect of one plant on the germination and growth of another through chemical substances (allelochemicals) released into the environment (Rice, 1984), and has significant potential for utilization in agriculture. Allelopathically active plants can suppress weed emergence and growth applied in various forms such as extracts, mulches, and residues (Singh et al., 2003). Current research is focused on plants with biologically active compounds, such as aromatic and medicinal plants (Islam et al., 2022), as well as invasive species which are considered to possess particularly strong allelopathic influence (Baličević et al., 2015, Moždzeń et al., 2020, Kato-Noguchi, 2022). Common milkweed (*Asclepias syriaca* L.) is a perennial invasive plant species from the Asclepiadaceae family. Its thick rhizomatic roots usually spread at 10–40 cm below the soil, while the stem is hollow, erect, up to 150 cm high, and covered in fine hairs. The leaves are opposite, oval to lanceolate, 15–25 cm long and 5–9 cm wide, with smooth margins and short stems. The upper surface is dark green and the underside pale green and finely hairy. Up to 100 small flowers, varying from pale pink to crimson red, are gathered in large umbels. The fruits are slightly curved, 8–10 cm long follicle-like capsules, covered in soft spines, and contain numerous flat seeds with tuft of long white hairs. All plant parts contain toxic white, milky sap (Knežević, 2006, Bagi, 2008, Nikolić et al., 2014). *Asclepias syriaca*, native to North America, was introduced to Europe in the 17th century as an ornamental plant and has since been recorded in over 20 European countries, including Croatia, as well as in other parts of the world such as Japan and the Middle East (Nikolić et al., 2014., Stef et al., 2022). It is found as weed in field

crops, vineyards and orchards, on ruderal habitats, along roadsides, forest edges, and on meadows (Knežević, 2006, Stef et al., 2022, Mihai et al., 2024). *Asclepias syriaca* possesses several competitive traits, including the ability to propagate vegetatively, drought resistance, and allelopathic properties (Mihai et al., 2024). Several studies reported allelopathic effects of *A. syriaca* on crops such as maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* (L.) Merr.) (Popov et al., 2021), oilseed rape (*Brassica napus* L.) (Bakacsy et al., 2024), as well as on weed species, e.g. redroot pigweed (*Amaranthus retroflexus* L.) and white goosefoot (*Chenopodium album* L.) (Gajić Umiljendić et al., 2025). The aim of the study was to assess allelopathic effect of water extracts from aboveground biomass of *A. syriaca* on seed germination and initial growth of lettuce (*Lactuca sativa* L.) seedlings.

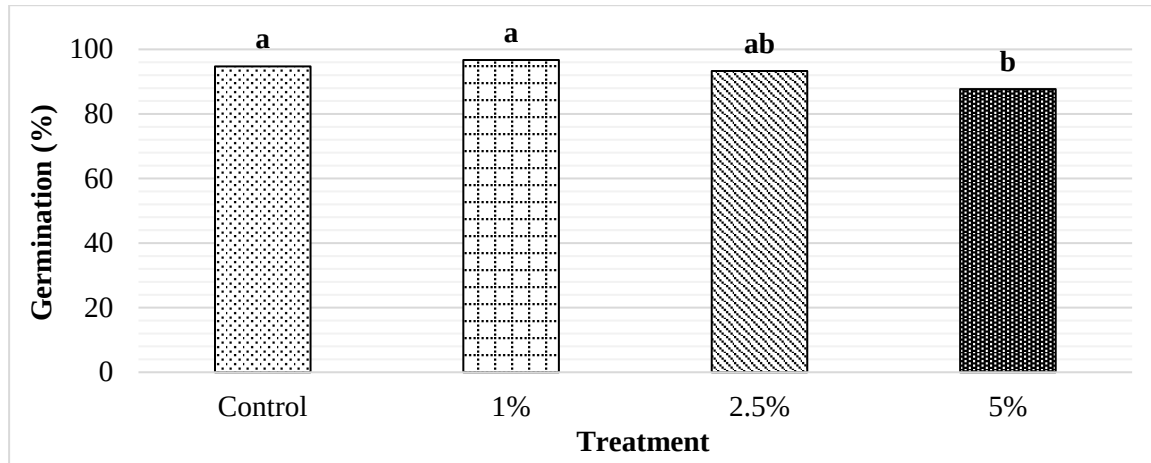
Material and methods

The fresh aboveground biomass of *A. syriaca* was gathered during the ripening stage in a vineyard located in the Vukovar-Srijem County area during the summer of 2023. In the laboratory, the collected plant material was cleaned, then air-dried for 72 hours, followed by additional drying in an oven at 40 °C for 72 hours. Once dried, the material was pulverized into a fine powder with a mill and kept in paper bags in a cool, dark place. Water extracts were prepared according to Norsworthy (2003), by combining 5 g of dried aboveground plant material with 100 ml of distilled water. The mixture was left to extract for 24 hours at room temperature (22 ± 2 °C), after which it was strained through muslin cloth to remove larger particles and then filtered through filter paper. The obtained 5% concentration extract was further diluted in order to give 2.5 % and 1% concentrations. The test species used in the experiment was lettuce (*Lactuca sativa* L.) cv. Atrakcija. Prior to the experiment, the lettuce seeds were surface sterilized with a 1% NaOCl solution for 20 minutes, then thoroughly rinsed with distilled water. The experiment was carried out under controlled laboratory conditions at the Faculty of Agrobiotechnical Sciences Osijek. It followed a completely randomized design with six replications. Each treatment involved 25 lettuce seeds placed on filter paper in Petri dishes. The filter paper was moistened with 3 ml of the extract at the specified concentration, while distilled water was used in the control. On the fifth day of the experiment, an additional 2 ml of extract or water was added to each Petri dish to prevent the seedlings from drying out. The seeds were germinated at a temperature of $22 (\pm 2)$ °C over a period of seven days. The allelopathic effect of *A. syriaca* was evaluated by measuring total germination (%), root and shoot length (cm), and total fresh weight of lettuce seedlings (mg). The collected data were processed in Microsoft Excel to calculate the mean values for each parameter, and statistical analysis was performed using one-way analysis of variance (ANOVA), with differences between treatment means tested using the LSD test at the 0.05 significance level.

Results and discussion

The results of the experiment revealed that *A. syriaca* water extracts showed different effect on lettuce germination (Graph 1). Although the increase in extract concentration reduced seed germination, the statistically inhibitory effect was observed only in 5% concentration treatment which resulted in 7.4% germination decrease. The effect of extracts on seed germination depends largely on the test species, plant part, as well as extract type and concentration. Reductions in maize, soybean and sunflower seed germination was observed by Popov et al. (2021), especially with higher concentrations of water extracts of *A. syriaca* roots, however no inhibition was recorded using methanolic extracts. Szilágyi et al. (2018) determined negative effect of *A. syriaca* on garden cress germination (*Lepidium sativum* L.), with extracts from fresh stems having greater inhibitory potential compared to fresh leaves. Plant parts contain different type and concentrations of allelochemicals which consequently may exhibit various allelopathic effect (Scavo et al., 2021). According to Gajić Umiljendić et al. (2025) total phenolic content

in *A. syriaca* amounted to 43.3 ± 1.5 mg GAE/g of dry leaf extract, while chemical profiling revealed presence of 15 phenolic compounds with delphinidin, 4-hydroxybenzoic acid and rutin as the most abundant. Total phenolic and tannin content in *A. syriaca* root extracts found by Popov et al. (2021) were 2.104 and 0.805 mg GAE/g.



Graph 1. Allelopathic effect of common milkweed (*Asclepias syriaca* L.) water extracts on lettuce seed germination

The application of *A. syriaca* water extracts exhibited allelopathic effect on root length of lettuce seedlings (Table 1). Lower concentrations of water extracts had no significant effect on root length, however root length was severely reduced by 69.5% in treatment with the highest extract concentration. *Asclepias syriaca* extracts showed significant detrimental effect on root length of several tests species with over 90% reductions compared to control (Csiszár et al., 2012, Nádasý et al., 2018, Popov et al., 2021, Gajić Umiljendić et al., 2025). Additionally, Bakacsý et al. (2024) demonstrated inhibitory effect of soil applied *A. syriaca* leaf residues on primary root length and lateral root number of oilseed rape.

Table 1. Allelopathic effect of common milkweed (*Asclepias syriaca* L.) water extracts on lettuce seedlings root and shoot length

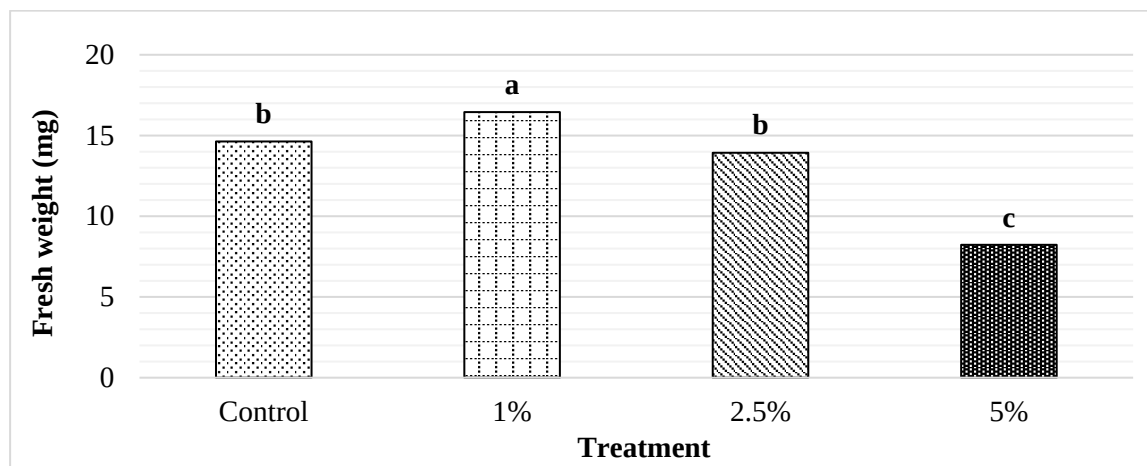
Extract concentration	root length (cm)	shoot length (cm)
control	2.20 ab	2.69 b
1%	2.35 a	3.35 a
2.5%	2.05 b	3.04 ab
5%	0.67 c	1.71 c

Mean values with the same letters are not statistically different at $P < 0.05$

Lettuce shoot length was inhibited by 36.4% at 5% concentration, while the lowest concentration increased shoot length by 24.5% compared to control (Table 1). Reduction in shoot length of maize seedlings up to 61% with *A. syriaca* root extracts and up to 36% with shoot extracts was also reported by Nádasý et al. (2018). Gajić Umiljendić et al. (2025) recorded severe reductions in shoot length of several weed species over 70% with the lowest concentration of *A. syriaca* leaf extract. On the other hand, according to Bakacsý et al. (2024) *A. syriaca* leaf residues in the highest concentration significantly reduced leaf number of oilseed rape seedlings, while none of the concentrations affected shoot length and leaf area in rhizotron system experiment.

Similar trend was found for fresh weight of lettuce seedlings (Graph 2). Significant positive effect was recorded with the lowest extract concentration which induced increase in fresh

weight by 12.4%, while the highest extract concentration caused reduction of fresh weight by 43.7%. Fresh weight of maize plumule and radicle was substantially decreased for over 50% with *A. syriaca* extracts as reported by Nádasy et al. (2018). Similarly, extracts in higher concentrations from other invasive species such as Canadian goldenrod (*Solidago canadensis* L.), giant goldenrod (*Solidago gigantea* Ait.) and velvetleaf (*Abutilon theophrasti* Med.) inhibited fresh and dry weight of test species (Baličević et al., 2015, Moždžen et al., 2020, Bakacsy et al., 2024).



Graph 2. Allelopathic effect of common milkweed (*Asclepias syriaca* L.) water extracts on lettuce seedlings fresh weight

On average, *A. syriaca* water extracts showed the highest allelopathic potential on root length and fresh weight of seedlings reducing them by 23.2% and 12%, respectively. These results are in agreement with other findings who reported greater inhibitory potential on root length of seedlings compared to other growth indices (Baličević et al., 2015, Szilágyi et al., 2018, Popov et al., 2021). Root length is usually more adversely affected due to its direct exposure of the roots to the water extract on the filter paper (Chon et al., 2000).

Conclusions

The results of the study indicated *A. syriaca* extracts possess both stimulatory and inhibitory potential on lettuce germination and seedlings growth depending on the concentration. Low concentration of extract may have potential as natural growth promoters of crops, while higher concentrations could be explored as natural herbicides for controlling weed species.

References

- Bagi, I. (2008). Common milkweed (*Asclepias syriaca* L.). In *The most important invasive species in Hungary*, Botta-Duká, Z., Balogh, L. (eds.), 151-160. Vácrátót, Hungary: HAS Institute of Ecology and Botany.
- Bakacsy, L., Kardos, L.V., Szepesi, Á., Nagy, K.N., Vasas, A., Feigl, G. (2024). Investigation of the Allelopathic Effect of Two Invasive Plant Species in Rhizotron System. *Life*, 14: 475.
- Baličević, R., Ravlić, M., Živković, T. (2015). Allelopathic effect of invasive species giant goldenrod (*Solidago gigantea* Ait.) on crops and weeds. *Herbologia*, 15(1): 19-29.
- Chon, S.U., Coutts, J.H., Nelson, C.J. (2000). Effects of light, growth media and seedling orientation on bioassays of alfalfa autotoxicity. *Agronomy Journal*, 92(4): 715-720.
- Csiszár, Á., Korda, M., Schmidt, D., Šporčić, D., Teleki, B., Tiborcz, V., Zagyvai, G., Bartha, D. (2012). Néhány inváziós és potenciálisan inváziós neofiton allelopátiás hatásának vizsgálata. *Botanikai Közlemények*, 99, 159-171.
- Gajić Umiljendić, J., Sarić-Krsmanović, M., Radivojević, Lj., Šantrić, Lj., Šćepanović, M., Šoštarčić, V., Đorđević, T. (2025). Phytochemical analysis of *Asclepias syriaca* L. leaf extract and its potential phytotoxic effect on some invasive weeds. *Scientific Reports*, 15: 6752.

- Hasan, M., Ahmad-Hamdani, M.S., Rosli, A.M., Hamdan, H. (2021). Bioherbicides: An Eco-Friendly Tool for Sustainable Weed Management. *Plants*, 10: 1212.
- Islam, A.K.M.M., Suttityut, T., Anwar, M.P., Juraimi, A.S., Kato-Noguchi, H. (2022). Allelopathic Properties of Lamiaceae Species: Prospects and Challenges to Use in Agriculture. *Plants*, 11: 1478.
- Kato-Noguchi, H. (2022). Allelopathy of knotweeds as invasive plants. *Plants*, 11: 3.
- Knežević, M. (2006). *Atlas korovne, ruderalne i travnjačke flore*. Osijek, Croatia: Sveučilište Josipa Jurja Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.
- Mihai, D.C., Cișlariu, A.G., Dumitrașcu, M., Georgescu, M.I., Toma, F. (2024). Current state of invasive *Asclepias syriaca* in Romania: Morphological and anatomical insights. *Acta Horti Botanici Bucurestiensis*, 50, 5-21.
- Możdżeń, K., Barabasz-Krasny, B., Zandi, P., Kliszcz, A., Puła, J. (2020). Effect of Aqueous Extracts from *Solidago Canadensis* L. Leaves on Germination and Early Growth Stages of Three Cultivars of *Raphanus sativus* L. Var. *radicula* Pers. *Plants*, 9: 1549.
- Nádasy, E., Pásztor, G., Béres, I., Szilágyi, G. (2018). Allelopathic effects of *Abutilon theophrasti*, *Asclepias syriaca* and *Panicum ruderalis* on maize. In *Tagungsband 28. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und – bekämpfung*, Nordmeyer, H., Ulber, L. (eds.), 454–458. Braunschweig, Germany: Julius-Kühn-Archiv.
- Nikolić, T., Mitić, B., Boršić, I. (2014). *Flora Hrvatske: invazivne biljke*. Zagreb, Croatia: Alfa d.d.
- Norsworthy, J.K. (2003). Allelopathic potential of wild radish (*Raphanus raphanistrum*). *Weed Technology*, 17: 307-313.
- Popov, M., Prvulović, D., Šućur, J., Vidović, S., Samardžić, N., Stojanović, T., Konstantinović, B. (2021). Chemical characterization of common milkweed (*Asclepias syriaca* L.) root extracts and their influence on maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) and sunflower (*Helianthus annuus* L.) seed germination and seedling growth. *Applied Ecology and Environmental Research*, 19(6): 4219-4230.
- Rice E.L. (1984). *Allelopathy*. 2nd Edition, Academic Press, New York.
- Scavo, A., Pandino, G., Restuccia, A., Caruso, P., Lombardo, S., Mauromicale, G. (2021). Allelopathy in Durum Wheat Landraces as Affected by Genotype and Plant Part. *Plants*, 11: 1021.
- Singh, H.P., Batish, D.R., Kohli, R.K. (2003). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *Critical Reviews in Plant Sciences*, 22, 239-311.
- Stef, R., Manea, D., Grozea, I., Chifan, R., Gheorghescu, B., Arsene, G. G., Carabet, A. (2022). *Asclepias syriaca* A new segetal species in Romania. *Scientific Papers. Series A. Agronomy*, 65(1): 703-712.
- Szilágyi, A., Radócz, L., Tóth, T. (2018). Allelopathic effect of invasive plants (*Eriochloa villosa*, *Asclepias syriaca*, *Fallopia* × *bohemica*, *Solidago gigantea*) on seed germination. *Acta Agraria Debreceniensis*, 74: 179-182.

ORIGINAL SCIENTIFIC PAPER

Perception and knowledge of wild poisonous plants among the student population of the Faculty of Agrobiotechnical Sciences Osijek

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Summary

Numerous wild plants are used in nutrition, cosmetics, and medicine; however, many are poisonous. A study was conducted with 80 students from the Faculty of Agrobiotechnical Sciences in Osijek to assess their perceptions of wild poisonous plants, their knowledge of symptoms of poisoning, and attitudes toward the need for education. The results revealed a significant lack of knowledge, as most students were uninformed about these plants and their associated symptoms. When asked about their familiarity with specific poisonous plants, less than 35% of the students were able to correctly identify specific species. However, the data show that students' perceptions largely supports education on wild poisonous plants. These findings highlight the critical need for additional education on the topic.

Key words: wild flora, poisonous plant symptoms, survey, knowledge gaps, education

Introduction

Wild plant species have long been utilized for their nutritional, medicinal, and industrial purposes. Rich in vitamins, minerals, and microelements, wild plants provide valuable nutrients through edible parts such as leaves, roots, and fruits, thus contributing to dietary diversity. Many wild species are valued for their therapeutic properties, providing natural remedies for ailments in traditional medicine, and are studied and used in modern medicine, as well as in the development of dietary supplements, due to their bioactive compounds and potential health benefits (Grlić, 2005, Filipović, 2021, Pentassuglia et al., 2025). While many wild plants offer beneficial uses, some species can be toxic and pose significant health risks. Inadequate plant identification skills, combined with a general lack of knowledge, contribute significantly to the accidental consumption of poisonous species, as individuals may misidentify toxic plants as edible (Fančovičová and Prokop, 2011). For example, edible wild garlic (*Allium ursinum*) can be confused with the autumn crocus (*Colchicum autumnale*), a poisonous plant that contains the toxic alkaloid colchicine. In their early stages of development, the leaves of these plants are similar, and their shared habitat and similar morphology increase the risk of misidentification, potentially resulting in fatal poisoning (Kolák et al., 2001, Žuntar and Bušić, 2018). Several studies have highlighted that young people often have limited knowledge of wild plants, including both edible and poisonous species. Despite the risks associated with poisonous plants, many students are unfamiliar with even the most common poisonous species. A significant proportion of students have difficulty identifying even a single poisonous plant, indicating a notable knowledge gap (Fančovičová and Prokop, 2011, Strgar et al., 2013, Burrows et al., 2015, Filipović, 2021). The aim of our study was therefore to assess the knowledge of first-year undergraduate students at the Faculty of Agrobiotechnical Sciences Osijek about wild poisonous plants, their awareness of poisoning symptoms, and their opinion on the need for further education and awareness about these plants.

Material and methods

The research was conducted in January 2022 at the Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek. The participants were first-year students enrolled in the undergraduate university studies in Agriculture. A total of 80 students participated in the study. The survey adhered to established scientific ethical guidelines, ensuring the confidentiality of participants and the anonymity of the collected data. The questionnaire contained both demographic and knowledge-based questions. In addition to multiple-choice and dichotomous questions, participants rated their agreement with various statements using a Likert scale from 1 to 5. The demographic questions included gender, age, level of completed secondary education, and place of residence. The Likert scale assessed participants' self-reported knowledge of poisonous wild plants and their associated symptoms on a scale of 1 (completely uninformed) to 5 (completely informed). Participants were also asked whether they had ever encountered any wild toxic plants and, if so, to specify which ones. The survey included well-known examples of wild poisonous plants (*Colchicum autumnale*, *Atropa belladonna*, *Convallaria majalis*, *Hedera helix*, *Taxus baccata*), with participants indicating their familiarity with these species. Furthermore, participants assessed their attitudes toward education on poisonous wild plants, the risk they present, and importance of recognizing specific poisonous species, rating their agreement on a scale of 1 (strong disagreement) to 5 (strong agreement).

Results and discussion

The sociodemographic characteristics of the participants, including gender, age, and education level across urban and rural areas, are presented in Table 1, based on a total of 80 individuals. The sample comprised of 32 female (40%) and 48 male (60%) students. A majority of respondents (97.5%) were aged between 18 and 25 years. More than half of the respondents (67.5%) were from rural areas, while 32.5% were from urban areas. Regarding educational background, the largest group of respondents (60%) had completed vocational-technical secondary school, followed by 33.8% who had attended gymnasium, and 6.3% who had completed an economic/commercial school prior to university enrolment.

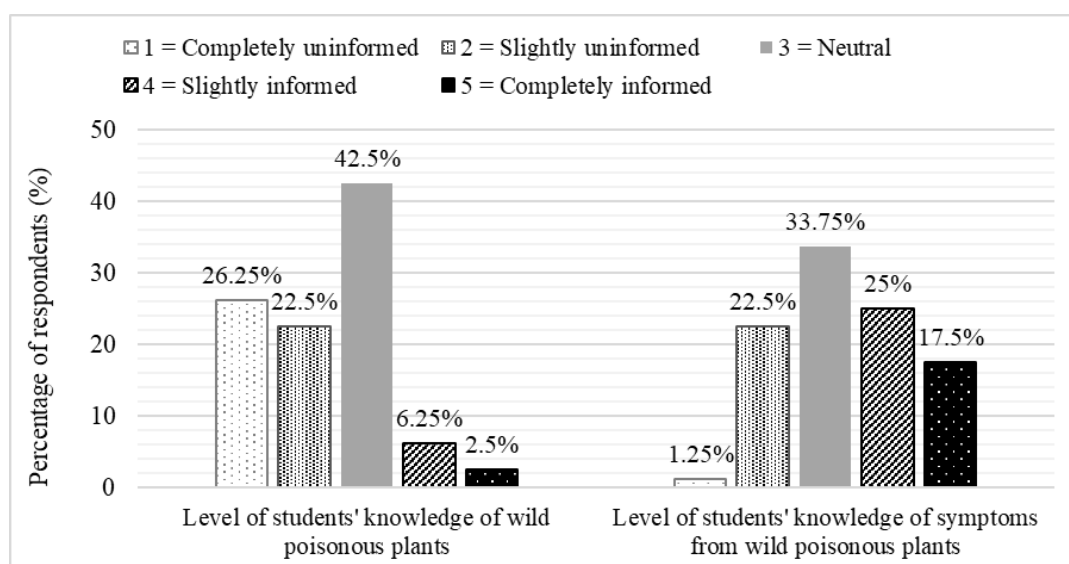
Table 1. Sociodemographic profile of students by gender, age and education

Sociodemographics	Total*	Urban Area	Rural Area
Gender			
Female	32 (40%)	9 (34.6%)	23 (42.6%)
Male	48 (60%)	17 (65.4%)	31 (57.4%)
Total	80	26	54
Age			
18-25	78 (97.5%)	26 (100%)	52 (96.3%)
26-30	1 (1.25%)	0 (0%)	1 (1.85%)
30+	1 (1.25%)	0 (0%)	1 (1.85%)
Total	80	26	54
Secondary School**			
Voc.-Tech.	48 (60%)	15 (57.7%)	33 (61.1%)
Gym.	27 (33.8%)	8 (30.8%)	19 (35.2%)
Econ./Comm.	5 (6.2%)	3 (11.5%)	2 (3.7%)
Total	80	26	54

*No. of total respondents (urban + rural area); **Completed secondary school; Vocational-Technical School; Gymnasium; Economic/Commercial School

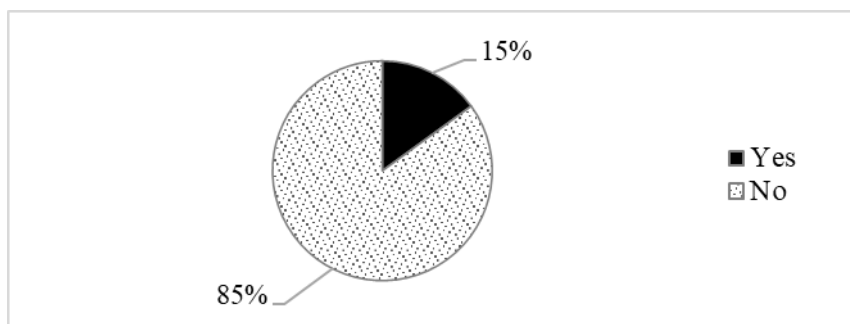
Subsequently, students were asked to assess their knowledge of wild poisonous plants and the symptoms resulting from exposure using a 5-point Likert scale (Graph 1). A large proportion

of respondents (42.5%) rated their knowledge as neutral (3), while almost half of the respondents considered themselves as uninformed. In contrast, only a small percentage felt somewhat or fully informed, suggesting that many respondents may have limited knowledge about these plants. When assessing their knowledge of symptoms caused by exposure to poisonous plants, 33.75% of respondents rated their knowledge as neutral. However, a higher percentage reported being informed, with 25% rating their knowledge of symptoms as slightly informed and 17.5% as completely informed. A clear discrepancy between knowledge of the plants and knowledge of their symptoms resulting from exposure to them may indicate that respondents, despite lacking detailed knowledge of the plants themselves, are more aware of the potential health effects associated with them. This difference may reflect the fact that while respondents might be familiar with the broader concept of poisonous plants, they lack detailed knowledge needed to identify specific species. Their awareness of the symptoms could stem from general education on the dangers of poisoning, media reports, or online resources, as symptoms of poisoning from wild plants are often more widely discussed or recognized in general terms, whereas identifying specific species requires more specialized knowledge. The findings of Filipović (2021) indicate that the largest percentage of respondents rated themselves as neutral (34%) and slightly uninformed (30.1%) regarding wild edible plants, with a smaller proportion considering themselves completely informed (10.9%), which is similar to the results of our research.



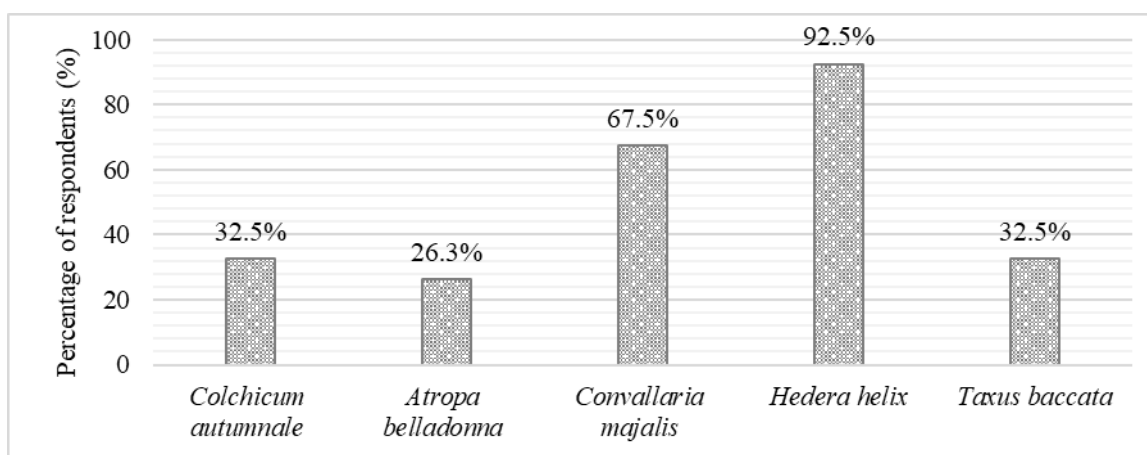
Graph 1. Students' knowledge of wild poisonous plants and their effects (Likert scale)

When participants were asked whether they had ever encountered poisonous wild plants, 85% reported no prior encounters (Graph 2). However, given the limited education on plant identification, it is plausible that some students may have unknowingly had contact with these species due to their inability to properly recognize them. In contrast, 15% indicated that they had encountered poisonous plants, with common ivy (*Hedera helix*) being the most frequently mentioned (83.3% of participants). Other species reported by students included lily of the valley (*Convallaria majalis*), black bryony (*Tamus communis*), yew (*Taxus baccata*) and Arum species. Overall, the average number of species listed per student among those who provided answers was approximately 1.6. Notably, differences in encounters were observed based on participants' place of residence: 66.7% of those who had come across poisonous plants were from rural areas, while 33.3% were from urban areas.



Graph 2. Proportion of students who have encountered poisonous wild plants

Following this, participants were presented with a list of several poisonous wild species along with their toxic parts, and their task was to indicate which of them they were familiar with. As shown in Graph 3, common ivy (*H. helix*) was the most recognized plant, identified by 92.5% of participants, followed by lily of the valley (*C. majalis*). Approximately one-third of participants identified autumn crocus (*C. autumnale*) and yew (*T. baccata*) as toxic, while only 26.3% reported familiarity with deadly nightshade (*Atropa belladonna*). Strgar et al. (2013) reported that secondary medical school students identified an average of 1.2 poisonous plant species, while grammar school students identified 0.7. A significant portion of students, 56.8% of grammar school and 32.4% of secondary medical school students, failed to identify a single poisonous plant. The most frequently cited species included hellebore (*Helleborus* sp.), common ivy (*H. helix*), yew (*T. baccata*), and deathly nightshade (*A. belladonna*). Similarly, Burrows et al. (2015) found that animal and veterinary science students as well as agriculture students demonstrated limited ability to identify poisonous plants, with average scores of 11.9% and 14%, respectively, for recognizing up to 40 species, despite many reporting a rural upbringing.



Graph 3. Students' familiarity with the listed species of poisonous wild plants

Participants' attitudes toward the importance of education on wild poisonous plants, their curiosity about poisonous species near their homes and awareness of the associated health and safety risks are presented in Table 2. A significant majority of participants (80%) agreed that investing in information and education about wild poisonous plants is necessary, while only 5% disagreed. Both male and female participants demonstrated similar levels of support. Similarly, 77.5% indicated that if they encountered a poisonous species near their home, they would be interested in learning about its origin, toxic components, and symptoms of poisoning, while 18.75% were uncertain. When comparing educational background, students with completed economic school showed the most support, followed by gymnasium graduates, and vocational

technical school graduates. Furthermore, being well-acquainted with poisonous and inedible fruits, which can pose a greater risk to health and life than edible plant species, was considered crucial by 87.5% of participants. Students from urban areas showed somewhat higher level of agreement with this statement than students from rural areas. According to Filipović (2021), nearly 80% of respondents agreed on the need to invest in information and education about wild plants, while only 7% disagreed.

Table 2. Students' attitudes towards education on wild poisonous plants (Likert scale)

Statements	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. It is necessary to invest in information and education about wild poisonous plants.	2.5%	2.5%	15%	26.25%	53.75%
2. If you discovered that a species commonly seen near your home was poisonous, you would be interested in its origin, toxic parts, and poisoning symptoms.	0%	3.75%	18.75%	28.75%	48.75%
3. For nature enthusiasts, it is more useful to be well-acquainted with poisonous and inedible fruits, as they can pose a greater risk to health and life compared to edible plant species.	0%	0%	12.5%	18.75%	68.75%

Conclusions

The study highlights a general lack of knowledge about wild poisonous plants among participants, with many showing limited awareness of plant identification but greater understanding of poisoning symptoms. While most had not encountered these plants, those who had were more likely to come from rural areas. The findings also show strong interest in education, with participants seeking more information on poisonous plant identification and health risks, underscoring the need for increased awareness and educational efforts.

References

- Burrows, G.E., Krebs, G.L., Kirchoff, B.K. (2015). 'Visual Learning – Agricultural Plants of the Riverina' – A New Application for Helping Veterinary Students Recognise Poisonous Plants. *Bioscience Education*, 1-13.
- Fančovičová, J., Prokop, P. (2011). Children's Ability to Recognise Toxic and Non-Toxic Fruits. *Eurasia Journal of Mathematics, Science & Technology Education*, 7(2): 115-120.
- Filipović, M. (2021). Poznavanje i upotreba samoniklog jestivog bilja. Graduate thesis, Faculty of Agrobiotechnical Sciences Osijek, Osijek.
- Grlić, L. (2005.). *Enciklopedija samoniklog jestivog bilja*. Rijeka, Hrvatska: Ex libris.
- Kolak, I., Šatović, Z., Popović, D., Rozić, I. (2001). Mrazovac (*Colchicum autumnale* L.). *Sjemenarstvo*, 18(5-6): 365-373.
- Pentassuglia, M., Lombardi, T., Bambi, G., Ventura, I., D'Ambrosio, B., Bertacchi, A., Pistelli, L. (2025). A Floristic Survey of Wild Edible Plants in Tuscan Maremma, Italy. *Plants*, 14: 976.
- Strgar, J., Piliš, M., Pogačnik, M., Žnidarčič, D. (2013). Knowledge of medicinal plants and their uses among secondary and grammar school students: a case study from Slovenia. *Archives of Biological Sciences*, 65(3): 1123-1129.
- Žuntar, I., Bušić, M. (2018). Trovanje mrazovcem i zamjena za medvjedi luk. *Farmaceutski glasnik*, 74(4): 273-286.

EXPERT PAPER

Agricultural land assessment in the Republic of Croatia with recommendations for amending existing legislation

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Summary

In order to protect agricultural land from conversion, the application of the valid " Ordinance on the measures for determining particularly valuable arable (P1) and valuable arable (P2) agricultural land" was analyzed on various examples of its valuation. The legal acts and methodology for land assessment that protect it from conversion in other Mediterranean countries with available legal acts were analyzed. There are high-quality agricultural soils in the area of mountainous and Adriatic Croatia, but they are insufficiently protected by legal acts. In order to amend them, it is recommended for the land to be assessed at representative locations as part of a multi-year project, and after that proposals will be made to amend the existing legal acts.

Keywords: agriculture, land assessment, legal acts

Introduction and aim

Since the seventies of the last century, methods and criteria recommended by experts of the FAO organization have been used to assess land suitability in Croatia (FAO, 1976; 1981, cor. Vidaček 1981). In the ex-state, agricultural land was valued according to the methodology developed by Kovačević et al. (1973), and it was later revised in accordance with the author's experience and knowledge of the valuation of agricultural land in the world (Kovačević et al. 1987). In the Republic of Croatia, agricultural land is rated based on the principles of the Ordinance for criteria for determining particularly valuable (P1) and valuable (P2) arable agricultural land (NN 23/2019). These particularly valuable and valuable agricultural lands are protected from conversion by law (Law on agricultural land, NN 57/2022). The said Ordinance was mostly drafted according to the previously valid criteria of land scoring, and the assessment of its suitability is made according to the same methodology that was used in the former state (FAO, 1976; 1981, cor. Vidaček 1981). Land grading is based on the scoring of the soil, climate and relief, as well as correction factors (rockiness, stoniness, terrain exposure, floods, etc.). The general assessment of the land is calculated on the basis of assessment points for soil, climate and relief, and is corrected by a correction equation containing negative points obtained by correction due to unfavorable natural conditions. The general land assessment corrected in this way is classified into spatial categories according to the final corrected assessment points of the land. In total, four spatial categories were distinguished according to the features of the soil, relief and climate, and the fourth is also the most unfavorable. For example, according to this Ordinance, with the aim of protecting particularly valuable and valuable agricultural land from conversion, its assessment rating was carried out in the area of Dalmatia, Gorski kotar and Posavina (Špoljar, 2023.a, 2023.b, 2023.c, 2024). The method of its evaluation earlier and now in Croatia, as already pointed out, is based on almost the same criteria. However, it does not prescribe the method of land assessment for crops or plantations registered in the cadastre, or that are recorded in other land information databases (eg. ARKOD land database). Cadastral crops include the following categories of land use: arable land, orchards, vineyards, meadows, pastures and forests. Today, the use of land parcels (cadastre parcels) is visible in the new digital

record system called ARKOD Browser (<https://preglednik.arkod.hr>). Kovačević (1992) on the basis of the general assessment of the land elaborates the evaluation of cadastral or agricultural crops in relation to their specific requirements. The author states that the assessment of plant production conditions in agriculture and forestry has a broad meaning and cannot be equated with land assessment. When the productive capacity of the land is determined based on natural conditions, then it is a question of its assessment. The Ordinance from 2019 prescribes the methodology of land assessment and, as already pointed out, it does not cover the scoring of land of cadastral or agricultural crops. Kovačević (1992) points out that, along with the introduction of vegetation areas instead of cadastral districts, the analysis of economic conditions for the selected agricultural production should also be introduced into the then method of land classification. Such a way of valuing agricultural land, as stated by the author, should serve as the basis of land value in the land consolidation assessment. In previous research into the assessment of agricultural land according to the current Ordinance, certain shortcomings of this legal act came to the fore, which mainly refers to the lands of the Mountainous and Adriatic Croatia. Namely, in these areas, there are quality soils on smaller production areas, and legal acts do not sufficiently protect them from conversion. Similarly, on plots of irregular shape, regardless of the difficulties in mechanized cultivation, it is possible to grow more profitable crops, and giving negative points reduces their value. It should also be pointed out that in the less favorable climatic conditions of the Mountainous Croatia, it is possible to organize successful agricultural production on smaller areas and on soils of higher quality. Of course, there are quality soils in Adriatic Croatia under the conditions of the Mediterranean climate, but due to some unfavorable natural conditions, they are insufficiently protected from conversion. Due to all of the above, and with the aim of protecting agricultural land from conversion, the available results so far were analyzed and appropriate recommendations were made for amending the legal acts that protect it.

Material and methods

The existing legal regulations in the Republic of Croatia (NN 39/2019; NN 57/2022) and the next legal acts of Italy and Spain were analyzed: The Cultural Heritage and Landscape Act and the General Urban Plan in Italy, as well as the method of evaluating agricultural land in Spain (Anno et al., 1997.). The most important results of land assessment according to the Ordinance on criteria for determining particularly valuable arable (P1) and valuable (P2) arable agricultural land are also presented (Špoljar 2023a; 2023b; 2023c) with recommendations for further research.

Results and discussion

Špoljar (2023c) evaluates agricultural land in the town of Vrbovsko and separates three systematic units at the level of soil type according to the valid soil classification in Croatia: rendzina, luvisol and anthropogenic soil from colluvium (Husnjak, 2014). The luvisol and anthropogenic soil from the colluvium has satisfactory physical and chemical characteristics, while the main limiting factor in plant production of rendzina is its depth. However, according to the equation for general land assessment, luvisol and anthropogenic soil from colluvium are lower ranked soils due to the unfavorable position in the relief and the fewer points awarded for the climate for all systematic units. According to the equation for the corrected land assessment, these soils were assigned negative points due to the small area they occupy. For these reasons, the mentioned soils fell into the spatial category that is not protected from conversion by the current Ordinance. Figures 1 and 2 show luvisol and anthropogenic soil from colluvium.

Since there are relatively few such high-quality soils on larger areas in Mountainous Croatia, regardless of their unfavorable position in relief and less favorable climatic conditions, the cultivation of crops that thrive in that climate can be recommended.

Table 1. Basic physical characteristics of the soil

Luvisol, Vrbovsko - Vujnovići						
Depth, cm	P		Wc		Ac	
	% vol.	Mark	% vol.	Mark	% vol.	Mark
0-40	51,89	porous	45,53	big	6,36	little
40-60	54,73	porous	42,19	medium	12,54	good
60-100	52,28	porous	44,93	medium	7,35	little
Anthropogenic soil from colluvium, Vrbovsko Vujnovići						
Depth, cm	P		Wc		Ac	
	% vol.	Mark	% vol.	Mark	% vol.	Mark
0-50	56,97	porous	48,34	big	8,63	medium
50-110	60,03	very porous	52,00	big	8,04	medium
110-150	54,78	porous	50,54	big	4,25	little
Colluvium medium deep, Tisno						
Depth, cm	P		Wc		Ac	
	% vol.	Mark	% vol.	Mark	% vol.	Mark
0-32	46,12	porous	35,00	medium	11,11	medium
>32			dolomite material			
>45			solid rock			

* abbreviation interpreter: P = total porosity, Wc = water soil retention capacity, Ac = soil capacity for air

Table 2. Basic chemical properties of soil

Luvisol Vrbovsko - Vujnovići					
Depth, cm	Soil reaction (pH), in				Humus
	Water	1M KCl	Mark	%	Mark for humus
0-40	6,82	5,68	slightly acidic	3,92	enough humus
40-60	6,45	5,45	acidic	-	-
60-100	6,30	5,27	acidic	-	-
Anthropogenic soil from colluvium, Vrbovsko - Vujnovići					
Depth, cm	Soil reaction (pH), in				Humus
	Water	1M KCl	Mark	%	Mark for humus
0-50	6,44	5,22	acidic	3,92	enough humus
50-110	6,63	5,60	slightly acidic	-	-
110-150	6,40	5,39	acidic	-	-
Colluvium medium deep, Tisno					
Depth, cm	Soil reaction (pH), in				Humus
	Water	1M KCl	Mark	%	Mark for humus
0-32	7,66	7,05	neutral	10,35	very much humus
32-45			dolomite material		
> 45			solid rock		

Špoljar (2023a) distinguishes three mapped/systematic units in the area of Tisno municipality: anthropogenic very shallow calcocambisol and shallow colluvium skeletal and colluvial medium deep skeletal soil. The colluvial soil has satisfactory physical and chemical characteristics, while in other isolated units the main limiting factor in plant production is the increased skeletal content and low ecological depth of the soil. However, regardless of these disadvantages, colluvial soil could be used to grow more profitable crops, Figure 3. The basic

physical and chemical characteristics of soils shown in figures 1 to 3 are given in tables 1 and 2. Due to the rocky nature, small production area or unfavorable position in relief, all considered soils received negative percentages of land assessment according to the Ordinance and are therefore ranked lower, so their conversion into construction land is possible. Some other Mediterranean countries protect their agricultural land more comprehensively with their legislation. Spanish spatial planning laws restrict the conversion of agricultural land to construction. Land use is regulated by the general urban plans of individual cities. Ano et al., (1997) develop a method for evaluating agricultural land in Spain, where they evaluate the external and internal features of the soil.



Figure 1. Luvisol



Figure 2. Anthropogenic soil from colluvium



Figure 3. Colluvium medium deep

Based on these features, the authors establish an index of land suitability and vulnerability. The vulnerability index shows the restrictions on the use of land for agricultural production. The mentioned indexes can be used, as stated by the authors, for planning land use. The Law on Cultural Heritage and Landscape in Italy restricts the conversion of land in protected rural areas (<https://shorturl.at/wIaoR>). It is only possible to build buildings that are directly related to agricultural activity, and the land is classified according to its potential for growing different agricultural crops. The general urban plan protects agricultural zones, especially in areas with high agricultural potential or cultural value (<http://www.urbanistica.comune.roma.it/prg.html>). Similarly, Kovačević et al., (1987) recommends in the elaborated new land assessment methodology for Croatia. The authors suggest carrying out assessment of cadastral or agricultural crops. They are working on assessment for arable land, orchards, olive groves, vineyards, meadows and pastures, as well as forest plants. Land assessment of cadastral crops enables the selection of the most favorable cadastral crop for the current and planned land use from an ecological point of view. Based on the analysis of the available results of land assessment of agricultural land in Croatia, it is possible to recommend amendments to existing legal acts, whereby special attention should be paid to the Ordinance on criteria for determining particularly valuable arable (P1) and valuable arable (P2) agricultural land. Before amending this legal act, it is recommended to carry out land assessment of representative agricultural land in the Republic of Croatia according to the methodology recommended by Kovačević et al., (1987) and according to the current Ordinance. With the aim of possible amendments and changes to existing legal acts, it is recommended that a multi-year soil assessment study be conducted in Croatia at several representative locations according to the existing Ordinance and the methodology recommended by Kovačević et al. (1987), in which the methodology for assessing cadastral or agricultural crops is elaborated. Based on this data, more specific proposals for correcting existing legal regulations can be made.

Conclusions

The analysis of the available results of the land assessment of agricultural land in Croatia found that it is insufficiently protected by the existing legal acts. This mainly applies to the area of

Mountainous and Adriatic Croatia, where there are quality agricultural soils on smaller areas, often of irregular geometric shape and less favorable position in the relief, and on which it is possible to organize successful agricultural production. Therefore, it is recommended to assess the land in representative locations as part of a multi-year project, based on which specific proposals will be made to amend and supplement the existing legal acts.

References

- Ano C., Sanches J., Antolin C. (1997). Land evaluation methodology for Mediterranean environments. Departamento de Biología Vegetal, Facultad de Farmacia, Universidad de Valencia, Spain.
- Kovačević P., Mihalić V., Miljković I., Licul R., Kovačević J., Martinović J., Bertović S. (1987). New method of land assessment in Croatia. *Agronomy journal*, 2-3: 47-75.
- Kovačević P. (1992). Land assessment in the function of preserving and increasing soil fertility. *Agronomy journal*, 4: 263-267.
- FAO. (1976). A framework for land evaluation. *FAO Soils bulletin* 32, First Printing, 66 p., Available from: <https://www.fao.org>.
- FAO. (1981). A framework for land evaluation. *FAO Soils bulletin* 32, Second Printing, 66 p., Available from: <https://www.fao.org>.
- Husnjak S. (2014). Soil classification of Croatia. Textbook, University of Zagreb, 373 p.
- Špoljar A. (2023a). Land assessment in the municipality of Tisno. Elaborate, 22 p. Križevci, Croatia: Križevci University of Applied Sciences.
- Špoljar A. (2023b). Land assessment in the cadastral municipality of Polača. Elaborate, 19 p. Križevci, Croatia: Križevci University of Applied Sciences.
- Špoljar A. (2023c). Land assessment in the city of Vrbovsko – Moravice. Elaborate, 19 p. Križevci, Croatia: Križevci University of Applied Sciences.
- Špoljar A. (2024). Land assessment in the cadastral municipality of Oborovo - Prevlaka. Elaborate, 32 p. Križevci, Croatia: Križevci University of Applied Sciences.
- Vidaček Ž. (1981). Assessment of production area and soil suitability for irrigation in Eastern Slavonia and Baranja. Dissertation, Agricultural Scientific Review, Zagreb, 57: 471-502.
- *** Ordinance on criteria for determining particularly valuable arable (P1) and valuable arable (P2) agricultural land (NN 23/2019.).
- *** Law on Agricultural Land, (NN 57/2022.)
- *** <https://preglednik.arkod.hr>
- *** <http://www.urbanistica.comune.roma.it/prg.html>
- *** <https://shorturl.at/wlaoR>

ORIGINAL SCIENTIFIC PAPER

Effects of soil amendments on Cd availability in soil

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Agrotechnical measures may reduce bioavailability of cadmium (Cd) from soil. This research investigated the effect of repeated addition of different soil amendments (lime (L), lime + zeolite (LZ), lime + organic matter (LOM)) in uncontaminated field where excessive Cd in carrot was determined in previous years. Regular use of amendments affected soil chemical properties and bioavailability of Cd. The lowest pH, at all samplings, was determined in control, the organic matter (OM) increased above all in the upper level of LOM, cation exchangeable capacity at control was lower than at other treatments. Consequently, the highest bioavailable Cd was determined at control. In uncontaminated soil with elevated levels of plant available Cd, the level of available Cd may therefore be effectively lowered by manipulating soil pH and OM.

Key words: cadmium, lime, zeolite, organic matter, pH, cation exchangeable capacity

Introduction

Cadmium (Cd) is one of the most ecotoxic metals, with harmful effects on humans, animals and plants. The presence of Cd in food is due to contamination of soil and water. Cd is transferred from soil and water to plants and aquatic organisms and accumulates in the food chain (NIJZ, 2023). European Commission Regulation 2023/915 of 25 April 2023 sets maximum levels for certain contaminants in food, including cadmium (EC, 2023). On average, Cd in soil is between 0.06 and 1.1 mg/kg and is an easily available element. Atmospheric deposition and the use of phosphate fertilisers are the main sources of cadmium inputs to agricultural soils, and can lead to elevated levels of cadmium in soils and crops, which can lead to increased dietary exposure to cadmium. Most of the cadmium contamination remains in the top 15 cm of the soil (Kabata-Pendias and Mukherjee, 2007).

In Slovenia the limit, alert and critical immission values for Cd in soil are written in the Regulation on limit, alert and critical immission values for dangerous substances in soil. The limit value (at which effects or impacts on human health or the environment are still acceptable) for Cd in soil is set at 1 mg/kg dry soil, the alert value (at which adverse effects or impacts on human health or the environment are likely to occur for certain types of land use) at 2 mg/kg dry soil and the critical value (at which contaminated soil is unsuitable for the production of plants for human or animal food and for water retention or filtration because of adverse effects or impacts on humans or the environment) at 12 mg/kg dry soil (UL RS, št. 68/96, 1996). In Slovenia soil Cd levels are higher than in other European countries due to the parent soil. According to the Geological Survey of Slovenia (n=817), the median Cd content of the surface layer (0-10 cm) is 0.47 mg/kg, so even a small increase in soil Cd (> 1 mg/kg) can pose a potential risk for Cd uptake into plants and further up the food chain (Gosar et al., 2019). Cd is most mobile in acidic soil, binding is weaker at pH higher than 5, and is relatively immobile in alkaline soil. Agronomic measures to reduce the Cd content in vegetable crops: higher pH, higher organic matter content, addition of Si- and clay mineral-rich materials contribute to a better cation exchange capacity (CEC) of the soil, soils with a higher CEC can bind more Cd,

which reduces its availability (Lo et al. 1992; Kabata-Pendias and Mukherjee 2007). The effect of amendment on Cd concentrations in plants also depends on the soil and the amount of the amendment, so it is necessary to select the amendment according to the soil type (Keller et al., 2005). Analyses carried out within the framework of the Public Service in Horticulture for crops grown in soils with Cd concentrations below the immission limit confirm that the Cd content of garlic crops is mainly related to the amount of bioavailable Cd in the soil, rather than the amount of total Cd in the soil and that the amount of bioavailable Cd is influenced by both the soil pH and the soil organic matter content, with variations between varieties (Ugrinović et al. 2022, Smolnikar et al. 2024). Research on Cd uptake by plants, both in Slovenia and globally, has been mainly related to cultivation in soils with elevated Cd levels, often carried out in pot experiments. To investigate the effects of soil amendments on soil Cd availability, we designed a multi-year field experiment and present the results of the first three years in this paper.

Material and methods

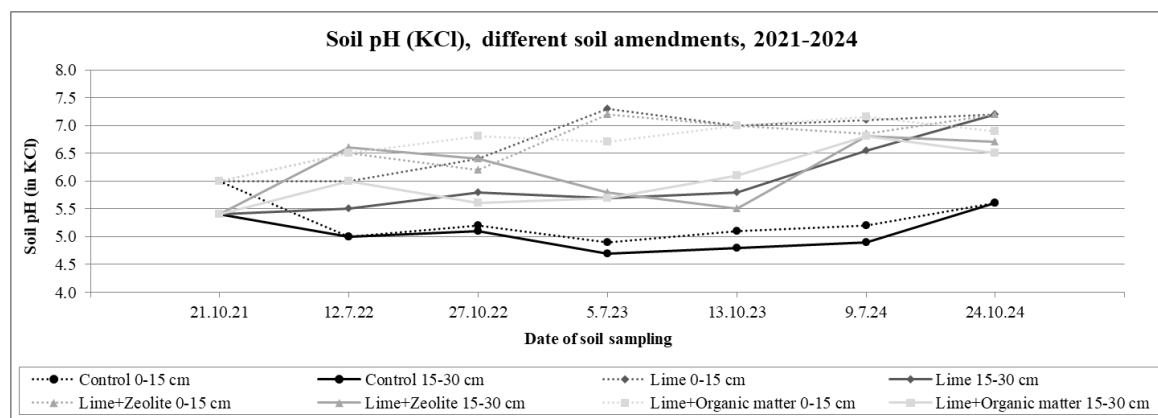
A multi-year trial was designed in autumn 2021 on a plot in Gradišče near Murska Sobota, where Cd levels have been exceeded in the past in carrot crop. The soil properties (0 to 30 cm) of the experimental plot before the experiment are reported in Smolnikar et al. (2024). In brief, the soil is moderately acidic (pH 5.4), with low organic matter (1.6 %) and low cation exchange capacity ($10.87 \text{ mmol } 100 \text{ g}^{-1}$), low total but high available Cd (0.294 mg kg^{-1} and 0.013 mg kg^{-1}). The experiment is designed as a split-plot, where the main factor, in addition to the control (C), where only mineral fertilisers containing N, K and P are added, is the different soil amendments: lime (L) with Calcevia Agro (Intercal) 3 t ha^{-1} , lime with Calcevia Agro 3 t ha^{-1} + zeolite flour (Montana) 1 t ha^{-1} (LZ) and lime with Calcevia Agro (Intercal) 3 t ha^{-1} + organic matter (LOM). The sub-factor in the trial is represented by 4 garlic varieties. The trial is conducted in a completely randomized design with 4 replications.

The previous crops and fertilisation in the growing seasons 2021/22 and 2022/23 are described in Smolnikar et al. (2024). In the growing season 2023/24 the previous crop was phacelia. In autumn 2023 the whole experimental plot was fertilised with $100 \text{ kg K}_2\text{O ha}^{-1}$ (fertiliser with potassium chloride) and 24 kg N ha^{-1} , $48 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $96 \text{ kg K}_2\text{O ha}^{-1}$. Each autumn the amendments were applied according to the experimental design and mechanically worked into the soil. To add organic matter to the main plot LO, green rye straw was incorporated in 2022, wheat straw was incorporated in 2023 and green mass of white mustard was incorporated in 2024. In March 2024, the trial was additionally fertilised with 54 kg N ha^{-1} , $14 \text{ kg CaO ha}^{-1}$ and $10 \text{ kg MgO ha}^{-1}$. Before planting (autumn 2021, 2022 and 2023) and after harvesting (summer 2022, 2023 and 2024) of garlic, soil samples were taken with a probe from each of the main plots at depths of 0-15 cm and 15-30 cm. Due to budget limitations pH, CEC, organic matter, available and total Cd were determined in composite samples of all repetitions together, so only trends may be observed while the statistical differences between the treatments could not be tested. Soil pH was analyzed according to ISO 10390, CEC according to ISO 13536, soil organic matter according to ISO 10694, and Cd in soil according to ISO 16965.

Results and discussion

Graph 1 shows soil pH fluctuations from October 2021 to October at different treatments. At all samplings, the lowest pH values at both depths were measured on the control plots, they ranged from 4.9 to 5.6 in the depth 0 to 15 cm and from 4.7 to 5.6 in the depth 15 to 30 cm, the maximum difference between the two layers was 0.3. For all treatments with soil amendments the pH values in the upper layer were higher (with the exception of LZ treatment in July 2022 where the values were almost equal), anyhow the differences between the two layers were much higher in the first two years (from 0.2 at LZ in October 2022 to 1.6 at L in July 2023) while in the 2024 they became much smaller or did not exist. The results show that the amendments

increased the pH of the soil and that the effect in the first two years was more pronounced in the upper layer while with time also lower layer was affected. Ibrahim et al. (2023) also observed that zeolite application significantly increased soil pH and EC. Li et al. (2019) examined 175 studies on the effects of liming rate, lime application method, and liming material type on various soil chemical properties and crop yield, and concluded that most important variables that drive changes in soil pH and crop yield are liming rate and crop species. Soil conditions, such as soil organic matter and pH, are important for increasing pH in field-based experiments. Amendment of lime, biochar, by-products, manure, straw and combinations of them significantly increased soil pH and increased yield respectively, but it varied among crop types (Zhang et al., 2023).

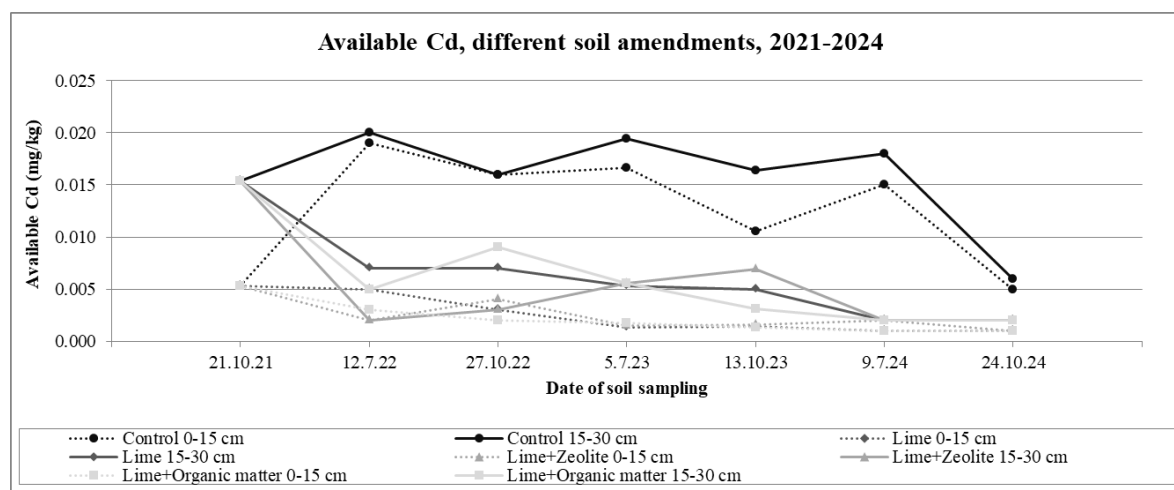


Graph 1. Soil pH (KCl), measured from October 2021 to October 2024

The highest soil OM (results are not shown) was, as expected, measured on LOM plots. Similarly, as pH, the OM first increased at the upper sampled layer and with time also at 15 to 30 cm.

CEC values (results are not shown) were the lowest at the control plots, with no difference between the layers and slight fluctuations over the time. The highest CEC values were determined at the upper layer of the plots with the amendments, and the increase of CEC is noticed over the time at both soil depths.

For total Cd (results are not shown), seasonal fluctuations may be observed, however on all plots, the measured content was $0.2 - 0.4 \text{ mg kg}^{-1}$.



Graph 2. Available Cd in soil, measured from October 2021 to October 2024

Graph 2 shows available Cd in soil, from October 2021 to October 2024. The highest concentrations of available Cd (0.02 mg kg^{-1}) were measured on the C plots. The lowest content (0.001 mg kg^{-1}) of available Cd was measured on 24.10.2024 at LZ and LOM plots. The results show that the amendments reduced available Cd in the soil and a trend of decreasing of available Cd over the time is noticeable at both depth of plots with amendments. It may be noticed (Graph 1 and 2) that with increasing pH and CEC bioavailable Cd content decreased. Li et al. (2024) reported that amendments increased soil pH and significantly decreased available Cd in the soil. Compared to the control, Cd bioavailability in soil was reduced by adding lime, lime+zeolite and lime+organic matter (Graph 1), what is in accordance with the expectations since lime and zeolite are known to elevate soil pH, and soil pH has significant role in Cd availability (Lu et al., 2017). Low pH leads to high solubility of Cd (Belimov et al., 2015). With increasing pH bioavailable Cd content linearly decreased (Shen et al., 2020).

Conclusions

This research investigated the effect of repeated addition of different soil amendments (lime, lime + zeolite, lime + organic matter) on Cd bioavailability in uncontaminated soil with elevated level of plant available Cd. Research showed that Cd bioavailability was reduced after the application of the studied soil amendments, with increasing pH and CEC bioavailable Cd content decreased. The repeated application of amendments resulted in decreasing soil available Cd over the time at both soil depths, 0 to 15 and 15 to 30 cm, but the changes at upper level were quicker.

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References

- Belimov A. A., Puhalsky I. V., Safronova V. I., Shaposhnikov A. I., Vishnyakova M. A., Semenova E. V., Zinovkina N. Y., Makarova N. M., Wenzel W., Tikhonovich I. A. (2015). Role of Plant Genotype and Soil Conditions in Symbiotic Plant-Microbe Interactions for Adaptation of Plants to Cadmium-Polluted Soils. *Water Air Soil Pollut*, 226. Available from: <https://doi.org/10.1007/s11270-015-2537-9>
- EC (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *OJ EU L* 119/103, 2023.
- Gosar M., Šajn R., Bavec Š., Gaberšek M., Pezdir V., Miler M. (2019). Geochemical background and threshold for 47 chemical elements in Slovenian topsoil. *Geologija*, 62(1), 7–59. Available from: <https://doi.org/10.5474/geologija.2019.001>
- Ibrahim E. A., El-Sherbini M. A. A., Selim E. M. (2023). Effects of biochar, zeolite and mycorrhiza inoculation on soil properties, heavy metal availability and cowpea growth in a multi-contaminated soil. *Scientific Reports*, Vol. 13, Article number 6621: 1-10. Available from: <https://doi.org/10.1038/s41598-023-33712-z>
- Kabata-Pendias A., and Mukherjee A. B. (2007). *Trace Elements from Soil to Human*. Berlin, Heidelberg, Germany: Springer-Verlag.
- Keller C., Marchetti M., Rossi L., Lugon-Moulin N. (2005). Reduction of cadmium availability to tobacco (*Nicotiana tabacum*) plants using soil amendments in low cadmium-contaminated agricultural soils: a pot experiment. *Plant and Soil*, Vol. 276: 69-84.
- Li Q., Chang J., Li L., Lin X., Li Y. (2024). Soil amendments alter cadmium distribution and bacterial community structure in paddy soils. *Science of The Total Environment*, Vol- 924. Available from: <https://doi.org/10.1016/j.scitotenv.2024.171399>.
- Li Y., Cui S., Chang S.X., Zhang Q. (2019). Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. *Journal of Soils and Sediments*, 19:1393–1406. Available from: <https://doi.org/10.1007/s11368-018-2120-2>

- Lo K. S. L., Yang W. F., Lin Y. C. (1992). Effects of organic matter on the specific adsorption of heavy metals by soil. *Toxicological & Environmental Chemistry*, Vol. 34: 139-153.
- Lu J., Yang X., Meng X., Wang G., Lin Y., Wang Y., ZHAO F. (2017). Predicting Cadmium Safety Thresholds in Soils Based on Cadmium Uptake by Chinese Cabbage. *Pedosphere*, Vol. 27 (3): 475-481. Available from: [https://doi.org/10.1016/S1002-0160\(17\)60343-6](https://doi.org/10.1016/S1002-0160(17)60343-6)
- NIJZ. (2023). Kadmij v živilih. Available from: <https://nijz.si/moje-okolje/varnost-zivil/kadmij-v-zivilih/>
- Shen B., Wang X., Zhang Y., Zhang M., Wang K., Xie P., Ji H. (2020). The optimum pH and Eh for simultaneously minimizing bioavailable cadmium and arsenic contents in soils under the organic fertilizer application. *Science of The Total Environment*, 711. Available from: <https://doi.org/10.1016/j.scitotenv.2019.135229>.
- Smolnikar E., Žnidaršič Pongrac V., Ugrinović K. (2024). Effects of different soil amendments on Cd content in garlic. 59. hrvatski i 19. međunarodni simpozij agronoma eZbornik radova. Available from: <https://sa.agr.hr/publication/29/59.+hrvatski+i+19.+me%C4%91unarnodni+simpozij+agronoma+eZbornik+radova.Full+text+str.175-182>
- Ugrinović K., Poplašen S., Vičar B., Žnidaršič Pongrac V., Škof M. (2022). Management strategies for the reduction of Cd bioavailability: 4th Workshop Fertilisation and Irrigation, EUVRIN, sep, 2022. Available from: https://vrtnarstvo.javnaslužba.si/wp-content/uploads/2024/05/UGRINOVIC_Management-strategies-for-the-reduction-of-Cd-ss.pptx
- Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (Uradni list RS, št. 68/96, 41/04 – ZVO-1 in 44/22 – ZVO-2). Priloga 1: Meje in kritične imisijske vrednosti snovi v tleh. (1996). Št. 68 – 29. XI: 5774.
- Zhang S., Zhu Q., de Vries W., Ros G. H., Chen X., Muneer M. A., Zhang F., Wu L. (2023). Effects of soil amendments on soil acidity and crop yields in acidic soils: A world-wide meta-analysis. *Journal of Environmental Management*, Vol. 345. Available from: <https://doi.org/10.1016/j.jenvman.2023.118531>

Session 4

Biodiversity Conservation

ORIGINAL SCIENTIFIC PAPER

Seed priming with KNO_3 improves early growth and development of poppy (*Papaver somniferum* L.) sprouts

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Summary

This study aims to test exogenous seed priming using 0.2% and 0.8% KNO_3 , as well as water, for durations of 6, 12, and 24 hours on the germination and sprout development of poppy seedlings. The experiment was set up in 4 replications, each containing 50 seeds in petri dishes. Total germination rate was determined on the 10th day, and also sprout length (root, stem, and total length), and sprout mass. The treatment of poppy seeds with 0.2% and 0.8% KNO_3 during 12 and 24 hours had mostly a negative effect or no significant effect on most of the tested parameters. Based on this study, it can be concluded that the exogenous application of 0.8% KNO_3 during a shorter period of time (6 h) could be used as a possible priming treatment for domestic poppy, in order to improve the early growth and development of sprouts.

Key words: germination rate, root, shoot, priming duration, seed

Introduction

Various traditional recipes with poppy seeds as the main ingredient are known. Opium poppy (*Papaver somniferum* L.), is mostly grown for the seed, which can be used in the household for the preparation of cakes, biscuits, pastries, fillings with poppy seeds, strudels, pies, and tarts (Lachenmeier et al., 2010; Brčić et al., 2016; Popović et al., 2017; Aslan Türker et al., 2021; Červeň et al., 2023; Burić et al., 2023). According to the U.S. Department of Agriculture (2025), whole poppy seeds are rich in protein and dietary fiber, as well as some essential vitamins and minerals, including calcium and magnesium (Table 1). A portion of poppy seeds, which is about 28 g (or three table spoons), covers 35% of Ca and 15% of Mg, the adult recommended daily value (U.S. Department of Agriculture, 2025).

Poppy seed is also a raw material for the production of alkaloids such as morphine, thebaine, codeine, etc. (Sproll et al., 2006; Powers et al., 2018). Edible oil is obtained by cold-pressing poppy seeds (Jevtić et al., 1986; Melo et al., 2022; Varga et al., 2025).

Potassium nitrate (KNO_3), is a white crystalline salt soluble in water. In nature, it is obtained by nitrification of organic compounds with nitrogen in the presence of potassium ions, while technologically it is obtained from sodium nitrate and potassium chloride. KNO_3 is most commonly used as an artificial fertilizer, especially in conditions where a source of highly soluble chloride-free nutrients is needed. All nitrogen is immediately available to the plant for uptake in the form of nitrate, without requiring any additional microbial action. In addition to nitrogen, KNO_3 is also a good source of potassium for plants, so fertilization with it is most often carried out before the vegetation period. A diluted solution is sometimes sprayed onto the leaves of plants (foliar application) to stimulate physiological processes or to overcome nutrient deficiencies.

Seed priming with potassium nitrate (KNO_3) is a widely used method to enhance seed germination by breaking seed dormancy and promoting better seedling growth. The exogenous application of priming agents to seeds plays a crucial role in facilitating the pre-sowing phases of germination. Moaaz et al. (2020) showed that seed treatment of two tomato cultivars with

0.75% KNO₃ had a positive effect on the germination and vitality of seedlings, as well as on the physiological and biochemical properties of the plant. Additionally, prior research (Ruttanaruangboworn et al., 2017; Mebratu, 2022; Zrig et al., 2023), has highlighted the beneficial effects of potassium nitrate (KNO₃) as a seed priming agent, promoting the seedling establishment and enhancing vigor.

Table 1. Nutrients of 100 grams (g) of raw, whole poppy seeds (U.S. Department of Agriculture (2025))

Nutrient	Amount
Protein	21.43 g
Total lipid (fat)	39.29 g
Carbohydrate	28.57 g
Fiber	25 g
Sugars	3.57 g
Calcium	1,250 mg
Iron	9.64 mg
Magnesium	357 mg
Zinc	8.04 mg
Fatty acids, total saturated	5.36 g

This study aimed to investigate the influence of water (hydropriming) and exogenous KNO₃ application in relation to different concentrations and durations on the germination and initial growth of domestic poppy seedlings.

Material and methods

In this study, seeds of a domestic poppy population were collected in 2021 in Podgrađe (Eastern Croatia), which was introduced into the CPGRD database (Croatian plant genetic resources database, CPGRD, <https://cpgrd.hapih.hr/>) with accession number IND00096.

The seed priming was conducted in plastic test tubes by soaking the seeds in: a) distilled water (hydropriming), and b) 0.2% and 0.8% aqueous solutions of potassium nitrate (KNO₃), produced by KEMIKA (Zagreb, Croatia), for durations of 6, 12, and 24 hours. The control treatment was done without priming. The basic sample size was 50 seeds per replication, and each treatment was performed in four replicates.

After soaking, the seeds were washed with tap water, and at the end with distilled water. After washing, the seeds were dried at room temperature for 48 hours. The dried seeds were germinated in Petri dishes $\phi=90$ mm on a filter paper substrate moistened with 3 mL of distilled water. Each Petri dish was wrapped in a plastic bag to prevent water evaporation during germination. The seeds were germinated in an ARALAB FitoClima 600 climate chamber (Rio de Mouro, Portugal), at a constant temperature of 20 °C and 50% relative humidity, without light. Seed germination, seedling weight, and morphological parameters (length of radicle, stem, and total seedling length) were measured after 10 days. A KERN ABP 200- 5DM analytical balance (Balingen, Germany) was used to weigh the seedlings, while statistical data processing was performed in the SAS Enterprise Guide 7.1 software package (SAS Institute Inc., Cary, North Carolina, USA).

Results and discussion

The germination rate was the highest without KNO₃ seed priming (Table 2). The highest germination rate was on the treatment with water soaking for 12 h (65.0%) and after that on the control treatment (61.5%), without seed priming. The lowest germination rate was with soaking poppy seeds for 12 h and 0.2% KNO₃ (40.5%).

Seed treatment with 0.2% KNO₃ at all treatments negatively influenced the stem length of poppy sprouts (Table 2). The highest average stem length was observed under conditions when seeds were treated with 0.8% KNO₃ for 6 h (4.14 cm), even though there were no significant differences compared to the 6 h soaking in water (4.05 cm). Treatment of domestic poppy seeds for 12 and 24 hours with both concentrations of KNO₃ had a significant negative effect on stem length compared to untreated seeds and water treatment (Table 2).

Table 2. Parameters of poppy sprouts of the experiment

Treatments		Germination rate	Stem length	Root length	Total length	Sprouts weight
		(%)		(cm)		(mg)
Control		61.50±8.54 A	3.72±0.33 BC	1.07±0.28 BC	4.79±0.52 C	6.42±0.78 BCD
Soaking 6 h	water	47.50±4.43 B	4.05±0.39 A	1.12±0.41 B	5.17±0.63 B	7.15±0.34 AB
	0.2% KNO ₃	43.00±2.58 B	2.29±0.37 H	0.32±0.11 F	2.61±0.40 I	5.04±0.53 EF
	0.8% KNO ₃	60.50±2.52 A	4.14±0.39 A	1.33±0.41 A	5.47±0.66 A	7.41±0.73 A
Soaking 12 h	water	65.00±3.46 A	3.83±0.37 B	1.31±0.56 A	5.14±0.78 B	6.97±0.82 ABC
	0.2% KNO ₃	40.50±1.00 B	2.64±0.47 G	0.82±0.26 D	3.46±0.66 H	4.35±0.16 F
	0.8% KNO ₃	47.50±4.12 B	3.52±0.50 D	0.97±0.30 C	4.49±0.60 D	6.22±0.41 CD
Soaking 24 h	water	46.00±5.88 B	3.62±0.32 CD	0.67±0.22 E	4.29±0.46 E	6.31±0.31 CD
	0.2% KNO ₃	57.50±5.26 A	3.00±0.48 F	1.00±0.28 C	4.00±0.67 F	5.69±0.46 DE
	0.8% KNO ₃	58.00±9.09 A	3.32±0.52 E	0.37±0.17 F	3.69±0.58 G	5.26±0.72 E

Means denoted by a different letter indicate significant differences between treatments ($p \leq 0.001$)

A significant positive effect on root length was observed in seeds treated for 6 hours with 0.8% KNO₃ compared to seeds treated with a lower concentration of KNO₃, seeds without treatment, and seeds treated with water (Table 2). A negative effect on root length was observed in seeds treated for 12 hours with 0.2% and 0.8% KNO₃ compared to seeds soaked in water for the same period. The highest value of average root length (1.33 cm) was determined in seeds treated for 6 hours in 0.8% KNO₃. The highest average weight of poppy sprouts (7.41 mg) was found in the treatment of soaking for 6 hours in 0.8% KNO₃.

Generally, the results of the present study indicate different responses to poppy sprouts development when soaking in KNO₃, but a very good response to soaking seeds only in water. Based on the regression analysis, a very significant relationship was determined between root and stem length, and it was determined that for each centimeter increment of sprout root length, the stem length increased by 0.36 cm (Figure 1).

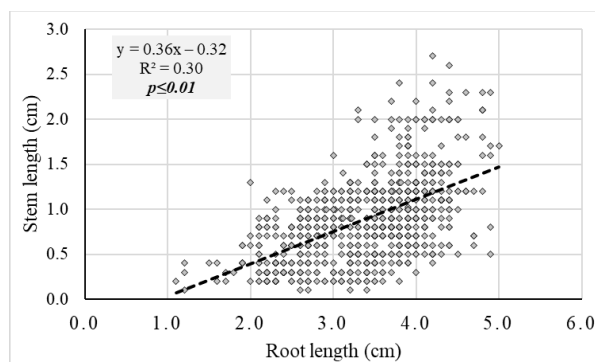


Figure 1. Regression analysis of poppy root and stem length (cm) (N=800)

Using KNO_3 of different concentrations (0.2, 0.4, and 0.6%) for 24 h, pretreatment of 0.4% KNO_3 soaking enhanced the seed total germination rate and germination index of holy basil (*Ocimum tenuiflorum*) as it is reported by Thongtip et al. (2022). For carrot seeds, Mahmood ur Rehman et al. (2024) found that in different treatments of KNO_3 seed priming (from 50 to 300) for 12 h, the seed priming with 50 mM of KNO_3 significantly enhanced seed germination (36%) compared to the control and that with increasing level of KNO_3 , seed germination, growth, and root biomass are reduced.

Conclusions

The study found that treatments of domestic poppy seeds with KNO_3 had a very significant ($p \leq 0.001$) effect on most of the tested parameters. Under conditions of seed soaking for 6 h, the best treatment was 0.8% KNO_3 , which had a positive effect on the length of the stem and radicle, and the total length and mass of the poppy sprouts, while it had no significant effect on the total germination rate. Under the same soaking conditions, seeds treated with a lower concentration of KNO_3 decreased all parameters tested compared to seeds without treatment. Exogenous application of 0.2% and 0.8% KNO_3 for 12 and 24 hours had a mainly negative effect or had no significant effect on most of the parameters tested. Based on the results of the study, it can be concluded that exogenous application of 0.8% KNO_3 for a shorter period could be used as a possible treatment of domestic poppy seeds to improve early growth and development of poppy sprouts.

References

- Aslan Türker D., Göksel Saraç M., Yetiman A. E., Doğan M. (2021). Interfacial properties of poppy seed protein (*Papaver somniferum* L.) as an alternative protein source at oil/water interface: influence of pH on stability, morphology and rheology. *European Food Research and Technology*. 247: 2545-2556.
- Brčić M., Pospíšil M., Pospíšil A., Butorac J., Škevin D., Obranović M. (2016). The agronomic traits of foreign cultivars and domestic populations of oilseed poppy. *Poljoprivreda*. 22 (2): 23-28.
- Burić M., Popović V., Iličković I., Bošković J., Petrović B., Vukomanović P, Šarčević Todosijević Lj. (2023). Challenges and possibilities of using *Papaver somniferum* L. in the food industry and toxicology. 13 Kongres udruženja Toksikologa Srbije, 1 Regionalna ToxSEE Konferencija, Sadašnjost i Budućnost Toksikologije: Izazovi i Prilike. 10-12.5.2023. Beograd, ISBN-978-86-917867-3-1. P.471. P. 319- 322
- CPGRD (2025). <https://cpgrd.hapih.hr/>
- Červen J., Vrbovský V., Horáček J., Bartas M., Endlová L., Pečinka P., Čurn V. (2023). New Low Morphine Opium Poppy Genotype Obtained by TILLING Approach. *Plants*. 12: 1077.

- Jevtić S., Milošević R., Šuput M., Mustapić Z., Uzunoski M., Gotlin J., Đorđevski J., Španring J., Miletić N. (1986). Posebno ratarstvo 2. IRO „Naučna knjiga“ Beograd, Republika Srbija.
- Lachenmeier D. W., Sproll C., Musshoff F. (2010). Poppy seed foods and opiate drug testing-where are we today?. *Therapeutic Drug Monitoring*. 32 (1): 11-18.
- Mahmood ur Rehman M., Liu J., Nijabat A., Alsudays I. M., Saleh M. A., Alamer K. H., Attia H., Ziaf K., Zaman Q., Amjad M. (2024). Seed priming with potassium nitrate alleviates the high temperature stress by modulating growth and antioxidant potential in carrot seeds and seedlings. *BMC Plant Biology*. 24 (1): 606.
- Mebratu A. (2022). Potassium Nitrate Priming Effect on the Germination of Tomato (*Lycopersicum esculentum*. Mill) cvs.“Mersa” and “Tekeze-1”. *International Journal of Agronomy*. 2022 (1): 4970107.
- Melo D., Álvarez-Ortí M., Nunes M. A., Espírito Santo L., Machado S., Pardo J. E., Oliveira M. B. P. (2022). Nutritional and chemical characterization of poppy seeds, cold-pressed oil, and cake: Poppy cake as a high-fibre and high-protein ingredient for novel food production. *Foods*. 11 (19): 3027.
- Popović V., Marjanović Jeromela A., Glamočlija Đ., Maksimović L., Ćurčić Ž., Kiprovska B., Jakšić S. (2017). Phenotypic characterization of the Serbian poppy (*Papaver somniferum* L.) population. COST WG1 / EPPN2020 workshop: Current and future applications of phenotyping for plant breeding, Institute of Field and Vegetable Crops, 29-30.9.2017. Novi Sad, ISBN 978-86-80417-77-6. p. 36-37.
- Powers D., Erickson S., Swortwood M. J. (2018). Quantification of Morphine, Codeine, and Thebaine in Home-Brewed Poppy Seed Tea by LC-MS/MS. *J Forensic Sci*. 63 (4): 1229-1235.
- Ruttanaruangboworn A., Chanprasert W., Tobunluepop P., Onwimol D. (2017). Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*. 16 (3): 605-613.
- SAS Institute Inc. Cary, North Carolina, USA
- Sproll C., Perz, R.C., Lachenmeier, D.W. (2006). Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. *J Agric Food Chem*. 54 (15): 5292-5298.
- Thongtip A., Mosaleeyanon K., Korinsak S., Toojinda T., Darwell C. T., Chutimanukul P., & Chutimanukul P. (2022). Promotion of seed germination and early plant growth by KNO₃ and light spectra in *Ocimum tenuiflorum* using a plant factory. *Scientific Reports*. 12 (1): 6995.
- U.S. Department of Agriculture, 2025, <https://fdc.nal.usda.gov/fdc-app#/food-details/375647/nutrients>, Accessed on 15 March 2025
- Varga I., Moslavac T., Flanjak I., Iljkić D., Pospišil M., Lončarić Z., Antunović M. (2025). White-Seeded Culinary Poppy (*Papaver somniferum* L.) Se Biofortification: Oil Quality, Fatty Acid Profile, and Seed Yield. *Plants*. 14: 95.
- Zrig A., Yousif Sidahmed Elsheikh S., Hamouda F., Najar B., A. Alsherif E., Magdy Korany S., Hassan A.H., AbdElgawad H. (2023). Potassium nitrate and ascorbic acid priming improved tissue chemical composition and antioxidant and antimicrobial activities of linseed (*Linum usitatissimum* L.) sprouts. *ACS omega*. 8 (39): 35975-35987.

PRELIMINARY COMMUNICATION

Preliminary research of the reproductive ability of foxes in the area of Karlovac and Zagreb counties

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Summary

Reproductive ability of the red fox (*Vulpes vulpes* L.) in Karlovac and Zagreb counties was analyzed with a focus on the fetal mass and age structure within populations. Forty-four females were sampled from January to March, 2024. The results showed the difference in fetal mass with male fetuses being heavier than female ones. This suggests possible different developmental and ecological strategies between sexes which may be related to adaptive advantages in habitats with variable resource availability. A higher representation of older individuals within the population was found compared to previous studies indicating population stability in the studied area. This research contributes to a better understanding of the reproductive biology of the red fox and provides a foundation for future studies of this species.

Key words: Red fox (*Vulpes vulpes* L.), reproductive ability, fetal mass, age structure

Introduction

The red fox (*Vulpes vulpes* L.) is known for its extraordinary adaptability to different habitats (Lloyd, 1980). It's inhabiting almost the entire European continent and is present in urban, rural and forest areas (Dumić, 2016; Zecchin et al., 2019). This species plays a key role as a predator in different ecosystems, and its presence and behavior can significantly affect the structure of populations of other species within these systems (Dumić, 2011; Lloyd, 1980).

Through detailed research into the reproductive characteristics of the red fox, it is possible to better understand the dynamics of its spread and survival, as well as the impact that this species has on the ecosystems it inhabits.

The aim of this study is to investigate the reproductive ability of red fox populations in Karlovac and Zagreb counties, with a special emphasis on the mass of fetuses and the age structure of the population. The thesis of the paper starts from the assumption that there are significant differences in the mass of fetuses between males and females, as well as differences in the age structure that may indicate population stability.

The research area includes the regions of Karlovac and Zagreb counties, which are characterized by diverse habitats suitable for the red fox. This area provides ideal conditions for research, given the richness of forest, rural and urban ecosystems that foxes inhabit. This diversity allows for the comparison of different populations within specific ecological conditions, which further contributes to the quality and relevance of the results obtained.

This research provides a deeper insight into the reproductive biology of the red fox in the local context, thereby contributing to a broader understanding of the population dynamics of this species and their ecological significance.

Material and methods

For the purposes of the research, 44 female foxes (*Vulpes vulpes* L.) were collected from the Karlovac and Zagreb counties in the period from January 1st to March 31st, 2024. The samples were collected as part of the regular fox cull and as a result of traffic collisions. Sex of the each individual was determined by visual inspection by hunters immediately after the cull. After collection, the weight of each fox was measured accurately in dekagrams using a scale manufactured by "WeiHeng".

An incision was made in the lower abdomen, in line with the midline of the body known as the white line or Linea alba. This approach allows the easiest examination of the internal organs. The uterus and ovaries were examined through the incision and the fertility of the fox was determined by visual inspection by recognizing the presence of fetuses or corpus luteum. If fetuses were present, first they were counted and then removed from the amniotic sacs with a single cut so that they could be analyzed in more detail.

After the fetuses were removed from the amniotic sacs, the first step was to visually determine whether the sex and weight of the fetus could be determined. Due to the fact that some fetuses were in an early stage of development, it was not possible to determine their gender or measure their mass, as the fetus could not be separated from the amniotic fluid without damage. In such cases, only the number of fetuses was determined. When the fetuses were in a later stage of development, their sex was determined and then they were weighed using a scale manufactured by "First" with an accuracy of one gram.

Also, the age of foxes was estimated based on the replacement of deciduous teeth with permanent teeth and the degree of tooth wear, according to the methods described by Andera and Roulichova (2007). Based on this, foxes were classified into three age categories: foxes up to 1 year old, foxes between 1 and 2 years old and foxes older than 2 years.

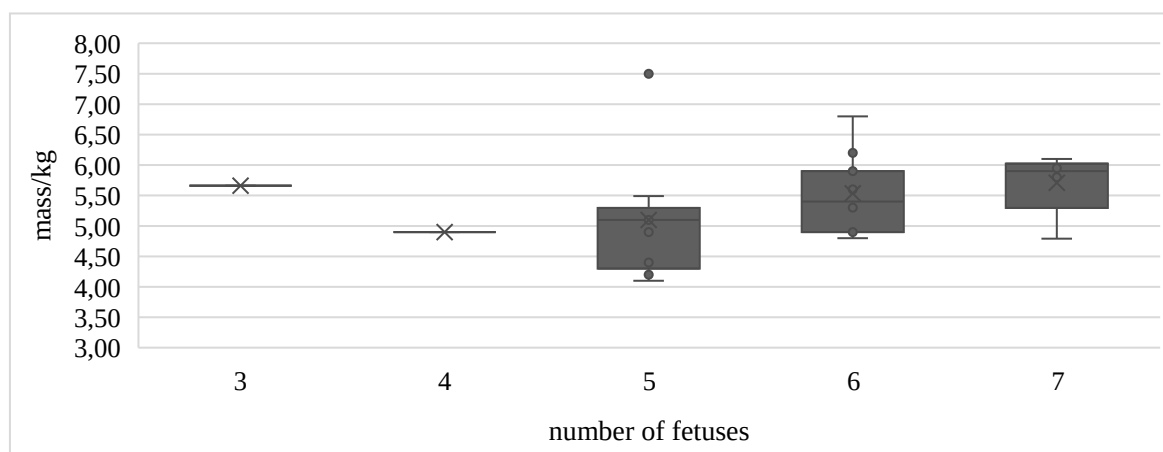
Results and discussion

Of the total of 44 foxes, 9 were collected from the Karlovac County area, while the remaining 35 were collected from the Zagreb County area. There were 5 foxes in the age group up to one year old, 26 foxes in the age group between 1 and 2 years old, and 13 foxes in the age group older than 2 years old. The smallest recorded mass was 3.7 kg, and the largest 7.5 kg. In foxes up to one year old, the mass varies from 3.7 kg to 5.6 kg, while the median is 4.5 kg. In the group of foxes aged 1 - 2 years, the mass ranges from 4.0 kg to 6.2 kg, with the exception of one individual reaching 7.5 kg. The median is 5.16 kg. In foxes older than 2 years, the mass range is from 4.3 kg to 6.8 kg, with a median of 5.44 kg. These data clearly indicate the expected trend of increasing mass of foxes with their age. According to Janicki (2004), the body mass of foxes in Croatia usually ranges between 6 and 8 kg, with the possibility of reaching up to 10 kg. This range overlaps somewhat with the findings from this study, especially in relation to the largest recorded mass. However, the average mass of foxes in this study is at the lower end of this range, which may indicate regional differences within Croatia or differences in sampling methods. Namely, previous studies included sampling of males and females throughout the year, while samples in this study were collected exclusively from January to March. In comparison with international data, Storm et al. (1976) state that in North America the heaviest male and female in their study weighed 7.05 kg and 5.81 kg, which is comparable to the masses recorded in this study. In Europe, data from studies in Great Britain (Hattingh, 1956; Southern, 1965; Fairley, 1970) show that adult males weigh an average of between 6 and 7 kg, while females have an average mass of between 5 and 6 kg. These results are also similar to the data from this study, with the upper limit of recorded mass in Croatian foxes being close to the highest recorded masses in Great Britain. Cavallini (1995) reports a mean mass of 5.45 kg $\pm$ 0.95 kg for foxes in Central Italy, which is comparable to the mean values from this study.

Similarly, Nowak (1999) records a median mass of 5.3 kg with a range of 3.6 to 6.5 kg, which also fits within the range of masses from this study. Taking all these data into account, it can be concluded that the masses of foxes in this study, which were conducted in the Karlovac and Zagreb counties, are generally consistent with those recorded in similar European studies, but differ from some North American studies. These differences may be due to geographical and ecological factors, including food availability, climatic conditions and seasonal variations, as well as differences in survey and sampling methods.

Of the 44 examined, 27 foxes had fetuses present. One fox had 3 fetuses, and its mass was 5.66 kg. A fox with 4 fetuses was also recorded, whose mass was 4.9 kg. Nine foxes had 5 fetuses, with masses varying between 4.1 kg and 7.5 kg, with a median of 5.1 kg. In addition, 11 foxes were recorded carrying 6 fetuses, with the smallest individual weighing 4.8 kg and the largest 6.8 kg. The median was 5.4 kg. In the end, 5 foxes with 7 fetuses were recorded, with the mass ranging from the smallest 4.79 kg to the largest 6.10 kg, while the median was 5.9 kg (Graph 1).

Similarly, Cavallini and Santini (1996) also showed that foxes with more placental scars and more fetuses had higher body mass. Their results showed that females with more fetuses (>4) were heavier (5.38 ± 0.61 kg) than females with fewer fetuses (<4 , 4.86 ± 0.55 kg), confirming the association between mass and reproductive success. These data indicate a certain trend of association between the number of fetuses and the weight of the fox. Heavier foxes generally carried more fetuses, which may indicate a relationship between body mass and reproductive capacity and fitness of the individual. However, it is important to note that body mass is not the only factor influencing the number of fetuses. Although there is a relationship between mass and number of fetuses, other factors such as food availability, health of the individual, stress, and seasonal and hunting ground variations should be taken into account, all of which can affect fecundity.



Graph 1. The relationship between the mass of foxes and the number of fetuses

In January, 25% of foxes, or 2 out of 8 collected individuals, had fetuses in the uterus. This relatively low percentage suggests that the reproductive season has just begun. In February, there was a significant increase in the number of fetuses present. As many as 50% of foxes, or 11 out of 22 collected individuals, had fetuses, which indicates an intensification of reproductive activities. In addition, in 27% of foxes, which is an additional 6 out of 22 individuals, a corpus luteum was observed, which also confirms their reproductive ability and preparation for pregnancy. March was marked by full reproductive activity, as all foxes collected in that month were pregnant. All samples, or more precisely 14 out of 14 foxes, contained fetuses in the uterus, which indicates the peak of the reproductive cycle.

On the other hand, the study by Ruetten and Albaret (2011) in western France shows a somewhat different pattern. Although reproduction also begins in February, the number of uteri with placental scars was relatively low in that month. This is explained by the fact that many foxes were just at the beginning of a new pregnancy, so placental scars could not be identified. These differences between this study and that of Ruetten and Albaret (2011) can be explained by regional variations in climatic conditions, which affect the onset and intensity of the fox reproductive season. In Croatia, reproduction intensifies earlier, while in western France reproductive activity may start somewhat later, depending on local conditions. In both cases, February and March are key months for fox reproductive activity, but differences in the timing of placental scar formation indicate that regional factors can significantly influence reproductive dynamics.

Among the 5 foxes collected up to one year old, fetuses were found in one and corpus luteum was observed in the other. This means that 20% of foxes up to one year old had fetuses, while an additional 20% showed the presence of corpus luteum. When these two groups are added together, we arrive at the data that 40% of foxes up to one year old showed signs of reproductive capacity. In foxes aged 1 - 2 years, the sample consists of 26 individuals. In 20 foxes fetuses were found, while in 3 foxes a corpus luteum was observed. This means that 77% of foxes aged 1 - 2 years had fetuses, and 12% showed the presence of corpus luteum, which in total makes a high 89% of foxes aged 1 - 2 years old that are reproductively capable.

Foxes older than 2 years make up a group of 13 individuals. In 6 foxes, fetuses were found, while in 2 foxes, a corpus luteum was found. These data show that 46% of foxes older than 2 years had fetuses, and 15% had a corpus luteum, which makes a total of 61% of foxes older than 2 years that are reproductively capable. These data clearly show that reproductive capacity increases with age, reaching a peak in foxes aged 1 - 2 years, while in individuals older than 2 years there is a slight decline. However, a significant proportion of foxes from all age groups show reproductive capacity, which is crucial for the maintenance and growth of the fox population in the observed areas. These results are consistent with the literature that suggests that young foxes, especially one-year-olds, have lower reproductive activity due to physiological immaturity. Ruetten and Albaret (2011) reported that one-year-old foxes produce fewer offspring compared to adults, confirming that young foxes are just entering the reproductive phase and therefore have lower reproductive success. Ruetten and Albaret (2011) emphasized that foxes aged 1 to 2 years have the highest reproductive capacity due to reaching physiological maturity and optimal health. The high reproductive capacity in this age group can be explained by the fact that these foxes are at the peak of their physiological maturity and health, while not yet affected by aging, which reduces reproductive capacity.

A total of 83 fetuses were collected, of which 40 were male, which makes up 48% of all fetuses, while 43 were female, which represents 52% of the total sample. For male fetuses, the lowest recorded mass was 9 g, while the highest reached 106 g, with a median of 43.73 g. In comparison, for female fetuses, the lowest mass was 8 g, while the highest recorded mass was 98 g, with a median of 40.36 g. From these data, it can be concluded that male fetuses are on average slightly larger than female fetuses, which may indicate different developmental dynamics of male and female fetuses.

Conclusions

The results showed that there are clear differences in fetal mass between the sexes, with male fetuses being heavier than female fetuses on average. These differences may be related to different physiological and developmental-biological needs of males and females, which is in line with theories of sexual dimorphism in mammals. Comparison with previous studies, such as Ćirko (2017), further supports the hypothesis of population stability in this area. While

previous studies indicated a higher presence of younger individuals, which may be a consequence of different seasonal influences or variability in sampling, this study suggests that the current red fox population in Karlovac and Zagreb counties shows long-term signs of stability and adaptability. The results of this study have important implications for future research and management of red fox populations in Croatia. Understanding sex and age structures within populations is crucial for proper fox management, especially in the context of increasing anthropogenic pressures and habitat changes. It is recommended to continue research with a focus on long-term monitoring of populations, including analysis of seasonal variations and changes in resource availability. In conclusion, this research provides a basis for further research on the reproductive ability of the red fox.

References

- Andera, M. and Roulichova, J. (2007). Age determination in the Red Fox (*Vulpes vulpes*): a comparative study. Praha: Department of Zoology, National Museum (Natural History).
- Cavallini, P. (1995). Variation in the body size of the red fox. *Annales Zoologici Fennici*, 32, 421-427.
- Cavallini, P. and Santini, S. (1996). Reproduction of the red fox *Vulpes vulpes* in Central Italy. *Annales Zoologici Fennici*, 33, 267-274.
- Čirko, I. (2017). Neke populacijske značajke lisice (*Vulpes vulpes* L.) na području južnog dijela Medvednice, diplomski rad. Zagreb: Šumarski fakultet Sveučilišta u Zagrebu.
- Dumić, T. (2011). Do kad ćemo uzgajati lisice? *Lovački vjesnik*, 120 (4): 26-27.
- Dumić, T. (2016). Lisica. *Lovački vjesnik*, 125 (1-2): 40-50.
- Fairley, J. (1970). The Food, Reproduction, Form, Growth and Development of the Fox *Vulpes vulpes* (L.) in North-East Ireland. *Proceedings of the Royal Irish Academy*, 70, 103-137.
- Hattingh, I. (1956). Measurements of foxes from Scotland and England. *Proceedings of the Zoological Society of London*, 126, 191-199.
- Janicki, Z. (2004). Lisica (*Vulpes vulpes* L.). In *Lovstvo*, Mustapić, Z (ed.), 111-115. Zagreb, Croatia: Hrvatski lovački savez.
- Lloyd, G. (1980). The Red Fox. London: B. T. Batsford LTD.
- Nowak, R. (1999). *Vulpes vulpes* (red fox). In *Walker's Mammals of the World*, Nowak, R. (ed.), 635-639. Baltimore, USA: The Johns Hopkins University Press.
- Ruette, S. and Albaret, M. (2011). Reproduction of the red fox *Vulpes vulpes* in western France: does staining improve estimation of litter size from placental scar counts? *European Journal of Wildlife Research*, 57, 555-564.
- Southern, S. (1965). The handbook of British mammals, 465-466. Oxford, Great Britain: Blackwell.
- Storm, G. I., Andrews, R. D., Phillips, R. L., Bishop, R. A., Siniff, D. B., Tester, J. R. (1976). Morphology, Reproduction, Dispersal and Mortality of Midwestern Red Fox Populations. *Wildlife Monographs* 49:(3-82).
- Zecchin, B., De Nardi, M., Nouvellet, P., Vernesi, C., Babbucci, M., Crestanello B., Milani, A. (2019). Genetic and spatial characterization of the red fox (*Vulpes vulpes*) population in the area stretching between the Eastern and Dinaric Alps and its relationship with rabies and canine distemper dynamics. *PLOS ONE*, 14(4), 1-16.

ORIGINAL SCIENTIFIC PAPER

Distribution of plant-parasitic nematodes in olive orchards treated with *Trichoderma atroviride*

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Summary

Trichoderma spp. are free-living fungi inhabiting soil and root ecosystems, widely used as biological control agents against plant-parasitic nematodes and other pathogens. A field trial was conducted at two locations using four olive varieties and four replications. Treatments included a control (C), and *T. atroviride* at 10⁶ (106) and 10⁸ (108) spores/mL. The aim was to assess effect of treatments on nematode genera. In I. bjelica variety, the Tukey HSD test ($p \leq 0.05$) confirmed a significant 23% reduction in *Rotylenchus* at higher dose, while no significant effect was observed for the dominant genus *Helicotylenchus*. *T. atroviride* has a positive effect on the reduction of plant-parasitic nematodes in olive orchards.

Key words: biological control, nematodes, olive varieties, plant health, *Trichoderma*

Introduction

The fungi of genus *Trichoderma* belong to cosmopolitan species, which means they are widely spread and can be found in various ecological areas around the world (Harman et al. 2004.). Strains of *Trichoderma* are collected worldwide, with 320 strains identified across 71 species (Braithwaite et al. 2017.). *Trichoderma* genus inhabits soil, plants, trees and various organic materials where they act as saprophytes or parasites. Some species of *Trichoderma* genus are also used in biological plant protection due to their ability to suppress pathogenic fungi (Singh et al. 2014.). In addition, *T. atroviride* enhances nitrogen and phosphorus availability, thereby improving soil nutrient composition (Liu et al. 2023.). The *Trichoderma* genus has significant potential as a biological control agent against plant-parasitic nematodes such as root knot nematodes and cyst-forming nematodes through direct parasitism of eggs and larvae. By increasing enzymes, such as chitinase and protease enables fungus to penetrate nematode eggs, by softening egg shell which results in reduction of eggs that are capable of hatching (Poveda et al. 2020.). Effectiveness of biopesticides made from *Trichoderma* species depends on various environmental factors such as location, temperature, humidity, and nutrient levels (Mukherjee et al. 2012.).

Material and methods

Experiment was set up at two different locations, in Nadin (Zadar County) and Vodnjan (Istria County). In Nadin there were two olive varieties that were used for experiment: *Oblica* and *Lastovka*, and in Vodnjan also two olive varieties: *Istarska bjelica* and *Buža*. Both locations were using principles of ecological farming. This experiment included three treatments, each with four replications; control (C), lower dose of *T. atroviride* spores at a concentration of 10⁶ spores/mL (106), higher dose of *T. atroviride* spores at a concentration of 10⁸ spores/mL (108). *Trichoderma* preparation was isolated from soil near Benkovac and Nadin, and applied a total

of five times (2022–2024), twice annually (in spring and autumn), at a rate of 2 liters per tree within a 1-meter radius around the olive tree canopy. Control group received water only, also applied twice per year. Each replicate consisted of 20 trees. Experimental material consists of soil samples for nematological and agrochemical analysis, collected from experimental plots. Soil samples for nematological analysis were taken with a probe at a depth from 0 to 30 cm. Extraction of nematodes was performed at a Nematology Laboratory, Faculty of Agrobiotechnical Sciences Osijek, using the method of Baermann funnel (Baermann 1917., Bezooijen 2006.). Afterward, nematodes were counted using a stereozoom magnifying glass and identified to genus level with a stereozoom microscope. Analysis included the distribution of plant-parasitic nematodes based on the classifications of Bongers (1994), Mai and Lyon (1975), and relevant nematological literature.

Results and discussion

The first figure illustrate distribution within genera of plant-parasitic nematodes across treatments, presenting data for olive varieties Lastovka and Oblica.

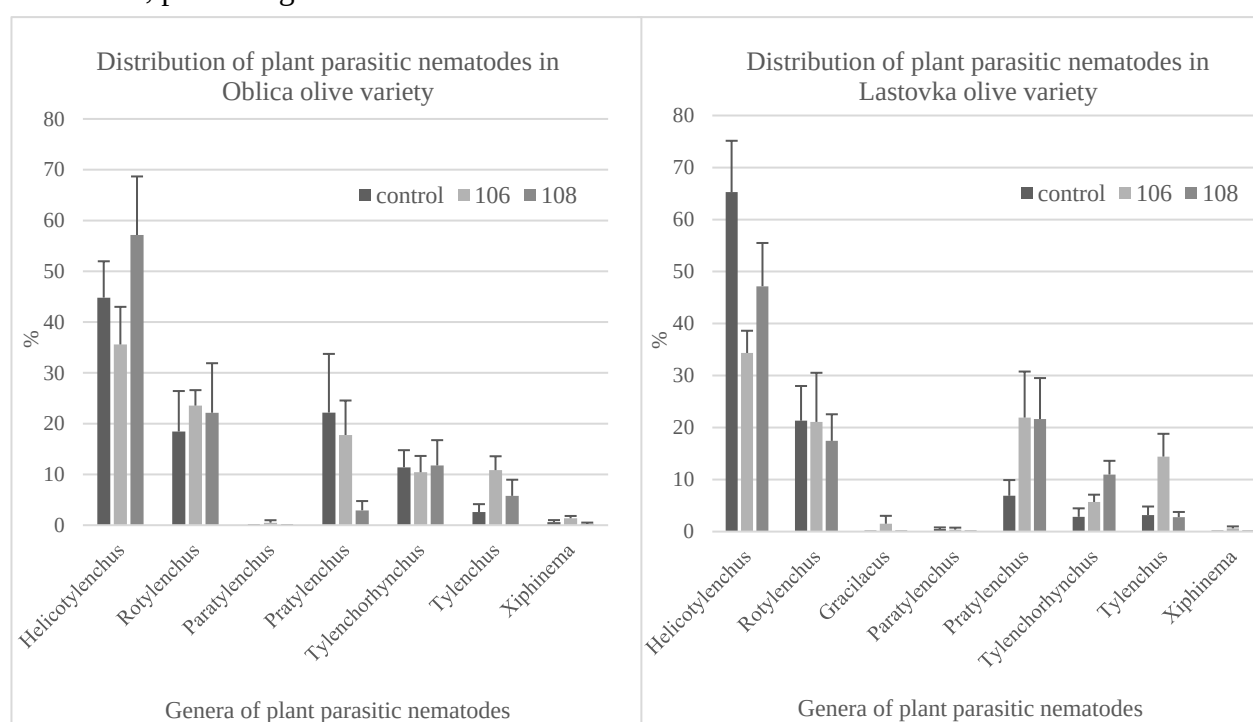


Figure 1. Percentage of plant parasitic nematodes in Nadin experimental field under different treatments. Olive varieties: Oblica (a) and Lastovka (b).

In Nadin experimental field, in Oblica variety, genus *Pratylenchus* exhibited a reduction under influence of treatment relative to the control, with a slight reduction in abundance of 4% at lower dose (10^6 spores/mL) of *T. atroviride* and 19% at higher dose (10^8 spores/mL) of *T. atroviride*. In Lastovka variety, genus *Rotylenchus* exhibited a slight reduction of 4% at higher dose of 10^8 spores/mL of *T. atroviride*. In both varieties, genus *Helicotylenchus* remained dominant, showing no statistically significant differences between treatments. Moreover, none of the plant-parasitic genera in Nadin exhibited statistically significant differences between treatments and the control, as determined by the Tukey HSD test ($p \leq 0.05$). Additionally, genus *Gracilacus* was not detected in Oblica variety.

Figure 2. illustrate distribution within genera of plant-parasitic nematodes across treatments, presenting data for olive varieties Istarska bjelica and Buža.

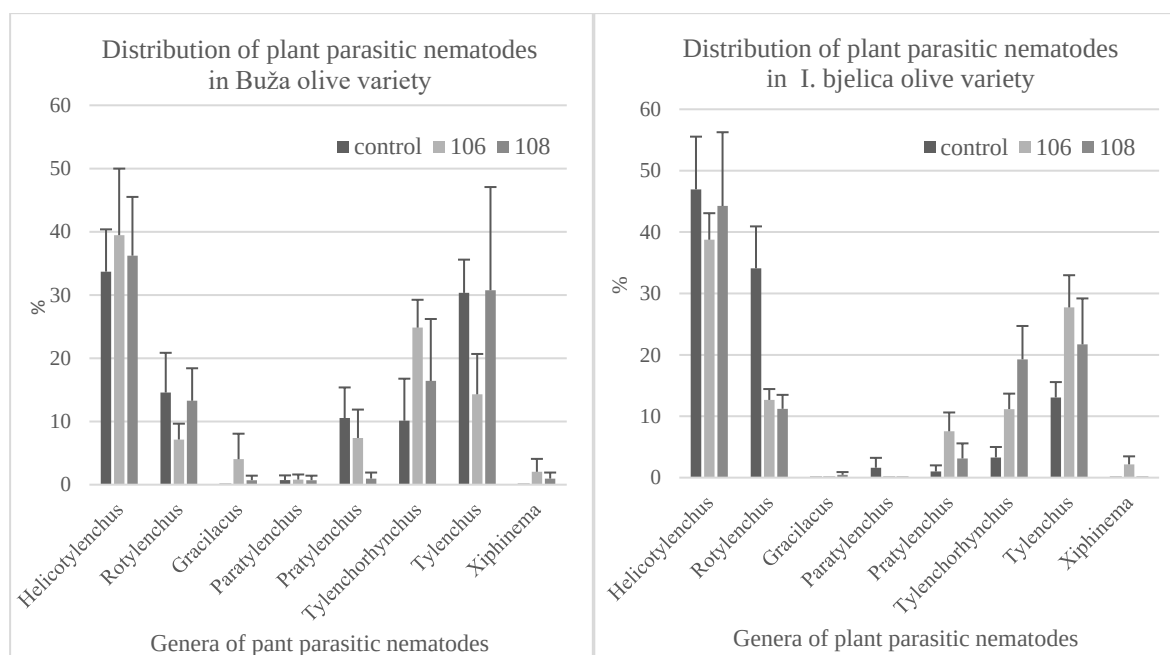


Figure 2. Percentage of plant parasitic nematodes in Vodnjan experimental field under different treatments. Olive varieties: I. bjelica (a) and Buža (b).

In Vodnjan experimental field, in Istarska bjelica variety, genus *Rotylenchus* showed notable and statistically significant reduction in abundance compared to control by 21% at lower dose (10^6 spores/mL) *T. atroviride* and 23% at the higher dose (10^8 spores/mL) *T. atroviride*. In Buža variety, at lower dose (10^6 spores/mL) of *T. atroviride*, genus *Tylenchus* showed a reduction of 16%, while genus *Rotylenchus* reduction by 6% compared to control, respectively. At higher dose (10^8 spores/mL) *T. atroviride*, a 10% reduction in abundance compared to control was recorded in genus *Pratylenchus*. In both varieties, genus *Helicotylenchus* remained dominant, showing no statistically significant differences between treatments. Moreover, with exception of genus *Rotylenchus* in I. bjelica, none of plant-parasitic genera in Vodnjan exhibited statistically significant differences between treatments and the control, as determined by the Tukey HSD test ($p \leq 0.05$).

Available literature provides no data on effects of fungi *Trichoderma* genus on plant-parasitic nematode genera examined in this study, whereas its efficacy against root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Globodera* spp.) is well established and documented. Reduction in egg production of the root-knot nematode *Meloidogyne arenaria* was observed by Windham et al. (1989) following treatment with *Trichoderma harzianum* and *Trichoderma koningii*. However, according to Meyer et al. (2000), a *Trichoderma virens* culture, which did not affect *Meloidogyne incognita* population levels, but did inhibit egg hatching and reduced the mobility of second-stage juveniles (J2). Similarly, Contina et al. (2017) utilized *Trichoderma harzianum* to investigate its interaction with *Globodera pallida* and confirmed a reduction in infection and reproduction of cyst nematodes, demonstrating the fungus's ability to affect both cysts and infective second-stage juveniles (J2).

Conclusions

Interactions between nematodes and *Trichoderma* fungi have a positive impact on reduction of plant-parasitic nematodes in olive orchards. The dominance of plant-parasitic nematode genus *Helicotylenchus* is noticeable in all four olive varieties. In Istarska bjelica, treatments significantly reduced genus *Rotylenchus*. In Buža, treatment with lower dose of *T. atroviride*

reduced abundance of genera *Tylenchus* and *Rotylenchus*, while higher dose reduced the genus *Pratylenchus*. These results suggest a stronger effect of the treatment at Vodnjan experimental field compared to Nadin, where differences are observed only at higher dose in genus *Pratylenchus*. The high standard error reflects the natural variability of nematodes in soil, which may hinder ability to draw firm conclusions for certain genera. Overall, it is shown that introduction of *Trichoderma* into agriculture system has improved the productivity and sustainability of olive production.

Acknowledgement

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References

- Bongers T. (1994). *De Nematoden van Nederland*. Utrecht, Netherlands: Koninklijke Nederlandse Natuurhistorische Vereniging, 408 pp.
- Braithwaite M., Johnston P.R., Ball S.L., Nourozi F., Hay A.J., Shoukouhi P., Chomic A., Lange C., Ohkura M., Nieto-Jacobo M.F., Cummings N.J., Bienkowski D., Mendoza-Mendoza A., Hill R.A., McLean K.L., Stewart A., Steyaert J.M., Bissett J. (2017) *Trichoderma* down under: species diversity and occurrence of *Trichoderma* in New Zealand. *Australas Plant Path*, 46, 11–30.
- Contina J.B., Dandurand L.M., Knudsen G.R. (2017) Use of GFP-tagged *Trichoderma harzianum* as a tool to study the biological control of the potato cyst nematode *Globodera pallida*. *Applied Soil Ecology*, 115, 31–37.
- Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. (2004) *Trichoderma* species - opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2 (1), 43–56.
- Liu K., Zhang Y.Z., Du H.Y., Wang Z.Y., Gu P.W., Liu Z.H., Yu Z.Y. (2023) Beneficial and biocontrol effects of *Trichoderma atroviride*, a dominant species in white birch rhizosphere soil. *Frontiers in Microbiology*, 14, 1265435.
- Mai W.F., Lyon H.H. (1975). *Pictorial Key to Genera of Plant-Parasitic Nematodes*. London, UK: Cornell University Press, 219 pp.
- Meyer S.L.F., Massoud S.I., Chitwood D.J., Roberts D.P. (2000) Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. *Nematology* 2(8), 871–879.
- Mukherjee M., Mukherjee P.K., Horwitz B.A., Zachow C., Berg G., Zeilinger S. (2012) *Trichoderma*-plant-pathogen interactions: advances in genetics of biological control. *Indian Journal of Microbiology*, 52, 522–529.
- Poveda J., Abril-Urias P., Escobar C. (2020) Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. *Frontiers in Microbiology*, 11, 992.
- Singh A., Shahid M., Srivastava M., Pandey S., Sharma A., Kumar, V. (2014) Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. *Virology & Mycology*, 3 (1), 1–7.
- Windham G.L., Windham M.T., Williams W.P. (1989) Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Disease*, 73(6), 493–494.

REVIEW ARTICLE

Ocean Acidification in the Adriatic Sea – Call for ActionSanja Grđan¹, Jelena Mandić²¹*Department of Applied Ecology, University of Dubrovnik, Croatia (sanja.grdjan@unidu.hr)*²*Laboratory of Chemical Oceanography and Sea Sedimentology, Institute of Oceanography and Fisheries, Split, Croatia***Summary**

Ocean acidification is a growing threat to marine ecosystems, affecting biodiversity, aquaculture and fisheries. The Adriatic Sea, a semi-enclosed basin of great ecological and economic importance, is particularly vulnerable to these changes. Despite its significance, research on ocean acidification in this area is still limited. This study uses a bibliometric analysis to highlight research gaps and calls for increased national investment in ocean acidification research. A bibliometric analysis identified 22 studies (2010-2025), 77% of which are from Italian institutions. Research focuses primarily on carbonate chemistry (59%), while biological studies are scarce. Spatial differences are also evident, with the northern Adriatic being the best-studied region. Resources on best practices in ocean acidification monitoring and experimental research became abundant and freely available in the past several years. This paper underlines the importance of directing research efforts to ocean acidification and highlights most important literature to begin with.

Key words: ocean acidification, Adriatic Sea, aquaculture, fisheries, biodiversity

Introduction

Since the industrial revolution, excessive anthropogenic gas emissions have been released into the atmosphere. The burning of fossil fuels, the expansion of cities and the intensification of agricultural production have led to an increase in atmospheric carbon dioxide (CO₂) concentrations of around 50% (Friedlingstein et al., 2022). The oceans play a crucial buffering role as they absorb about 27% of the emitted CO₂. However, as atmospheric concentrations increase, oceanic absorption also increases, changing the carbonate chemistry of seawater (Doney et al., 2009). This process, known as ocean acidification (OA), is accompanied by simultaneous changes in the concentrations of hydrogen ions (H⁺), dissolved CO₂, bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) leading to a decrease in seawater pH (Hurd et al., 2018). Over the past 250 years, an average decrease of 0.11 pH units has been observed in surface seawater and projections indicate a further decrease of up to 0.4 pH units by 2100 (Friedlingstein et al., 2022).

Coastal carbonate chemistry is also influenced by biological processes such as photosynthesis, respiration and the precipitation/dissolution of calcium carbonate, which can serve as a source or sink for CO₂. Freshwater inputs, organic runoff and natural upwelling events exacerbate fluctuations in pH and pCO₂ in coastal regions (Osborne et al., 2020). Understanding these fluctuations and their effects on marine organisms and ecosystems is critical, as the effects of OA have been shown to influence development, survival, reproduction, calcification, behavior, and feeding at multiple trophic levels (Figuerola et al., 2021). The effects of OA are further complicated by interactions with additional stressors such as eutrophication and rising sea surface temperatures (Hurd et al., 2018).

To standardize experimental data Riebesell et al. (2011) proposed a three-scenario approach based on present-day conditions and International Panel for Climate Change (IPCC) projections

for 2050 and 2100. However, these projections apply primarily to the open ocean, while coastal ecosystems are subject to natural pH fluctuations and that is where most of the studied organisms live. Local populations may have adaptive mechanisms that influence their sensitivity to OA (Vargas et al., 2017). Therefore, experimental designs should incorporate natural pH variability rather than relying solely on predefined scenarios. Studies suggest that there is no universal response to OA; different populations of the same species may show different responses depending on environmental factors and habitat conditions (Asnicar et al., 2021). Therefore, research efforts should focus on commercially important species and key species within their specific habitats (Saba et al., 2019).

Despite increasing awareness and institutional commitment in the Mediterranean, research to date is still limited in certain areas (Hassoun et al., 2022). The Adriatic Sea is the northernmost part of the Mediterranean; a semi-enclosed basin connected to the Mediterranean Sea by the 72 km wide Strait of Otranto (Lipizer et al., 2014). Six countries surround its coasts and rely on activities such as fishing, tourism, maritime transport and aquaculture (Hala & Bakiu, 2024). In addition, the biodiversity that provides ecosystem services is of great value. Studying emerging stressors in the area with their potential impact on the habitat and species that are both commercially and ecologically important should become a priority. The Croatian Ministry of Economy and Sustainable Development has recognized ocean acidification as a major threat in its *Climate Change Adaptation Strategy for the Republic of Croatia until 2040, with a vision for 2070* (Official Gazette, 47/2020). The document highlights the potential vulnerability of European flat oyster *Ostrea edulis* Linnaeus, 1758 and Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 to the projected decline in pH by 0.1 to 0.2. This paper aims to assess the current state of research on ocean acidification in the Adriatic Sea, draw attention to the importance of monitoring and biological experiments and to provide baseline recommendations for future studies.

Material and methods

A bibliometric analysis was performed using Web of Science (WOS), Scopus and the Zotero OA-ICC (Ocean Acidification International Coordination Centre) literature databases. Key search terms included "ocean acidification", "low pH" and "Adriatic Sea," combined with Boolean operators (AND, OR, *):

- WOS returned 65 articles, of which 24 remained after screening for relevance to carbonate chemistry parameters or experimental studies in the Adriatic Sea.
- Scopus provided 31 articles, all of which were already indexed in WOS.
- The Zotero OA-ICC library, which contains 11,108 OA-related articles, was filtered for "Adriatic Sea" and yielded 34 articles. After screening and merging with the cleaned WOS database, 22 articles remained for analysis.

The final dataset was analyzed using R Statistical Software (v.4.4.1; R Core Team 2024).

Results and discussion

Current status of OA research in the Adriatic Sea

Preliminary results based on a bibliometric analysis revealed 22 articles on ocean acidification in the Adriatic Sea for the period from 2010 to 2025. Most research papers originate from Italy (77%), followed by Croatia (5%), Greece (5%) and Slovenia (5%). The most studied region of the Adriatic is the northern Adriatic (77%), which is probably due to existing monitoring programs and Italian institutions on the western part of the Adriatic. The southern Adriatic has received much less attention, while there are no published studies on ocean acidification in its central part. The focus of research is on carbonate chemistry and CO₂ fluxes (59%) and is mainly conducted in the northern Adriatic Sea. A few published studies on biological response

focused on species from the phylum Mollusca. Croatian contributions are sparse and so far consist of two experimental studies. This research gap underlines the need for increased national investment in OA studies and urgent collaboration with neighboring countries. In 2015, the United Nations adopted the 2030 Agenda and the Sustainable Development Goals (SDGs), including SDG 14, which calls for the conservation and sustainable use of the oceans. Target 14.3 aims to minimize and address ocean acidification impacts through enhanced scientific cooperation, with Indicator 14.3.1 tracking marine acidity at representative sampling stations (IOC-UNESCO, 2024). The Croatian Institute of Oceanography and Fisheries, as the national marine reference center, works closely with the Croatian authorities in adopting and implementing the EU acquis and has started implementing SDG indicator 14.3.1 from 2024. Monitoring includes climate quality data on total alkalinity and spectrophotometric pH measurements at 40 stations in the Adriatic Sea. The implementation of mitigation measures first requires the identification of vulnerable areas and the initiation of monitoring programs. Experimental studies on commercially important species should complement these efforts.

OA future studies recommendations

To assess ocean acidification in the Adriatic, research institutions should initiate monitoring programs at multiple levels. The Global Ocean Acidification Observing Network (GOA-ON) has defined data quality objectives as: (1) “climate” - defined as measurements of sufficient quality to assess long-term trends with a defined level of confidence, and (2) - “weather” - defined as measurements of sufficient quality to detect relative spatial patterns and short-term variability, especially in nearshore regions where variability is higher (Tilbrook et al., 2019). While achieving climate-quality data is resource-intensive, initial efforts can focus on simpler methods. Many institutions already collect data on temperature, salinity, oxygen and nutrients on a regular basis or as part of other projects - these could be expanded to include carbonate chemistry parameters. To describe the carbonate system in seawater in relation to ocean acidification, four parameters are used: pH (expressed on a total scale), partial pressure of CO₂ (pCO₂, ppt or μatm), total alkalinity (TA, $\mu\text{mol kg}^{-1}$) and dissolved inorganic carbon (DIC, $\mu\text{mol kg}^{-1}$) (Dickson et al., 2007). By measuring two of these parameters, the most common choices being pH_T and total alkalinity, the remaining parameters can be calculated using either CO2 SYS software (Lewis and Wallace, 1998) or using the R package seacarb (Gatusso et al., 2021). Standard operating procedures can be found in the *Guide to best practices for Ocean CO₂ Measurements* (Dickson et al., 2007). With growing interest for the subject matter it has become clear that a simplified methodology for carbonate chemistry (weather quality data) is needed to allow more researchers to study ocean acidification, especially from the biological point of view. Over the last five years, a number of papers, videos and manuals have been published (Table 1).

Table 1. List of best practices resources for ocean acidification research

Parameter(s)	Method(s)	In brief	Reference
pH _T , pCO ₂ , DIC, TA	Standard operating procedures	Starting point	Dickson et al., 2007.
pH _T , TA	Spectrophotometer; open cell titration	Set of instructional videos	GOA-ON in a box, 2020
pH _T	Potentiometric	TRIS buffer – simplified method	Paulsen and Dickson, 2020

pH_T	Potentiometric	Set of instructional videos	IAEA, 2022
pH_T , TA	Spectrophotometer; open cell titration	Practical best practices for OA monitoring	Currie et al., 2024

Once the local pH variability has been determined, a series of experiments should be conducted to assess the effects of low pH on the species of interest. As the industry relies on a limited number of species, it is important to know their physiological vulnerabilities and acclimatization potential. Given the urgency of the issue, experiments must be carefully designed to provide meaningful insights. *A Guide to best practices in ocean acidification research* (Riebsell et al., 2011) is the best starting point for conducting experiments. The manipulation of carbonate chemistry in the experimental system is explained in great detail, with advantages and disadvantages of each method.

Key considerations for experimental design should include:

- Defining the specific research question to be answered.
- Ensuring pH values reflect natural variability
- Accurately measuring and reporting carbonate chemistry parameters.
- Selecting appropriate experimental durations and endpoints

Conclusion

National and regional cooperation is essential to close identified research gaps. Extending existing monitoring efforts to carbonate chemistry parameters and integrating standardized methods will provide important data for informed decision-making. Failure to act could have serious consequences for biodiversity, marine food webs and the economic stability of fisheries and aquaculture. Therefore, a proactive approach — through targeted research, policy development and transboundary cooperation - is needed to mitigate the effects of ocean acidification and secure the future of the Adriatic Sea.

References

- Asnicar D., Novoa-Abelleira A., Minichino R., Badocco D., Pastore P., Finos L., Munari M., Marin M. G. (2021). When site matters: Metabolic and behavioural responses of adult sea urchins from different environments during long-term exposure to seawater acidification. *Marine Environmental Research*, 169, 105372. <https://doi.org/10.1016/j.marenvres.2021.105372>
- Currie K., Sabine C., Lowder K., Hassoun A. E. R., Meléndez M., Chu S., Easley R., García-Ibáñez M. I., Schoo K., Isensee K., Lebrech M., Valauri-Orton A. (2024). Practical best practices for ocean acidification monitoring (1.1). The Ocean Foundation. <https://doi.org/10.5281/zenodo.13876198>
- Dickson A. G., Sabine C. L., Christian J. R. (2007). Guide to best practices for ocean CO₂ measurements. PICES Special Publication 3, IOCCP Report No. 8.
- Doney S., Fabry V., Feely R., Kleypas J. (2009). Ocean acidification: The other CO₂ problem. *Annual Review of Marine Science*, 1, 169-192. <https://doi.org/10.1146/annurev.marine.010908.163834>
- Figuerola B., Hancock A. M., Bax N., Cummings V. J., Downey R., Griffiths H. J., Smith J., Stark J. S. (2021). A review and meta-analysis of potential impacts of ocean acidification on marine calcifiers from the Southern Ocean. *Frontiers in Marine Science*, 8, 584445. <https://doi.org/10.3389/fmars.2021.584445>
- Friedlingstein P., O'Sullivan M., Jones M. W., Andrew R. M., Gregor L., Hauc J., Le Quéré C., Luijkx I. T., Olsen A., Peters G. P., Peters W., Pongratz J., Schwingshackl C., Sitch S., Canadell J. G., Ciais P., Jackson R. B. (2022). Global Carbon Budget 2022. *Earth System Science Data*, 14, 4811–4900. <https://doi.org/10.5194/essd-14-4811-2022>
- GOA-ON in a box, 2020. GOA-ON in a box instructional videos. <https://www.youtube.com/playlist?list=PLkDCbxtte-tKWKyKAJhvAQ9ZzyQli7wz>

- Hala E., Bakiu R. (2024). Adriatic Sea fishery product safety and perspectives in relation to climate change. *Fishes*, 9(5), 160. <https://doi.org/10.3390/fishes9050160>
- Hassoun A. E. R., Bantelman A., Canu D., Comeau S., Galdies C., Gattuso J.-P., Giani M., Grelaud M., Hendriks I. E., Ibello V., Idrissi M., Krasakopoulou E., Shaltout N., Solidoro C., Swarzenski P. W., Ziveri P. (2022). Ocean acidification research in the Mediterranean Sea: Status, trends and next steps. *Frontiers in Marine Science*, 9, 892670. <https://doi.org/10.3389/fmars.2022.892670>
- Hurd C. L., Lenton A., Tilbrook B., Boyd P. W. B. (2018). Current understanding and challenges for oceans in a higher CO₂ world. *Nature Climate Change*, 8, 686-694. <https://doi.org/10.1038/s41558-018-0211-0>
- IAEA. (2022). How to measure pH in biological experiments. <https://www.iaea.org/services/oa-icc/building-capacity/how-to-measure-pht-in-biological-experiments>
- IOC-UNESCO. (2024). Ocean acidification research for sustainability – A community vision for the Ocean Decade. Paris, IOC-UNESCO. (IOC Technical Series, 185.) <https://doi.org/10.25607/YPE3-0H04>
- Lewis E., Wallace D. W. R. (1998). Program developed for CO₂ system calculations. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.
- Lipizer M., Partescano E., Rabitti A., Giorgetti A., Crise A. (2014). Qualified temperature, salinity and dissolved oxygen climatologies in a changing Adriatic Sea. *Ocean Science*, 10, 771-797. <https://doi.org/10.5194/os-10-771-2014>
- Osborne E. B., Thunell R. C., Gruber N., Feely R. A., Benitez-Nelson C. R. (2020). Decadal variability in twentieth-century ocean acidification in the California Current Ecosystem. *Nature Geoscience*, 13, 43-49. <https://doi.org/10.1038/s41561-019-0499-z>
- Paulsen M. L., Dickson A. G. (2020). Preparation of 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS) pHT buffers in synthetic seawater. *Limnology and Oceanology: Methods*, 19(9), 504-515. <https://doi.org/10.1002/lom3.10383>
- R Core Team. (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Riebesell U., Fabry V. J., Hansson L., Gattuso J. P. (2011). Guide to best practices for ocean acidification research and data reporting. Luxembourg, Publications Office of the European Union. <https://doi.org/10.25607/OBP-1374>
- Saba G. K., Goldsmith K. A., Cooley S. R., Grosse D., Mesecke S. L., Miller A. W., Phelan G. B., Poach M., Rheaulz R., Laurenti K. S., Testa J. M., Weis J. S., Zimmerman R. (2019). Recommended priorities for research on ecological impact of ocean acidification and coastal acidification in the U.S. Mid-Atlantic Estuarine. *Estuarine, Coastal and Shelf Science*, 225, 106188. <https://doi.org/10.1016/j.ecss.2019.04.022>
- Tilbrook B., Jewett E. B., DeGrandpre M. D., Hernandez-Ayon J. M., Feely R. A., Gledhill D. K., Telszewski M. (2019). An enhanced ocean acidification observing network: From people to technology to data synthesis and information exchange. *Frontiers in Marine Science*. <https://doi.org/10.3389/fmars.2019.00337>
- Vargas C., Lagos N., Lardies M. A., Duarte C., Manriquez P. H., Aguilera V. M., Broitman B., Widdicombe S., Dupont S. (2017). Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. *Nature Ecology Evolution*, 0084. <https://doi.org/10.1038/s41559-017-008>

ORIGINAL SCIENTIFIC PAPER

Characteristics of the ichthyofauna of Aljmaš Rit

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Summary

The study aimed to assess the ichthyofauna composition and habitat status of two oxbow lakes in the Aljmaš Rit floodplain. A total of 1,680 fish from 16 species and 9 families were recorded, with four non-native species identified. The most abundant species were bleak (*Alburnus alburnus*), topmouth gudgeon (*Pseudorasbora parva*), Prussian carp (*Carassius gibelio*), and roach (*Rutilus rutilus*). Only two protected species, Balkan loach (*Cobitis elongata*) and weatherfish (*Misgurnus fosillis*), were found. Biodiversity indices indicate low species richness and diversity, with dominant invasive species suggesting ecological stress. Habitat degradation is ongoing, emphasizing the need for revitalization measures to prevent further decline.

Key words: ichthyofauna, Aljmaš Rit, invasive species, habitat degradation

Introduction

One of the greatest challenges in the management of aquatic ecosystems is finding simple ways to evaluate their condition (Rombouts et al., 2013). Ichthyofauna composition and anthropogenic impacts are tightly connected. Therefore, fish are considered appropriate indicators of trends in aquatic biodiversity because their enormous variety reflects a wide range of environmental conditions (Moyle & Leidy, 1992). The use of fish species as indicators of ecological changes in aquatic ecosystems assumes that fish communities are sensitive to changes within these systems and that fish abundance and species diversity can provide a good measure of the impacts and pressures to which a particular system is exposed (Karr, 1981; Zhang et al., 2019). The Aljmaš Rit encompasses the area of the Drava River from Nemetin, through Bijelo Brdo and Sarvaš, all the way to the confluence of the Drava and the Danube. This area includes several channels (Žuta Voda, Malo Renovo, Stankov Bogaz, Aljmaški Bogaz, and Paljevinski Bogaz) as well as two oxbow lakes (Sarvaša Bara and Bijelobrdska Bara). The Aljmaš Rit is a floodplain of the Drava River, located on its right bank along the last 12 kilometers of the river's course before it merges with the Danube. It is an integral part of the Mura-Drava Regional Park, aimed at protecting the ecosystem and preserving natural habitat types and species that inhabit the area. The goal of this study was to identify fish species present in the two largest water bodies of the Aljmaš Rit and, based on the collected data, assess the habitat status.

Material and methods

Sampling was performed at two selected locations (Figure 1.) on two occasions: September 22, 2020, and May 7, 2021. The sampling aimed to collect data on the fish community in the old river arms, now small stagnant water bodies. Electrofishing was performed from a boat using a single-pass method with the AGK EL65 II electrofisher, connected to a round stainless-steel anode with a diameter of 50 cm and a mesh size of 10 mm. The electrofishing survey was conducted over a period of 30 minutes, covering a distance of 500 meters, with the aim of capturing the main habitat types present at the sites, including variation in sediment type, flow velocity, availability of fish refuges, and other relevant features. All caught individuals were identified to the species level based on external morphological traits (Kottelat & Freyhof, 2007),

and all individuals were counted. The fish caught during electrofishing was kept in aerated water. Upon completion of the measuring process, native species were returned to the sampling location. The fish were released into the water near the shore and, after adapting to the conditions, swam away voluntarily. Non-native fish species from the catch were not returned to the water in accordance with the Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act (2018) and Amendment to the Act (2019) and were euthanized using clove oil, following the instructions of Woody et al. (2002). A Simpson Index, Shannon-Weiner Index and Margalef Index were used to analyse biodiversity (Table 3.). Data on the classification of strictly protected species was prepared in accordance with the Strictly Protected Species Act (2013) and the Amendments to the Strictly Protected Species Act (2016).



Figure 1. Sampling area and sampling locations (1 – Sarvaška bara, 2 – Bijelobrdská bara)

Results and discussion

In the Aljmaški rit, a total of 1680 fish belonging to 16 species and 9 families were caught, with four species identified as non-native (Table 1).

Table 1. Fish composition during sampling at the sites in Aljmaš Rit

Species composition	Sarvaška bara		Bijelobrdská bara	
	September 22, 2020	May 7, 2021	September 22, 2020	May 7, 2021
Balkan spined loach, <i>Cobitis elongata</i>	2	2	2	4
Weatherfish, <i>Misgurnus fosillis</i>	1	-	1	-
European bitterling, <i>Rhodeus amarus</i>	10	36	-	-
Prussian carp, <i>Carassius gibelio</i>	5	6	54	42
Common carp, <i>Cyprinus carpio</i>	1	1	8	-
Topmouth gudgeon, <i>Pseudorasbora parva</i>	91	16	29	24
Bream, <i>Abramis brama</i>	1	17	-	-
Bleak, <i>Alburnus alburnus</i>	712	72	248	8
Asp, <i>Leuciscus aspius</i>	22	6	1	7
Ide, <i>Leuciscus idus</i>	18	6	1	6
Roach, <i>Rutilus rutilus</i>	30	12	10	37
Rudd, <i>Scardinius erythrophthalmus</i>	3	18	9	-

Silver carp, <i>Hypophthalmichthys molitrix</i>	-	-	1	14
Northern pike, <i>Esox lucius</i>	17	6	4	5
Pumpkinseed, <i>Lepomis gibbosus</i>	6	36	1	2
Perch, <i>Perca fluviatilis</i>	4	-	5	-
TOTAL	923	234	374	149

The most abundant family was Leuciscidae, with six species. Cobitidae and Cyprinidae were represented by two species each, while Acheilognathidae, Gobionidae, Xenocyprinidae, Esocidae, Centrarchidae, and Percidae were represented by one species each. In terms of ecological preference and habitat type, eurytopic species were dominant, while in terms of reproduction and spawning substrate, phytophilic species were dominant. This is not surprising, as the locations are characterized by stagnant or slow-moving water and abundant aquatic vegetation. There was also one ostracophilic species (*Rhodeus amarus*) present in Sarvaška bara (Table 2).

Table 2. Number of species and ecological characteristics for the sampling site in Aljmaš Rit (according to Aarts & Nienhuis, 2003; Kottelat & Freyhof, 2007; Froese, & Pauly, 2020)

Species	Ratio in sample (%)	Ecological habitat preference	Water column	Spawing substrate	Feeding strategy
Balkan spined loach	0.60	Rheophilic	Bentic	LITO	INS/INV
Weatherfish,	0.12	Eurytopic	Bentic	FITO	INV
European biterling	2.74	Eurytopic	Water column	OSTR	OMNI
Prussian carp	6.37	Eurytopic	Bentic	FITO	OMNI
Common carp	0.60	Eurytopic	Bentic	FITO	OMNI
Topmouth gudgeon	9.52	Eurytopic	Water column	FITO/LITO	OMNI
Bream	1.07	Eurytopic	Bentic	FITO	OMNI
Bleak	61.90	Eurytopic	Water column	FITO/LITO	OMNI
Asp	2.14	Eurytopic	Water column	FITO/LITO	PISC
Ide	1.85	Rheophilic	Water column	FITO/LITO	OMNI
Roach	5.30	Eurytopic	Water column	FITO/LITO	OMNI
Rudd	1.79	Limnophilic	Bentic	FITO	OMNI
Silver carp	0.89	Limnophilic	Water column	PEL	PHYT
Northern pike	1.90	Eurytopic	Water column	FITO	PISC
Pumpkinseed	2.68	Limnophilic	Water column	FITO/LITO	INS/INV
Perch	0.54	Eurytopic	Water column	FITO/LITO	INV/PISC

Abbreviations: LITO – lithophile, FITO – phytophile, OSTR – ostracophile, PEL – pelagophile, INS – insectivore, INV – invertivore, OMNI – omnivore, PHYT – phytoplanktivore, PISC – piscivore

The most abundant fish caught during sampling were bleak (61.90%), topmouth gudgeon (9.52%), Prussian carp (6.37%), and roach (5.30%). The ratios for the other species were less than 3% (Figure 2). Bleak has a rapid generation time and can tolerate high levels of pollution. It is a common fish species in open waters and lakes of all sizes (Kottelat & Freyhof, 2007). Its high adaptability explains its high abundance in the study area. Topmouth gudgeon is an alien species that poses a significant threat to the native fish community and is a serious food

competitor for native species. This species is widespread across Europe, is most abundant in well-vegetated stagnant waters, and can become numerically dominant in the absence of adequate predators. Prussian carp is also an alien species, well known for its invasiveness, and is considered one of the most hazardous fish species for native fish communities (Crivelli, 1995; Kalous et al., 2004; Tsoumani et al., 2006; Leonardos et al., 2008). Its invasive success was also facilitated by its unusual tolerance of a wide spectrum of ecological conditions (Vetemaa et al., 2005; Grabowska and Przybylski, 2015). The Prussian carp can survive and thrive under adverse environmental conditions where other species rarely succeed. Therefore, it can easily become one of the dominant species in stagnant and slow-running waters and may alter the flow of nutrients in the entire ecosystem (Paulovits et al., 1998). The Roach is a native fish that forms shoals and feeds near the bottom. It is most abundant in nutrient-rich lakes and is frequently caught in recreational fisheries. At the Sarvaška and Bjelobrdská Bara sites, only two endangered fish species were recorded: the Balkan loach (*Cobitis elongata*) and the weatherfish (*Misgurnus fossilis*).

Table 3. Number of species, individuals, species richness and biodiversity indices for the sampling site in Aljmaš Rit

	Sarvaška bara (cumulative)	Bjelobrdská bara (cumulative)
Species number	15	14
Number of individuals	1157	523
Number of native species	12	10
Number of Alien species	3	4
% of Alien species	20 %	28.57 %
Number of protected species	2	2
Margalef index	1.985	2.077
Shannon-Wiener index	1.339	1.674
Simpson index	0.474	0.293

All three analyzed indices used to assess species diversity and species richness indicate low species richness, diversity, and uniformity of the fish population. The Shannon-Wiener index clearly shows that the abundances of different species are not similar, meaning that the population is dominated by easily adaptable species (bleak, topmouth gudgeon, and Prussian carp). Human activities usually shift the balance among fish species, causing the extirpation of many indigenous species and the dominance of a reduced set of often introduced fish species (Balirwa et al., 2003).

Conclusions

At the observed locations, the results indicate ecological stress, ecosystem instability, recent disturbance and reduced resilience to future changes. Unfortunately, this is normal for water bodies that are declining over years and have increasingly rare contact with the river due to the absence of flooding. Without serious habitat revitalization, these water bodies will continue to degrade.

References

- Aarts, B. G., Nienhuis, P. H. (2003). Fish zonations and guilds as the basis for assessment of ecological integrity of large rivers. *Hydrobiologia*, 500, 157-178.
- Amendments to Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act, Official Gazette, 14 (2019)
- Amendments to the Strictly Protected Species Act, Official Gazette, 73 (2016)

- Balirwa, S. J., Chapman, A. C., Chapman, J. L., Cowx, G. I., Geheb, K., Kaufman, L., Lowe-McConnell, H. R., Seehausen, O., Wanink, H. J., Welcomme, L. R., Witte, F. (2003). Biodiversity and Fishery Sustainability in the Lake Victoria Basin: An Unexpected Marriage? *BioScience* 53(8): 703-715.
- Crivelli, A. J. (1995). Are fish introductions a threat to endemic freshwater fishes in the northern Mediterranean region? *Biol. Conserv.* 72, 335–337. [https://doi.org/10.1016/0006-3207\(94\)00092-5](https://doi.org/10.1016/0006-3207(94)00092-5)
- Froese, R., Pauly, D. (2020). FishBase. <https://www.fishbase.org>
- Grabowska, J., Przybylski, M. (2015). Life-history traits of non-native freshwater fish invaders differentiate them from natives in the Central European bioregion. *Reviews in Fish Biology and Fisheries* 25: 165–178. <https://doi.org/10.1007/s11160-014-9375-5>
- Kalous, L., Memis, D., Bohlen, J. (2004): Finding of triploid *Carassius gibelio* (Bloch, 1782) (Cypriniformes, Cyprinidae) in Turkey. *Cyprum* 28, 77–79.
- Karr, J. R. (1981). Assessment of biotic integrity using fish communities. *Fisheries*, 6(6), 21–27.
- Kottelat, M., Freyhof, J. (2007). Handbook of European freshwater fishes (Vol. 13). Cornol, Switzerland: Publications Kottelat.
- Leonardos, I. D., Tsikliras, A. C., Eleftheriou, V., Cladas, Y., Kagalou, I., Chortatou, R., Papigiotti, O. (2008). Life history characteristics of an invasive cyprinid fish (*Carassius gibelio*) in Chimaditis Lake (northern Greece). *Journal of Applied Ichthyology*, 24(2): 213–217. <https://doi.org/10.1111/j.1439-0426.2007.01031.x>
- Moyle, P. B., Leidy, R. A. (1992). Loss of Biodiversity in Aquatic Ecosystems: Evidence from Fish Faunas. *Conservation Biology*. pp. 127.
- Paulovits, G., Tatrai, I., Matyas, K., Korponai, J., Kovats, N. (1998). Role of Prussian carp (*Carassius auratus gibelio* Bloch) in the nutrient cycle of the Kis-Balaton Reservoir. *International Review of Hydrobiology*, 83, 467–470.
- Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act, Official Gazette, 15 (2018)
- Rombouts, I., Beaugrand, G., Artigas, L. F., Dauvin, J. C., Gevaert, F., Goberville, E., ... & Kirby, R. R. (2013). Evaluating marine ecosystem health: case studies of indicators using direct observations and modelling methods. *Ecological Indicators*, 24, 353–365.
- Strictly Protected Species Act, Official Gazette, 80 (2013)
- Tsoumani, M., Liasko, R., Moutsaki, P., Kagalou, I., Leonardos, I. (2006). Length–weight relationships of an invasive cyprinid fish (*Carassius gibelio*) from 12 Greek lakes in relation to their trophic states. *J. Appl. Ichthyol.* 22, 281–284. <https://doi.org/10.1111/j.1439-0426.2006.00768.x>
- Vetemaa, M., Eschbaum, R., Albert, A., Saat, T. (2005). Distribution, sex ratio and growth of *Carassius gibelio* (Bloch) in coastal and inland waters of Estonia (north-eastern Baltic Sea). *Journal of Applied Ichthyology*, 21(4), 287–291. <https://doi.org/10.1111/j.1439-0426.2005.00680.x>
- Woody, C. A., Nelson, J., Ramstad, K. (2002). Clove oil as an anaesthetic for adult sockeye salmon: field trials. *Journal of fish Biology*, 60(2), 340–347. DOI10.1006/jfbi.2001.1842
- Zhang, Y., Wang, X. N., Ding, H. Y., Dai, Y., Ding, S., Gao, X. (2019). Threshold responses in the taxonomic and functional structure of fish assemblages to land use and water quality: A case study from the Taizi River. *Water*, 11(4), 661.

ORIGINAL SCIENTIFIC PAPER

Comparative study of viral presence in tomato collection under open field and greenhouse cultivation

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Summary

During 2024 tomato (*Solanum lycopersicum* L.) collection from the Gene Bank of Republic of Srpska at the Institute of Genetic Resources was surveyed for virus presence. The same 30 accessions were tested in the greenhouse and under open field condition. All samples were tested for the presence of 3 viruses: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus), ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) with ELISA (Bioreba) test.

Eleven samples in the open field and four samples in the greenhouse were positive for TSWV presence and negative for other two viruses and only one accession was positively confirmed in both production conditions. In future, surveys on more accessions could perceive a better sanitary status of all autochthonous tomato varieties from the Gene Bank.

Key words: Gene Bank, virus, autochthonous, TSWV

Introduction

Tomato spotted wilt virus (TSWV) is one of the most economically important virus and also has a particularly broad host-range. The hosts range of TSWV include around 1300 crop and weed species both monocotyledonous and dicotyledonous plants (Moyer, 1999). TSWV is a member of the genus *Orthotospovirus*, a group of plant viruses vectored by arthropods, usually thrips. TSWV is known to cause wilting, chlorosis, and fruit deformities. TSWV is transmitted by thrips in a circulative and propagative manner (Pappu, 2008). This virus is one of the most destructive viruses, responsible for numerous epidemics across the globe leading to heavy economic losses (Parrella et al., 2003) in a wide range of agricultural sectors. Until now, in the Republic of Srpska TSWV was detected in pepper from greenhouses and tobacco from open field (Delić et al., 2017) and also confirmed the presence of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) and tobacco thrips, *Thrips tabaci* (Linderman) in greenhouses in Herzegovina region (Trkulja et al., 2013; Kohnić et al., 2006). The highly polyphagous nature of both virus and vector (the thrips) along with the virus's ability to efficiently spread and mutate, makes it especially difficult to manage. Regular removal of weed populations can reduce the sources of infection since the weeds can serve as reservoirs for TSWV and its vector. Also, growing crops that are resistant to TSWV can significantly reduce infection and this is especially important for high-risk crops like tomatoes and peppers. These integrated pest management strategies, combining biological, chemical, and cultural control methods, are critical for managing TSWV and minimizing its impact on agricultural crops.

Tomato black ring virus (TBRV) is a member of the *Nepoviruses* group (nematode-transmitted virus), has a broad host range, affecting a variety of plant families, including both annual and perennial species, as well as woody plants and economically important crop species like:

grapevine, cherry, apricot, peach, berry-fruits, different ornamental plants and weeds, and solanaceous species like: potato, pepper, tobacco and tomato (Edwardson et al., 1997). TBRV is transmitted both through seeds and by free-living nematodes *Longidorus elongatus* and *Longidorus attenuatus* (Harrison et al., 1961; Brown et al., 1989) carrying the virus from infected to healthy plants often over large distances. Nematode larvae and adults are able to transmit but the virus is not retained after moulting, nor is it passed to progeny through the egg (Murant, 1970). Nematodes acquire virus from infected plants after feeding for about one hour and retain the ability to transmit for many weeks in soil without host plants. TBRV infections cause overall plant health decline and yield reduction, yellowing, stunting, and poor fruit quality. Wide host range of TBRV combined with its ability to be transmitted by root-feeding nematodes, seed and pollen makes them severe problem, hard to eradicate and control (Murant, 1981; Card et al., 2007). Additionally, the virus can be dispersed by transport of soil containing TBRV-infected nematodes and TBRV-infected seed. In perennial plants, virus may be distributed in material vegetatively propagated from infected plants. The virus has been reported in various region in Europe, North and South America, India and Japan (Harper et al., 2011), Australia, New Zealand (Šneideris et al., 2012). Known until now, Tomato black ring virus (TBRV) was previously detected on potato and grapevine in some parts of Yugoslavia. Isolates from sugar beet, pepper and tobacco were found in North Bosnia, the second tobacco isolate in Herzegovina, and the potato isolate in West Bosnia (Buturović et al., 1979). TBRV is concerned as a quarantine worldwide (Šneideris et al., 2012). Quarantine regulations aim to prevent its spread by limiting the movement of infected plants, seeds, or soil, especially to regions that are currently free from the virus.

Tomato brown rugose fruit virus (ToBRFV) is a member of *Tobamovirus* genus, and has been identified from tomato plants (Luria et al., 2017; Salem et al., 2016) and primarily affects *Solanum lycopersicum*. ToBRFV can cause fruit deformation (become misshapen), discoloration on both fruits and leaves, and reduced yield. ToBRFV is known to spread through physical contact between infected and healthy plants, making it highly contagious in areas with dense tomato cultivation. The virus is stable and can persist on surfaces such as tools, equipment, and even human hands, which can inadvertently transfer the virus from plant to plant. Additionally, it can be transmitted through seeds, further complicating efforts to prevent its spread. ToBRFV is transmitted by mechanical contact, propagation material, plant debris, contaminated soil, growing media, circular water, workers farming activities and tools (Oladokun et al., 2019). ToBRFV was discovered in greenhouse tomato plants grown in Jordan and its first outbreak was in Israel (Salem et al., 2016). The virus has been reported in at least 35 countries across four continents in the world. Recently, viruses has caused devastating disease outbreaks in tomato production areas in many countries, resulting in a severe reduction in yield (Avni et al., 2021; EPPO, 2020; Jones, 2021; Oladokun et al., 2019). Italy and Slovenia are the nearest counties to Bosnia and Herzegovina that have identified the presence of this virus (Panno et al., 2019). EPPO Working Party on Phytosanitary Regulations and Council agreed that ToBRFV should be added to the A2 List of pests recommended for regulation as quarantine pests in 2020.

Material and methods

Open field and greenhouse production

The research was conducted on 30 tomato accessions (Table 1) from the Gene Bank of the Republic of Srpska. During the growing season of 2024, tomato accessions were planted both in the open field and in the greenhouse (tunnel-type polypropylene) at the Institute of Genetic Resources, University of Banja Luka. Plants were maintained using standard agricultural practices, applying fertilization and drip irrigation systems.

Sample preparation

During 2024 monitoring and sample collection were carried out on leaf samples randomly from symptomatic and asymptomatic plants in both open fields and greenhouses environment. Bioreba extraction bags were used to homogenize the fresh leaf samples for DAS-ELISA testing. One sample for DAS-ELISA testing includes leaves of two plants per accession. Prepared samples were stored at temperature 4°C overnight.

Serological analysis

Prepared samples were analyzed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercial diagnostic kits (Bioreba AG, Reinach, Switzerland) for detecting specific viral antigens: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus) and ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) according to manufacturer's instructions. Positive and negative controls are included in each assay to validate ELISA procedure. The positive control confirms the assay's ability to detect the virus, while the negative control ensures no false positives are generated by contamination or procedural errors. ELISA reactions were read for absorbance at 405 nm using a HiPo MPP-96 Microplate Reader (BioSan, Lithuania). Yellow color development was assessed visually after 30 and 60 minutes and overnight.

Results and discussion

A total of 30 selected tomato samples were collected in greenhouse and in the open field and checked for the presence of 3 viruses (TSWV, TBRV and ToBRFV). The mild symptoms of leaf chlorosis and nerve yellowing that were observed on some accessions could be linked to certain viruses, but other factors like nutrient deficiencies or environmental stress could also cause similar symptoms. So, confirming the presence of the specific viruses was done by serological testing.

DAS-ELISA positive tests resulted in 36.7% (11/30) of TSWV infected plants in the open field and 13.33% (4/30) of TSWV infected plants in the greenhouse. These results show a high infection of TSWV in the open field where spread is possible by thrips. The fact that only one tested accession (GB01498) was positive for TSWV presence in both the field and the greenhouse suggests a few important points of checking potential sources of infection and additional testing. *F. occidentalis* as a vector of TSWV has already been detected in Bosnia and Herzegovina, opening up opportunities for the rapid spread of this virus.

Other two viruses, TBRV and ToBRFV were negative in DAS-ELISA test for all 30 tomato accessions. All nepoviruses are transmitted through soil by free-living nematodes and considering that the soil substrate Klasmna TS3 (Germany) was used for plant production in the greenhouse, there is a small opportunity for infection. The fact that TBRV is transmitted by seeds and pollen is important because it highlights the risk of cross-contamination within seed collections. Also, virus has been reported in some countries in Europe in the RNQP (Regulated non-quarantine pests) status, so the goal was to check whether the virus is present in the seed collection from the Gene Bank of the Republic of Srpska. Tomato brown rugose fruit virus (ToBRFV) was important to test, especially given its A2 quarantine pest status, its potential to spread easily through seeds and propagation material, and the fact that there have been reports of infections in neighboring counties.

Table 1. Gene bank code, local name, and TSWV presence on plants grown in the open field and greenhouse

No.	Gene bank code	Local name	TSWV in the open field	TSWV in the greenhouse
1.	GB01485	‘Volovsko srce crveno’	-	-
2.	GB01486	‘Kruševački’	-	+
3.	GB01487	‘Black cherry’	-	+
4.	GB01488	‘Amerikanac’	-	-
5.	GB01489	‘Crnković Yugoslavia’	-	-
6.	GB01490	‘Žuto putnički’	+	-
7.	GB01491	‘Ohridski dolgaves’	+	-
8.	GB01492	‘Rozi starinski’	-	+
9.	GB01493	‘Manastirac’	-	-
10.	GB01494	‘Konare Bugarski jabučar’	+	-
11.	GB01495	‘Bosanski žuti’	-	-
12.	GB01496	‘Džinovski starinski jabučar’	-	-
13.	GB01497	‘Novosadski žuti jabučar’	-	-
14.	GB01498	‘Pogačar’	+	+
15.	reference variety	‘Volovsko srce’	+	-
16.	GB01499	‘Džinovsko crveno volovsko srce’	-	-
17.	GB01500	‘Jabučar iz Hercegovine’	-	-
18.	GB01501	‘Bečejski’	+	-
19.	GB01502	‘Pusta kolox’	+	-
20.	GB01503	‘Mađarsko volovsko srce’	-	-
21.	GB01504	‘Žuti putnički’	-	-
22.	GB01505	‘Brački jabučar’	+	-
23.	GB01506	‘Woolly Kate’	+	-
24.	GB01507	‘Moneymaker’	-	-
25.	GB01508	‘Woods famous brimer’	-	-
26.	GB01509	‘Kosovsko volovsko srce’	-	-
27.	GB01510	‘Volovsko srce roza’	+	-
28.	GB01511	‘Rumunski jabučar’	-	-
29.	GB01512	‘Mali šeb čeri Dalmacija’	-	-
30.	reference variety	‘Novosadski jabučar’	+	-

Conclusions

Gene Banks have established procedures to test the collections to eliminate infections from accessions or for ensuring that only healthy, virus-free seeds are multiplied and distributed. During multiplication of seeds collection, it is important to ensure that plants are grown in a way that prevents the spread of harmful diseases before storage in the Gene Bank. This study represents background for a wider survey of TSWV, TBRV and ToBRFV presence or absence in selected tomato accessions before multiplication.

References

- Avni B. Gelbart, D., Sufrin-Ringwald, T, Zinger, A, Chen, L., Machbash, Z. (2021). Tomato genetic resistance to tobamoviruses in compromised, *Acta Horticulturae*, Vol. 1316, pp. 89-98.
- Brown D. F., Murrant A. F., Trudgill D. L. (1989). Differences between isolates of the English serotype of tomato black ring virus in their transmissibility by an English population of *Longidorus attenuatus* (Nematoda: Dorylaimoidea), *Revue Nematologie*, Vol. 12, pp. 51–56.
- Buturović, D., Grbelja, J., Jelić, Ž. (1979). On the geographical distribution of tomato black ring virus in Yugoslavia, *Acta Botanica Croatica*, Vol. 38, No. 1., pp. 9-12.
- Card S. D., Pearson M. N., Clover G. R. G. (2007). Plant pathogens transmitted by pollen, *Australasian Plant Pathology*, Vol. 36, pp. 455–461.
- Delić, D., Balech, B., Radulović, M., Đurić, Z., Lolić, B., Santamaria, M., Đurić, G. (2017). Molecular identification of Tomato spotted wilt virus on pepper and tobacco in Republic of Srpska (Bosnia and Herzegovina), *European Journal of Plant Pathology*, Vol. 134, No. 3.
- Edwardson J. R., Christie R. G. (1997). Viruses infecting peppers and other Solanaceous crops. Gainesville, USA, Vol. 2, pp. 337–390.
- EPPO (2020). Tomato brown rugose fruit virus, *EPPO Bulletin*, Vol. 50, pp. 529-534.
- Harper S. J., Delmiglio C., Ward L. I., Clover G. R. G. (2011). Detection of Tomato black ring virus by realtime one-step RT-PCR. *Journal of Virological Methods*, Vol. 171, No.1, pp. 190–194.
- Harrison B. D., Mowat P., Taylor C. E. (1961). Transmission of a strain of tomato black ring virus by *Longidorus elongatus* (Nematoda). *Virology*, Vol. 4, No. 4, pp. 480–485.
- Jones, R.A.C. (2021). Global plant viruses disease pandemic and epidemic, *Plants*, Vol. 10, pp. 233.
- Kohnić, A., Ostojić, I., Karić, N. (2006). Vegetable pests in greenhouses in territory of Herzegovina. *Radovi Poljoprivrednog Fakulteta Univerziteta u Sarajevu*, Vol.51, No.2, 139–140.
- Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., Elad, N., Tam, Y., Sela, N., Abu-Ras, A., Ezra, N., Haberman, A., Yitzhak, L., Lachman, O., Dombrovsky A. (2017). A new Israeli Tobamovirus isolate infects tomato plants harboring tm-22 resistance genes. *PLoS One*, Vol. 12, No.1.
- Moyer, J.W. (1999). *Encyclopedia of virology*, Second edition, pp. 1803-1807.
- Murrant, A.F. (1970). Tomato black ring virus. CMI/AAB Descriptions of Plant Viruses No. 38. Association of Applied Biologists, Wellesbourne, UK.
- Murrant A. F. (1981). Nepoviruses, *Handbook of plant virus infections/Comparative Diagnosis* – Amsterdam, Netherlands, pp. 197–238.
- Oladokun, J.O., Halabi, M.H., Barua, P., Nath, P.D. (2019). Tomato brown rugose fruit disease: current distribution. Knowledge and future prospects, *Plant Pathology*, Vol. 8, pp. 1579-1586.
- Panno, S., Caruso, A.G., Davino, S. (2019). First report of tomato brown rugose fruit virus on tomato crops in Italy, *Plant Disease*, Vol. 103, pp. 1443.
- Pappu, H.R. (2008). Tomato Spotted Wilt Virus, *Encyclopedia of Virology (Third Edition)*, pp.133-138.
- Parrella G., Gognalons P., Gebre-Selassie K., Vovlas C., Marchoux G. (2003). An update of the host range of tomato spotted wilt virus. *Journal of Plant Pathology*. Vol 85 (No 4). Special Issue: 227-264.
- Salem, N., Mansour, A., Ciuffo, M., Falk, B.W, Turina, M. (2016). A new tobamovirus infecting tomato crops in Jordan, *Archives of Virology*, Vol. 161, pp.503-506.
- Šneideris, D., Zitkaitė, I., Žižytė, M., Grigaliūnaitė, B., Staniulis, J. (2012). Identification of nepoviruses in tomato (*Lycopersicon esculentum* Mill.), *Agriculture*, Vol. 99, No. 2, pp. 173–178.
- Trkulja, V., Mihić Salapura, J., Ćurković, B., Stanković, I., Bulajić, A., Vučurović, A., Krstić, B. (2013). First report of tomato spotted wilt virus on gloxinia in Bosnia and Herzegovina, *Plant Disease*, Vol. 97, pp. 429.

EXPERT PAPER

Green Waste Management Practices of Invasive Plant Species in Croatia

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Summary

The introduction of invasive alien plant species (IAPS) threatens biodiversity and ecosystems and poses a challenge to their effective management. The aim of this study was to investigate the management of green waste and IAPS by municipalities in north-west Croatia. Despite a 90% awareness rate, 72.7% of municipalities lack specific protocols for dealing with IAPS, and none of the municipalities reported having a curated selection of ornamental plant species for public planting. As many as 86.4% of respondents were in favour of improved waste management for green waste containing IAPS. In addition, 68.2% of respondents had not considered composting for circular economy purposes, while 63.6% recognised that there were gaps in education about IAPS disposal.

Key words: green waste, alien plant species, municipalities, survey

Introduction

Invasive alien plant species (IAPS) pose a major threat to biodiversity by disrupting ecosystems and species of EU-wide importance, as shown by numerous studies and legislation (Kourantidou et al., 2022). These species spread accidentally or intentionally via vectors such as human activities, transport, contaminated agricultural products, trade, horticulture, etc. (Novak, 2018). Once introduced, they can proliferate, disrupt ecological processes, harm flora and fauna and contribute to biodiversity loss (Keller et al., 2011). Although some IAPS are prohibited, many of them have been introduced as ornamentals, but their accidental or deliberate spread has led to ecological concerns. Addressing the problems of IAPS is both costly and complex (Haubrock i sur., 2021), especially for species that are considered beneficial or appealing by certain sectors. For example, ornamental horticulture has unwittingly contributed significantly to the introduction of alien species. Although most of them are confined, some species have proven to be highly invasive and harmful to the environment (Beaury et al., 2021). Despite the increasing prevalence of IAPS, public awareness remains low, while many people unknowingly cultivate and spread these species. Municipalities and waste management companies often lack knowledge or protocols for the disposal of IAPS, which can further spread and inhibit the growth of native species (Bumbalová et al., 2024).

The LIFE+ programme co-funded the project "Prevention and management of adverse impact of ornamental invasive alien plant species on endangered habitat types and species of EU importance". This initiative, aims to reduce the harmful effects of IAPS on biodiversity in the two neighbouring countries of Croatia and Slovenia. One of the main objectives of the project is to develop effective garden waste management practises to prevent the introduction and unintentional spread of IAPS through composting and disposal methods for green waste from yards, gardens and homesteads of residents of settlements and public areas of individual municipalities (Dujmović Purgar et al., 2024).

This study focuses on analysing the existing methods of green waste management in municipalities in north-western Croatia. It emphasises the importance of user-friendly waste

management practises, circular economy approaches and the safe use of composted materials to curb the spread of IAPS.

Material and methods

In order to analyse the existing methods of garden waste management and composting, surveys were conducted among municipalities and waste management companies in north-western Croatia, including Zagreb, Krapina-Zagorje, Varaždin and Međimurje counties. The main objectives were to assess current practices in dealing with green waste and IAPS. In addition, the surveys aimed to capture the views of users, including municipalities and waste management companies, on the management of garden waste and the potential threats posed by IAPS. The questionnaire was developed using Google Forms and distributed digitally to target municipalities in Zagreb, Krapina-Zagorje, Varaždin and Međimurje counties, more precisely counties near the border with Slovenia, in order to include the planned research project area. The data was collected from April to June 2024. The target sample size was achieved with 22 completed survey forms. The survey comprised a total of 20 questions (Table 1).

Table 1. Overview of survey questions

Nr.	Question
1.	Who is responding to the survey - city or municipality? Please state the name.
2.	How many inhabitants does your municipality have?
3.	How many households is your municipal company responsible for?
4.	Is the same municipal company (MC) responsible for the collection of biodegradable waste and municipal waste from households? Please enter the name(s).
5.	Does the municipal company have its own composting plant?
6.	Does the municipal company in your municipality collect organic household waste together with green and garden waste (e.g., cut branches, mown grass, etc.)? Please enter the name of MC.
7.	Is there an organised collection of green and garden waste at recycling centres for the residents of your municipality?
8.	How is the collection of garden or green waste organised for households?
9.	How many recycling centres are there in your municipality?
10.	What are the working hours at the recycling centres?
11.	How many cubic metres (m ³) of garden and residual waste can each household dispose of free of charge at the recycling centres?
12.	Are you familiar with the term "invasive alien plant species"?
13.	Indicate whether the municipal company has specific rules/protocols for handling green and garden waste containing invasive plant species (e.g., ragweed, tree of heaven, Japanese knotweed, milkweed, Himalayan balsam, etc.).
14.	Does the municipality have specific rules for handling green and garden waste containing invasive plant species?
15.	Indicate whether the municipality has specific rules or agreements for the selection of ornamental plant species for public areas, especially with regard to invasive plant species.
16.	Is the municipality confronted with the problem of the proliferation and spread of invasive plant species on in municipal public areas?
17.	Are you interested in ways to properly process green and garden waste from invasive plant species to prevent them from being harmful to the environment?
18.	Is an efficient system for collecting, disposing of and processing invasive plant species of interest to the municipality?
19.	Do you see potential in the processing of invasive plant species (e.g., into compost or other forms of circular economy)?
20.	Do you think additional educational measures for dealing with invasive plant species in your municipality to be necessary (e.g. lectures, workshops, information brochures, etc.)?

Questions 1 to 11 concerned information about the municipalities / cities themselves and the municipal and waste management companies but also their attitude to garden waste and the current systems for the management, collection and disposal of green waste. Questions 12 to

20 related to their awareness of IAPS, threats to biodiversity and their protocols for dealing with such waste. Participants were given the opportunity to further explain their answer to each question. This comprehensive approach aimed to collect data on current practises to support the creation of guidelines and management plans for IAPS waste for municipalities, waste management municipal companies and residents in the following phases of the LIFE OrnamentalIAS project.

Results and discussion

Survey results showed, as illustrated in the subsequent pie charts represented in Figure 1 (questions 1–20), that there is a notable gap in the management of IAPS waste in municipalities in north-western Croatia, despite widespread familiarity with the term IAPS among respondents. From a total of 22 respondents, only four cities (18%) and 18 municipalities (82%) participated in the survey (Q1).

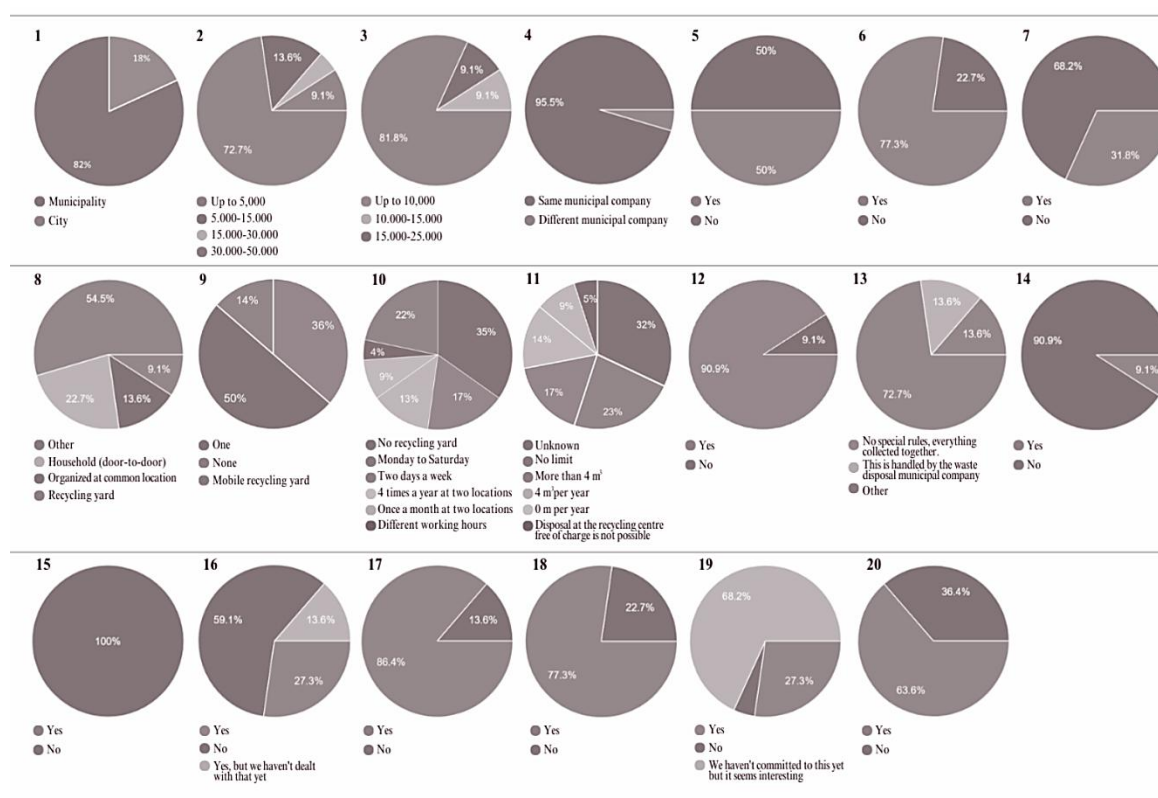


Figure 1. Graphical representation of the results of the survey (Each question is represented graphically and is numbered 1-20 in Figure 1)

The majority of responses (72.7%) were from municipalities with up to 5,000 inhabitants, while 13.6% of responses came from municipalities with 5,000 to 15,000 inhabitants and less than 15% from municipalities with more than 15,000 inhabitants (Q2). In 81.8% of cases, the municipal companies were responsible for waste collection for up to 10,000 households, while the remaining municipalities were responsible for a larger numbers of households, namely up to 25,000 (Q3). Regarding responsibilities for waste management, most respondents (95.5%) indicated that the same municipal company is responsible for both biodegradable waste and municipal waste, with the exception of the city of Prelog in Međimurje County (4.5%), which uses separate companies (Q4). In addition, 50% of municipalities stated that the municipal company responsible for their waste has its own composting plant, while the other half use external facilities or do not compost biodegradable waste at all (Q5). When asked about the collection of organic and green garden waste, 77.3% of municipalities reported collecting these

types of waste together, while 22.7% collected them separately and some households disposed of them independently. Certain companies were named for the disposal of biodegradable waste, including Eko-flor d.o.o. (for four municipalities), GKP Prekom d.o.o. and GKP Čakom d.o.o. (for a total of five municipalities), Energija Gradec d.o.o. and Čistoća d.o.o. (for two municipalities each) and Agroproteinka Energija (for one municipality) (Q6). However, 68.2 % of the municipalities did not have organised collection systems for green garden waste at recycling centres, and only 31.8 % had introduced such systems (Q7). In terms of organising green waste collection for households, the majority (54.5%) said that they relied on alternative approaches, such as composting waste in their yards or gardens. Door-to-door collection was reported by 22.7% of municipalities, while 13.6% organised seasonal collection in spring and autumn and 9.1% allowed disposal at recycling centres (Q8). Nevertheless, 50% of municipalities had at least one recycling centre, 36% had none and 14% had occasionally available mobile recycling centres (Q9). Where there were recycling centres (65%), the opening hours varied greatly. Some were open all week, while others were only open a few times a year (Q10). The amount of green and garden waste that households could dispose of free of charge also varied. Around 32% of municipalities did not specify, probably because there are no recycling centres, while 23% placed no restrictions on the amount of waste accepted. Others allowed limited quantities, with 14% accepting up to 4m³, while 5% of respondents stated that free disposal at the recycling centre was not possible (Q11). The survey revealed that 90.9% of respondents were familiar with the term "invasive alien plant species", while 9.1% were not (Q12). However, the lack of specific rules for dealing with IAPS was notable - 72.7% of municipalities stated that the responsible municipal companies do not have specific protocols for dealing with green waste containing IAPS and treat it in the same way as other green waste. On the other hand, 13.6% of the municipalities stated that this is done by an external company for the disposal of biodegradable green and garden waste, so no further information is available to the municipalities, while another 13.6% stated "other" and further stated that waste containing IAPS is disposed of in private gardens (Q13). Similarly, 90.9% of municipalities had no specific rules for dealing with IAPS in general, while 9.1% had limited measures in place but did not further explain what the rules were for dealing with this type of waste (Q14). None of the municipalities surveyed had rules or agreements for the selection of ornamental plant species for public areas with regard to their invasiveness (Q15). Interestingly, 59.1% of municipalities had no problems with the spread of IAPS on public areas, while 27.3% were confronted with such issues. A further 13.6% were aware of the problem but had taken no action to address it (Q16). Nonetheless, 86.4% of municipalities expressed an interest in learning more about the proper management of IAPS to prevent environmental damage (Q17). In addition, 77.3% stated that the establishment of efficient systems for the collection, disposal and treatment of IAPS would be beneficial for municipalities (Q18). When asked about the potential for processing IAPS waste in line with circular economy principles, 68.2% stated that they had not yet looked into the topic but found it interesting. A further 27.3% thought it was an innovative concept and would like to learn more about it, while less than 5% were not interested (Q19). Furthermore, 63.6% of municipalities felt that additional educational measures such as lectures and workshops were necessary to tackle IAPS, while 36.4% felt that such measures were unnecessary (Q20).

Conclusions

This study reveals a significant gap in the management of IAPS waste in municipalities in north-western Croatia, although most municipalities and cities are familiar with the term IAPS. A large proportion of municipalities do not have specific protocols or regulations for handling green waste containing IAPS. This lack of regulations is reinforced by the fact that none of the municipalities and cities that participated in the survey have specific systems for the collection

and disposal of garden waste containing IAPS. Although the municipalities recognise the environmental threat posed by IAPS and express a desire for improved waste management systems (in relation to IAPS), the study shows a clear need for further education, awareness raising and the development of formal protocols. More than 63% of municipalities believe that additional education is needed on how to deal with IAPS. In addition, while there is interest in exploring the potential for processing IAPS into compost or other circular economy solutions, most municipalities have not yet considered these options.

These findings emphasise the importance of educational initiatives, the creation of specific waste management protocols and the integration of circular economy approaches to curb the spread of IAPS and protect local biodiversity, which are some of the objectives of the LIFE OrnamentalIAS project. The results of this study could serve as a basis for the development of guidelines and strategies for municipalities, waste management companies and local residents to tackle the challenge of IAPS in an environmentally sustainable way.

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References

- Beaury E. M., Patrick M., Bradley B. A. (2021). Invaders for sale: the ongoing spread of invasive species by the plant trade industry. *Frontiers in Ecology and the Environment*. 19(10): 550-556.
- Bumbalová M., Fehér A., Prčík M., Marišová E. (2024). Who Holds the Keys to the Management of Municipal Waste and Which Locks of Municipal Sustainability do they Fit Into?. *Polish Journal of Environmental Studies*. 33(2): 1017-1031.
- Dujmović Purgar D., Šic Žlabur J., Peter A., Kušen M., Šipek Penić M., Masten Milek T., Basrek L., Židovec V. (2024). LIFE OrnamentalIAS - new project in Croatia. In *59th Croatian and 19th International Symposium on Agriculture*, Carović-Stanko K., Kljak K. (eds.), 117-117. Zagreb, Croatia: University of Zagreb Faculty of Agriculture.
- Haubrock P. J., Turbelin A. J., Cuthbert R. N., Novoa A., Taylor N. G., Angulo E., Ballesteros-Mejia L., Bodey T. W., Capinha C., Diagne C., Essl F., Golivets M., Kirichenko N., Kourantidou M., Leroy B., Renault D., Verbrugge L., Courchamp F. (2021). Economic costs of invasive alien species across Europe. *NeoBiota*. 67: 153-190.
- Keller R.P., Geist J., Jeschke J.M., Kühn I. (2011). Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe*. 23(23): 1-17.
- Kourantidou M., Haubrock P. J., Cuthbert R. N., Bodey T. W., Lenzner B., Gozlan R. E., Nunez M. A., Salles J. M., Diagne C., Courchamp F. (2022). Invasive alien species as simultaneous benefits and burdens: trends, stakeholder perceptions and management. *Biological Invasions*. 24(7): 1905-1926.
- Novak M. (2018). Alelopatski potencijal invazivne alohtone vrste pajasena (*Ailanthus altissima* (Mill.) Swingle). Doktorski rad. Zagreb, Croatia: University of Zagreb Faculty of Agriculture.

ORIGINAL SCIENTIFIC PAPER

Shellfish biodiversity on larvae mesh bag collectors in the ecological network Natura 2000 site Šćedro – undersea (HR3000119)Zvezdana Popović Perković¹, Jelena Kurtović Mrčelić², Josip Boban², Ivan Dominis¹¹University Department of Marine Studies, University of Split, Ruđera Boškovića 37, Split, Croatia (zvjezdana.popovic@unist.hr)²Public Institution for the Management of Protected Areas in the County of Split and Dalmatia “Sea and Karst”, Prilaz braće Kaliterna 10, Split, Croatia**Summary**

A research of the shellfish community on shellfish larvae collectors was conducted in the ecological network Natura 2000 site Šćedro – undersea (HR3000119). The collectors were set up to collect juveniles of critically endangered species *Pinna nobilis* Linnaeus 1758 as a method to monitor recruitment and juvenile survival, collect data on bivalve biodiversity, and record non-native species. Analysis of the shellfish larvae collectors resulted in the determination of 17 species from 12 families. During collector processing, none of the *P. nobilis* juveniles were found among 883 analysed individuals on six mesh bag larvae collectors on 3 locations at this Natura 2000 site.

Keywords: *Pinna nobilis*, *Pinctada radiata*, Šćedro, shellfish larvae collectors, biodiversity

Introduction

Since 2016, there has been a widespread mass mortality event affecting the species *P. nobilis*, with mortality rates recorded at 80-100% in less than three years. The causative agent identified is *Haplosporidium pinnae* (Cabanellas-Reboredo et. al, 2019; Kersting et al., 2019). This parasite was first detected in the Adriatic Sea in May 2019, specifically on the outer side of Mljet Island. Observations indicated that the parasites spread from the open sea towards locations closer to the mainland, moving from the southern to the northern parts of the region. In 2019, no deaths were reported in the northern Adriatic; however, six months later, the causative agent reached the coast of Istria, leading to high mortality rates among local populations (Mihaljević et al., 2021). Šćedro Island, a small and isolated island near Hvar, has been protected as a Significant Landscape since 1968 in its land part. It is located in the central part of the Eastern Adriatic within the Split-Dalmatian County. The marine site surrounding Šćedro Island, which includes an area approximately 250 meters from the coastline, was declared as a Site of Community Importance (SCI) in 2014 and designated as a Special Area of Conservation (SAC) in 2023 due to its importance for *Posidonia* beds and reefs (ZZOP, 2025). The Public Institution “Sea and Karst” manages both the land and marine areas of the Šćedro region. Prior to the mass mortality events, populations of *P. nobilis* have been recorded by the institution (Sea and Karst, 2017). Research on shellfish biodiversity using a larvae mesh bag collector can help document the recovery of native species such as *P. nobilis* while also providing an early warning system for invasions (Kersting and Hendriks, 2019).

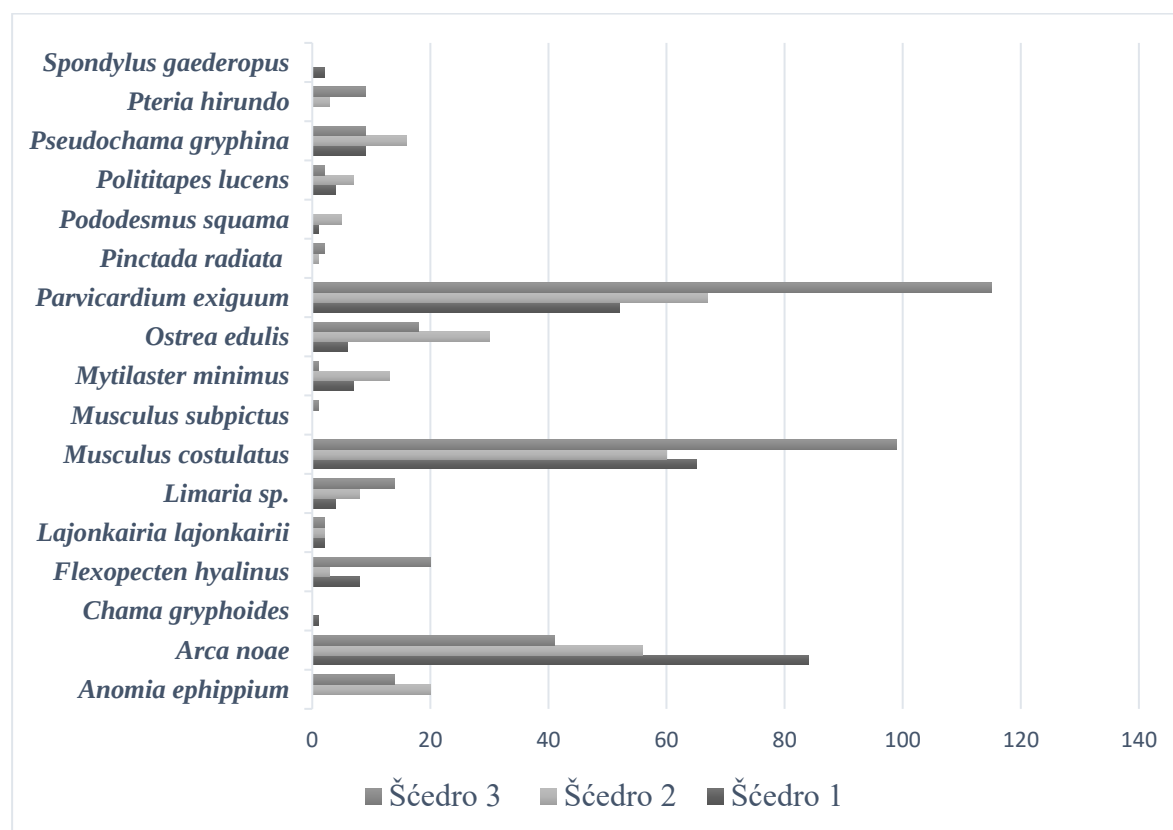
Material and methods

In the area of the ecological network Natura 2000 site Šćedro – undersea (HR3000119), the larvae mesh bag collectors were placed at three locations of Porat Bay at different depths: Šćedro 1 (43°05'37.5"N 16°42'08.5"E) - collectors 1 and 2 at a depth of 3,4 and 2,9 m; Šćedro 2 (43°05'30.4"N 16°41'48.6"E) - collectors 3 and 4 at a depth of 1,6 and 2,6 m; Šćedro 3

(43°05'28.3"N 16°41'57.1"E) - collectors 5 and 6 at a depth of 3,1 and 6,1 m. The mesh bag collectors were placed on the main rope lines of the nautical anchoring in Porat Bay. One mesh bag collector consists of polyethylene bag in which 3 more polyethylene bags are installed. The mesh bag collectors were collected by SCUBA divers after being submersed for around 6 months from June to November 2024. As soon as collected, the mesh bag collectors were placed in plastic containers with seawater with a volume of 80 L. All fouling organisms found on the mesh bag collectors were manually separated and placed in a container following the filtration on a 500 µm sieve and conservation in 500 ml vials with 96% ethanol for further laboratory analyses. In the laboratory all the species were sorted, counted and determined with the help of an Olympus SZX10 stereo microscope. For the need of classification and nomenclature the European register of marine species was implemented (Costello et al., 2001). Margalef index, Pielou index, Shannon–Wiener index and Simpson index were calculated in Primer 6 and Permanova+ program.

Results and discussion

The results of the analysis of shellfish biodiversity on a larvae mesh bag collectors at all three studied locations from the Šćedro – undersea (HR3000119) Natura 2000 site are presented in Graph 1. Indices of species diversity according to location and depth are and presented in Table 1.



Graph 1. Biodiversity of bivalves on the mesh bag collectors in the Natura 2000 ecological network site Šćedro – undersea (HR3000119).

Table 1. Indices of species diversity according to the location and the depth (S=number of species; N=number of individuals, d=Margalef index, J'=Pielou index, H'=Shannon–Wiener index, $1-\lambda$ =Simpson index).

	S	N	d	J'	H'(loge)	1-Lambda'
ŠĆEDRO 1	10	115	1,896761	0,710866	1,63683	0,763997
ŠĆEDRO 2	10	130	1,848985	0,738871	1,701312	0,761717
ŠĆEDRO 3	12	203	2,070313	0,796296	1,978721	0,830122
ŠĆEDRO 4	12	88	2,456818	0,85934	2,135379	0,867555
ŠĆEDRO 5	12	167	2,14928	0,688331	1,710437	0,754852
ŠĆEDRO 6	11	180	1,925685	0,797864	1,913195	0,811794

During the research a total of 883 individuals were recorded on the larvae mesh bag collectors. A total of 17 bivalve species from 12 families were determined, with no juvenile individuals of the *P. nobilis* species being recorded. The non-native species *Pinctada radiata* (Leach, 1814) (Bratoš Cetinić et. al., 2023) was found at the collectors Šćedro 1 and Šćedro 5 at a depth of 2,9 and 3,1 m. Species with commercial value such as *Ostrea edulis* and *Arca noae* were recorded. The most numerous species were *Parvicardium exiguum*, *Musculus costulatus*, and *A. noae*. The smallest number of bivalves was recorded at the Šćedro 1 location, while the largest number was recorded at the Šćedro 3 location. A total of 347 bivalves were counted at the Šćedro 3 location, and the bivalve species *P. exiguum*, *M. costulatus*, *A. noae* and *Flexopecten hyalinus* were the most numerous. A total of 291 individuals were recorded at the Šćedro 2 location, and the *P. exiguum*, *M. costulatus*, *A. noae* and *O. edulis* were the most prevalent species. Biodiversity indices were calculated, of which the highest value of the Margalef index was 2,456818 for the Šćedro 4 collector at a depth of 2,6 m, while the lowest value was 1,848985 for the Šćedro 2 collector at a depth of 2,9 m. The highest value of the Pielou index was 0,85934 for the Šćedro 4 collector at a depth of 2,6 m, while the lowest value was 0,688331 for the Šćedro 5 collector at a depth of 3,1 m. The highest value of the Shannon-Wiener biodiversity index was 2,135379 for the Šćedro 4 collector at a depth of 2,6 m, and the lowest was 1,63683 for the Šćedro 1 collector at a depth of 3,4 m. The Simpson index had the highest value at the Šćedro 4 collector at a depth of 2,6 m and was 0,867555, and the lowest Simpson index value was 0,754852 for the Šćedro 5 location at a depth of 3,1 m. Before this research, there were no studies on the settlement of bivalve larvae on the mesh bag collectors in the area of Šćedro and Hvar. Similar study was conducted within Brijuni National Park in North Adriatic and in Luka Cove on Dugi Otok Island in the Central Adriatic in 2019 and 2020 (Bakran-Petricioli et al., 2023). In that research, bivalves dominated in number of species and abundance, but the reduction in the number of juvenile pen shells was explained by the explosion of the invasive bivalve *Anadara transversa* population which was not recorded in this study. Three additional non-bivalve invasive species were present in mentioned study *Styela plicata*, *Bugula neritina* and *Paraleucilla magna* proving collectors as a very convenient method for monitoring the appearance and progression of non-native species. Another study conducted in the Adriatic Sea in the Bay of Bokotor with mesh bag collectors (Peraš et al., 2018) detected a total of 15 species of bivalves including *Acanthocardia* sp., *Anomia ehippium*, *Aequipecten opercularis*, *Chlamys varia*, *Chlamys multistriata*, *Flexopecten glaber*, *Lima hians*, *Hiattella rugosa*, *Modiolarca subpicta*, *M. galloprovincialis*, *O. edulis*, *P. nobilis*, *Pecten jacobaeus*, *P. radiata* and *Tapes decussatus*, of which five are the same as in this research. Marčeta et al. (2022) conducted research in the northwestern Adriatic where they analyzed the settlement of bivalve larvae on collectors that were installed at two locations, Pellestrina and Caleri. A total of 28 species of bivalves were determined on 140 installed collectors. The most abundant species in the spring-summer period were *M. galloprovincialis*, *F. glaber*, *M. varia* and *A. opercularis*,

and in the summer-autumn period *A. transversa* and *F. glaber*. In addition, the non-indigenous species *A. senhousia* was discovered. In this research non-native bivalve species *P. radiata* was found on 2 collectors installed in two locations in Porat Bay. Because the settling of *P. nobilis* larvae on the mesh bag collector is a complex process under the influence of abiotic and biotic parameters that generate high variability in settlement rates, larval settlement trends can be assessed only through longer data sets (Kersting and Garcia-March, 2017) with further research in non-native species presence.

Conclusions

This is the first conducted research presenting both qualitative and quantitative data on bivalve larvae settlement using mesh bag collectors installed in the ecological network Natura 2000 site Šćedro – undersea (HR3000119). The data obtained are significant for assessing biodiversity in the area and for providing early warnings about potential non-native species. The results are valuable for program planning and implementing habitat protection in sites that are also known as *P. nobilis* habitat. Further research, analysis, and monitoring are recommended.

References

- Bakran-Petricioli, T., Kujundžić, D., Naranda, M., Petricioli, D., Petricioli, L., Kipson, S. (2023). Fouling Community on *Pinna nobilis* Larval Collectors in the Adriatic—Impact of Invasive Species. *Journal of Marine Science and Engineering*, 11(3), 618. Available from: <https://doi.org/10.3390/jmse11030618>
- Bratoš Cetinić, A., Grdan, S. i Bolotin, J. (2023). Rayed pearl oyster *Pinctada radiata* (Leach, 1814) (Bivalvia: Pteriidae) in the eastern Adriatic Sea – recent observations. *Naše more*, 70 (3 Special issue): 184-188. Available from: <https://doi.org/10.17818/NM/2023/SI7>
- Cabanellas-Reboredo M, Vázquez-Luis M, Mourre B, Álvarez E, Deudero S, Amores A, Addis P, Ballesteros E, Barraón A, Coppa S, García-March JR, Giacobbe S, GiménezCasalduero F, Hadjioannou L, Jiménez Gutiérrez SV, Katsanevakis S, Kersting D, Mačić V, Mavrič B, Patti FP, Planes S, Prado P, Sánchez J, Tena-Medialdea J, de Vaugelas J, Vicente N, Belkhamssa FZ, Župan I, Hendriks IE. (2019). Tracking a mass mortality outbreak of pen shell *Pinna nobilis* populations: A collaborative effort of scientists and citizens. *Scientific reports*, 9: 1-11.
- Costello, M.J., Embrow C., White, R.J. (2001). European register of marine species: a check-list of the marine species in Europe and a bibliography of guides to their identification. Collection Patrimoines Naturel, 50. Muséum national d'Histoire naturelle, Paris, 463 pp.
- Kersting DK, García-March JR. (2017). Long-term assessment of recruitment, early stages and population dynamics of the endangered Mediterranean fan mussel *Pinna nobilis* in the Columbretes Islands (NW Mediterranean). *Marine Environmental Research*, 130: 282-292.
- Kersting DK, Benabdi M, Čizmek H, Grau A, Jimenez C, Katsanevakis S, Öztürk B, Tuncer S, Tunesi L, Vázquez-Luis M, Vicente N, Otero Villanueva M. (2019). *Pinna nobilis*. The IUCN Red List of Threatened Species 2019. IUCN, 22 str.
- Kersting DK, Hendriks IE. (2019). Short guidance for the construction, installation and removal of *Pinna nobilis* larval collectors. IUCN, 6 str.
- Sea and Karst, (2017). Public institution for the management of protected areas of nature in the Split-Dalmatia County Sea and Karst: Study on the protection of the underwater environment of the Natura 2000 ecological network at the anchorages of the Split-Dalmatia County.
- Marčeta T, Marin MG, Codognotto VF, Bressan M. (2022). Settlement of Bivalve Spat on Artificial Collectors (Net Bags) in Two Commercial Mussel Parks in the North-Western Adriatic Sea. *Journal of Marine Science and Engineering*, 10: 210.
- Mihaljević Ž, Pavlinec Ž, Zupčić IG, Oraić D, Popijač A, Pečar O, Sršen I, Benić M, Habrun B, Zrnčić S. (2021). Noble Pen Shell (*Pinna nobilis*) Mortalities along the Eastern Adriatic Coast with a Study of the Spreading Velocity. *Journal of Marine Science and Engineering*, 9: 764.
- Peraš I, Gvozdenović S, Petović S, Mandić M. (2018). Comparative Analysis of Bivalves Diversity on Experimental Spat Collectors. *Water Research and Management*, 8: 25-31.
- ZZOP, Zavod za zaštitu okoliša i prirode Ministarstva zaštite okoliša i zelene tranzicije (2025): Biportal – Zaštićena područja. Available at <http://www.biportal.hr/>.

ORIGINAL SCIENTIFIC PAPER

Biodiversity of nematodes in an agroforestry system with black locust (*Robinia pseudoacacia* L.) and sunflower (*Helianthus annuus* L.)

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Summary

The diversity of nematode genera and community structure are important indicators of soil health and condition. In this study, nematode communities were analysed in an agroforestry system with black locust and sunflower in three different soil ecosystems: 1) degraded soil (D), 2) agricultural soil (A) and 3) agricultural soil with green manure (Agm). Each plot had three treatments: 1) control (C), 2) mineral fertilisation (M) and 3) organic fertilisation (O). In ecosystem D, D-O had the highest total nematode diversity, in ecosystems A and Agm, A-O and Agm-O had the highest total number of nematode genera, the lowest percentage of phytoparasitic nematodes and the highest percentage of bacterivores, fungivores, omnivores and predators, indicating higher soil fertility and stability.

Key words: nematode community, genus, trophic group, consociation

Introduction

Soils harbour numerous organisms and many of these are beneficial and contribute to the development of a healthy soil ecosystem by participating in various soil processes (Ferris and Tuomisto, 2015). These organisms influence organic matter decomposition, nutrient conversion, biocontrol and some have characteristics that characterise them as ecological indicators (Kibblewhite et al., 2008). Such organisms are nematodes. They have the characteristics required for a good bioindicator: they are numerous, diverse, abundant, occur in almost every habitat and respond quickly to changes in the ecosystem (Brmež et al., 2018). In addition, the study of their community structure provides information on soil processes, soil condition, soil disturbance and other biodiversity in the ecosystem (Bongers, 1990). The trophic groups of nematodes belong to the primary, secondary and tertiary decomposers of organic matter. Phytoparasitic nematodes feed directly on plants, while the others, as consumers of microflora, omnivores or predators, indirectly regulate the decomposition of organic matter and the distribution of nutrients (Urzelai et al., 2000). Their composition reflects the disturbances in the soil caused by fertilisation, cultivation, liming, heavy metal accumulation, pollution, but also by other disturbances in an ecosystem (Neher et al., 1998). The consumers of microflora (bacterivores and fungivores) reflect the bacterial and fungal conditions in the soil as well as their structure and activity (Ingham et al., 1985).

Anthropogenic influences on the soil can change soil quality and resilience as well as soil-related processes and functions (Wagg et al., 2014). Agroforestry, a combination of woody plants and crops, has the potential to mitigate these impacts on soil health and biodiversity. The shading and protection provided by woody plants reduces the negative environmental impacts on agricultural production and has a positive effect on soil fertility, disease infestation, pest control, nutrient and water retention and biodiversity (Dupraz et al., 2005).

The aim of this study was to determine the effects of an agroforestry system with black locust (*Robinia pseudoacacia* L.) and sunflower (*Helianthus annuus* L.) on the abundance of nematodes and trophic groups.

Material and methods

The field work was carried out at the Tenja trial site (Croatia) in an agroforestry ecosystem with black locust in short rotation coppice and sunflowers. It included three different soil ecosystems: 1) degraded soil (D), 2) agricultural soil (A) and 3) agricultural soil with green manure (Agm). Each plot had three treatments: 1) control (C), 2) mineral fertilisation (M) and 3) organic fertilisation (O), with samples taken in three replicates.

The 27 soil samples were taken during vegetation for nematological analyses with a nematological probe in the arable layer. The nematodes were extracted from the soil using the Baermann funnel method (Van Bezooijen, 2006), were counted under a stereozoom microscope (Olympus BX 16). In each sample, at least 100 nematodes were determined to genera using a microscope (Olympus BX 50) and measured with Promicra - QuickPHOTO MICRO 3.1 programme. Identification was performed according to the keys: Bongers, 1988, Andrassy, 2005, 2007 and 2009, and biodiversity of nematode genera and trophic groups (Yeates et al., 1993) were analyzed. Statistical analyses were done using SAS 9.4 Software.

Results and discussion

In this study, nematode communities were analysed in an agroforestry system with black locust (*Robinia pseudoacacia* L.) and sunflower (*Helianthus annuus* L.). The highest total genus diversity (Table 1) and average genus diversity (Figure 1a) in degraded soils was found in the D-O ecosystem, in agricultural soils in the A-O ecosystem, while the highest total genus diversity in agricultural soils with green manure was found in the Agm-O ecosystem (Table 1). Organic fertilisation proved to be the best in all three soil types, but it should be emphasised that species diversity in degraded soils under organic fertilisation was not statistically different from that in agricultural soils. Ekschmit et al. (2001) argue that the diversity of nematode genera reflects both the abiotic conditions and the biotic activity of the soil and show consistent correlations with the activity and mass of nematofauna and microflora and is a good indicator of the rate of organic matter decomposition.

Bacterivores are the most numerous and diverse trophic group in all ecosystems studied, which is extremely important in agricultural land, as a higher percentage of bacterivores is significantly related to soil fertility (Michiels and Traunspurger, 2005). The genera recorded are *Acrobeloides*, *Acrobeles*, *Cephalobus*, *Cervidellus*, *Chiloplacus*, *Diploscapter*, *Eucephalobus*, *Geomonhystera*, *Heterocephalobus*, *Panagrolaimus*, *Plectus* and *Wilsonema* as well as the family *Rhabditidae*. The listed genera are widely distributed in agroforestry systems in the Republic of Croatia (Puškarić, 2022), with the exception of the genus *Wilsonema*, which favours degraded soils in this study. (Table 1.).

In the study by Bai et al. (2018), the phytoparasitic genus *Tylenchus* was widely distributed in the agroforestry ecosystem, and in this study, *Tylenchus* was slightly more dominant in the degraded soil ecosystems (D), but the most dominant genus in the other systems was *Pratylenchus* (Table 1.).

The lowest percentage of phytoparasitic nematodes in the community in degraded soils was found in ecosystem D-C, in agricultural soils in ecosystem A-O and in agricultural soils with green manure in ecosystem Agm-O. The highest percentage of microbivores, i.e. bacterivores and fungivores, was also observed in these ecosystems (Figure 1b). Bacterivores and fungivores contribute to ecosystem services in the mineralisation of nutrients and are also food for predatory nematodes that regulate phytoparasites and act as biological control (Zhang et al., 2017). Large numbers of prey support predatory nematodes abundance (Sánchez-Moreno et al., 2011), and this correlation is visible in A-O and Agm-O ecosystems, which have the highest percentage of predators and, in general, persists in the nematode community, i.e. predators and omnivores together. Although Li et al. (2016) argue that omnivores and predators are sensitive to environmental change and negatively associated with nutrient-rich resources, our

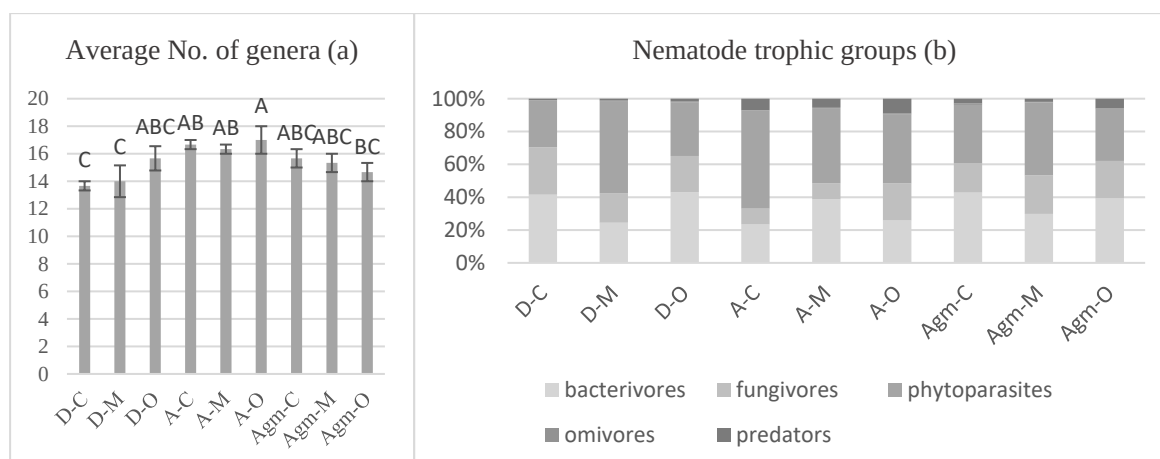
studies confirm the sensitivity of omnivores to disturbance but not to resource availability. The most dominant family among omnivores in the topsoil of the studied ecosystems was the family *Dorylaimidae* with the species *Epidorylaimus*, *Eudorylaimus*, *Microdorylaimus* and *Prodorylaimus*. *Aporcelaimellus* and *Pungentus* were also detected (Table 1.).

Table 1. Percentage of nematode genera diversity under different ecosystems and treatments

	Genus \ Treat.	D-C	D-M	D-O	A-C	A-M	A-O	Agm-C	Agm-M	Agm-O
Bacterivores	<i>Acrobeloides</i>	29,03	9,83	15,32	4,81	7,06	8,19	14,90	15,49	13,70
	<i>Acrobeles</i>	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	<i>Cephalobus</i>	2,51	0,49	0,90	3,48	0,31	0,00	1,15	0,52	2,23
	<i>Cervidellus</i>	0,72	0,00	1,80	0,27	0,00	0,00	0,00	0,00	0,00
	<i>Chiloplacus</i>	0,36	0,00	0,00	4,55	0,00	0,29	0,00	0,00	0,00
	<i>Diploscapter</i>	0,00	0,00	0,30	0,27	0,00	0,00	0,00	0,00	0,00
	<i>Eucephalobus</i>	0,00	0,00	0,30	0,00	0,00	0,58	2,01	1,05	1,59
	<i>Geomonhystera</i>	0,72	0,74	0,30	0,00	0,31	0,00	0,00	0,00	0,32
	<i>Heterocephalobus</i>	4,30	5,65	7,21	4,55	6,44	6,73	5,16	3,15	1,27
	<i>Panagrolaimus</i>	0,00	0,00	0,00	0,00	0,00	0,58	0,00	0,00	0,32
	<i>Plectus</i>	0,00	0,25	0,00	0,00	1,53	0,58	1,15	1,05	0,00
	<i>Rhabditidae</i>	1,79	2,95	10,81	1,87	8,59	0,88	2,87	2,89	7,33
	<i>Wilsonema</i>	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	<i>dauer larvae</i>	3,58	4,42	6,61	3,21	15,03	8,48	15,47	5,51	13,70
Fungivores	<i>Aphelenchoides</i>	6,81	8,11	4,50	2,94	0,92	2,92	2,01	5,51	1,59
	<i>Aphelenchus</i>	12,54	7,62	9,91	3,74	5,22	14,04	15,19	12,60	17,52
	<i>Diphtherophora</i>	0,00	0,00	1,20	0,00	0,00	0,00	0,00	0,00	0,00
	<i>Ditylenchus</i>	8,96	2,21	6,01	2,14	3,07	3,51	0,29	5,25	1,91
	<i>Filenchus</i>	1,08	0,00	0,30	0,53	1,23	2,05	0,29	0,00	2,55
Pytoparasities	<i>Gracilacus</i>	0,36	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,32
	<i>Helicotylenchus</i>	0,36	5,90	1,50	31,02	15,65	21,05	14,33	32,28	20,70
	<i>Paratylenchus</i>	0,72	0,00	0,00	0,00	0,31	0,29	0,86	2,89	0,00
	<i>Pratylenchus</i>	6,45	25,31	6,01	9,63	2,76	4,39	6,88	2,89	3,50
	<i>Rotylenchus</i>	0,72	7,37	0,00	0,00	3,37	0,00	0,00	0,00	0,00
	<i>Tylenchorhynchus</i>	3,58	9,83	3,30	3,74	11,04	2,34	6,88	2,62	0,32
	<i>Tylenchus</i>	13,98	7,37	21,62	16,04	11,66	13,45	6,88	4,20	4,78
Omnivores	<i>Aporcelaimellus</i>	0,36	0,25	0,60	0,27	0,31	0,29	0,57	0,00	0,00
	<i>Epidorylaimus</i>	1,08	0,49	1,20	3,21	2,15	2,34	1,43	0,52	4,46
	<i>Eudorylaimus</i>	0,00	0,00	0,00	0,27	0,00	0,88	0,00	0,00	0,64
	<i>Microdorylaimus</i>	0,00	0,00	0,30	2,94	0,92	4,68	1,43	0,26	0,00
	<i>Prodorylaimus</i>	0,00	0,00	0,00	0,00	0,31	0,58	0,00	0,00	0,00
	<i>Pungentus</i>	0,00	0,00	0,00	0,00	0,00	0,00	0,29	0,26	0,32
Predators	<i>Anatonchus</i>	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,52	0,32
	<i>Clarkus</i>	0,00	0,49	0,00	0,53	1,53	0,58	0,00	0,26	0,32
	<i>Mylonchulus</i>	0,00	0,00	0,00	0,00	0,00	0,29	0,00	0,26	0,32
	<i>Tripyla</i>	0,00	0,25	0,00	0,00	0,31	0,00	0,00	0,00	0,00
	Σ genera	13	14	15	16	16	17	19	20	22

D - degraded soil, A - agricultural soil, Agm - agricultural soil with green manure, C - control, M - mineral fertiliser, O - organic fertiliser

Agroforestry ecosystems play an important role in preventing soil loss and improving land use efficiency. In addition, the presence of trees in an agroforestry system can maintain soil moisture longer (Meng and Zhang, 2004), which is beneficial to the diversity of nematode communities, and organic fertilisation has further promoted the development of nematode communities in all three ecosystems.



D - degraded soil, A - agricultural soil, Agm - agricultural soil with green manure, C - control, M - mineral fertiliser, O - organic fertiliser. Values are means of three replicates $\pm$ the standard error (SE). Different letters above the bars indicate significant differences according to Fisher's LSD test ($p \leq 0.05$) among different ecosystems and treatments.

Figure 1. Average number of nematode genera (a) and Nematode trophic groups (b) under different ecosystems and treatments.

Conclusions

In all three agroforestry ecosystems, the ecosystem with degraded soils (D), the ecosystem with agricultural soils (A) and the ecosystem with agricultural soils with green manure (Agm), the highest total diversity of nematode genera was found in the ecosystems with organic fertilisers (D-O, A-O, Agm-O). The lowest percentage of phytoparasitic nematodes and the highest percentage of microbiovores in degraded soils was found in ecosystem D-C, in agricultural soils in ecosystem A-O and in agricultural soils with green manure in ecosystem Agm-O.

Agroforestry ecosystems play an important role in preventing soil loss, improving land use efficiency and maintaining soil moisture, which has a favourable effect on the diversity of nematode communities. In addition, organic fertilisation further promoted the development of nematode communities in all three agroforestry ecosystems.

References

- Andrassy I. (2005). *Pedozoologica Hungarica* No. 3.: Free-living nematodes of Hungary (Nematoda errantia) Volume I, Hungarian National History Museum, Budapest, Hungary
- Andrassy I. (2007). *Pedozoologica Hungarica* No. 4.: Free-living nematodes of Hungary (Nematoda errantia) Volume II, Hungarian National History Museum, Budapest, Hungary.
- Andrassy I. (2009). *Pedozoologica Hungarica* No. 5.: Free-living nematodes of Hungary (Nematoda errantia) Volume III, Hungarian National History Museum, Budapest, Hungary.
- Bai P. H., Liu Q. Z., Li X. Y., Liu Y. B., Zhang L. L. (2018). Response of the wheat rhizosphere soil nematode community in wheat/walnut intercropping system in Xinjiang, Northwest China. *Applied entomology and zoology*, 53(3): 297-306.
- Bongers T. (1988). *De nematoden van Nederland* (KNNV Bibliotheekuitgave 46). 408. Pirola, Schoorl.
- Bongers T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83(1): 14-19.
- Brmež M., Puškarić J., Siber T., Raspuđić E., Grubišić D., Popović B. (2018). Influence of liquid chicken manure preparation on soil health and agrochemical soil properties. *Poljoprivreda*, 24(1): 3-9.
- Dupraz C., Burgess P., Gavaland A., Graves A., Herzog F., Incoll L., ... van der Werf W. (2005). Synthesis of the silvoarable agroforestry for Europe project. INRA-UMR System Editions, Montpellier, 254.

- Ekschmitt K., Bakonyi G., Bongers M., Bongers T., Boström S., Dogan H., ...Wolters V. (2001). Nematode community structure as indicator of soil functioning in European grassland soils. *European Journal of Soil Biology*, 37(4): 263-268.
- Ferris H., Tuomisto H. (2015). Unearthing the role of biological diversity in soil health. *Soil Biology and Biochemistry*, 85: 101-109.
- Ingham R. E., Trofymow J. A., Ingham E. R., Coleman D. C. (1985). Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological monographs*, 55(1): 119-140.
- Kibblewhite M. G., Ritz K., Swift M. J. (2008). Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1492): 685-701.
- Li X.Y., Lewis E. E., Liu Q., Li H., Bai C., Wang Y. (2016). Effects of long-term continuous cropping on soil nematode community and soil condition associated with replant problem in strawberry habitat. *Scientific reports*, 6(1): 1-12.
- Meng P., Zhang J. S. (2004). Effects of pear-wheat inter-cropping on water and land utilization efficiency. *Forest Research-Chinese Academy Of Forestry*, 17(2): 167-171.
- Michiels I. C., Traunsperger W. (2005). Impact of resource availability on species composition and diversity in freshwater nematodes. *Oecologia*, 142(1): 98-103.
- Neher D. A., Easterling K. N., Fiscus D., Campbell C. L. (1998). Comparison of nematode communities in agricultural soils of North Carolina and Nebraska. *Ecological Applications*, 8(1): 213-223.
- Puškarčić J. (2022). Nematode kao bioindikatori stanja ekosustava tla u konsocijaciji drvenastih vrsta i poljoprivrednih kultura, doktorska disertacija, Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Osijek.
- Sánchez-Moreno S., Ferris H., Young-Mathews A., Culman S. W., Jackson L. E. (2011). Abundance, diversity and connectance of soil food web channels along environmental gradients in an agricultural landscape. *Soil Biology and Biochemistry*, 43(12), 2374-2383.
- Urzelai A., Hernández A. J., Pastor J. (2000). Biotic indices based on soil nematode communities for assessing soil quality in terrestrial ecosystems. *Science of the Total Environment*, 247(2-3): 253-261.
- Van Bezooijen J. (2006). Methods and techniques for nematology. Wageningen: Wageningen University.
- Wagg C., Bender S. F., Widmer F., Van Der Heijden M. G. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, 111(14): 5266-5270.
- Yeates G. W., Bongers T., De Goede R. G., Freckman D. W., Georgieva S. S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of nematology*, 25 (3): 315-331.
- Zhang X., Ferris H., Mitchell J., Liang W. (2017). Ecosystem services of the soil food web after long-term application of agricultural management practices. *Soil Biology and Biochemistry*, 111: 36-43.

ORIGINAL SCIENTIFIC PAPER

The Importance of Slavonian Oak Forests and Field Hedges for the Biodiversity of Slavonian Fields

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Summary

The aim of the paper is to present oak forests (*Quercus robur* L.) and field hedges as guardians of biodiversity and as determinants of the anthropogenic agricultural landscape of Slavonia. The drying of oak forests began in 1900 and continues to this day. Within the agricultural landscape of Slavonia, they appear as patches and are its main landscape feature. The biological diversity of forests in Slavonia is currently endangered. Field hedges are also a determinant of the agricultural landscape of Slavonia and form a dividing line between individual plots or roads and canals. They are guardians of biodiversity, but with the expansion of arable land, they are disappearing today. 38 plant species of trees, shrubs and climbers have been determined within field hedges.

Key words: Slavonia, agriculture, biodiversity, endangered

Introduction

Within the agricultural landscape of Slavonia, pedunculate oak forests and field hedges appear as its basic features. With the increase in arable land, for the purpose of growing mostly field crops, they disappear. With them, the necessary biodiversity is also lost. Croatia belongs to the Pannonian Basin. The first National Forest Inventory in the Republic of Croatia was published in 2010 and it lists categorical variables of stand, habitat and economic characteristics (Čavlović, 2010). According to the Regulation on the List of Habitat Types and Habitat Map (Narodne novine, broj 27/21, 101/22), and areal distribution according to Plišo Vusić et al., (2019.) the following pedunculate oak associations are found in the area of Slavonia: Floodplain forests of pedunculate oak, black alder and field ash; Floodplain forests of pedunculate oak; Pedunculate oak forest with large yellow sedge, subassociation with scattered sedge (subas. *Genisto elatae Quercetum roboris caricetosum remotae* Horvat 1938). - the area of this habitat type occupies the lowland areas of Croatia; Pedunculate oak forest with large yellow sedge, subassociation with quaking sedge (subassociation *Genisto elatae-Quercetum roboris caricetosum brizoides* Horvat 1938), Pedunculate oak forest with large yellow sedge, subassociation with Tatarian maple (subassociation *Genisto elatae-Quercetum roboris aceretosum tatarici* Rauš 1975); Mixed oak-hornbeam and pure hornbeam forests (*Erythronio-Carpinion* associations /Horvat 1958/ Marinček in Mucina et al. 1993 and *Carpinion betuli* Isler 1931); Pedunculate oak and common hornbeam forest, typical subassociation (subas. *Carpino betuli-Quercetum roboris typicum* Rauš 1975) – Occurs throughout the entire range of pedunculate oak in the Pannonian part of Croatia; Pedunculate oak and common hornbeam forest, subassociation with beech (subas. *Carpino betuli-Quercetum roboris fagetosum* Rauš 1975) – this habitat type develops in lowlands exclusively at the highest positions outside the reach of floodwater; Pedunculate oak and common hornbeam forest, subassociation with

Turkey oak (subassociation. *Carpino betuli-Quercetum roboris quercetosum cerris* Rauš 1971) Distributed - in eastern Croatia, western Srijem, Baranja, and locally in Podravina; Pedunculate oak and common hornbeam forest, subassociation with silver-leaved linden (subassociation. *Carpino betuli Quercetum roboris tilietosum tomentosae* Rauš 1971). Although the drying of oak forests has been recorded since the 19th century, a more significant drying began with the extinction of the lowland elm (due to Dutch disease), and repeated severe attacks of pests and diseases (gopher, goldenrod, cuckoo's eye, etc.) in the first half of the 20th century (Plišo Vusić et al., 2019). The factors of drying are the increasing anthropization of forests that began in the early 19th century, inappropriate deforestation. Immunologically weakened plant organisms are attacked by diseases and pests. The expansion of agricultural land and intensive agriculture are the main causes of the disappearance of field hedges.

The aim of this paper is to show the importance of forests and field hedges for the preservation of biodiversity in the area of intensive agriculture.

Material and methods

The available literature was studied. The research for field hedges included 10 locations near the city of Osijek and the determination of trees, shrubs and climbers within field hedges. The analysis within the scope of the intervention was carried out on an area of 1m², using the method of random squares (Kershaw 1973). The plant species are presented in a table with Latin names, families, life form and flora element. The observation was carried out in the period from 01. 03. 2023 to 12. 11. 2024. The determination was carried out according to Domac (1994) and Knežević (2006), the nomenclature of plant species was harmonized with the Flora Croatica Database (Nikolić, 2022). The life-form of the mature plant is classified according to the system of Raunkiaer (1934), as modified by Rauš and Šegulja (1983). The following designations were used for life forms: H-Hemicryptophyta, P-Phanerophyta, G-Geophyta, N-Nanofanerofita. Plant species are classified according to Gajić (1980) into the following floral elements (FE): Eur.-Eurasian; subeur.-subeurasian; Cirkeur.-m.-Circum-European Mediterranean; circ.-circumpolar; subcirc.-subcircumpolar; se.- Central European; subse.-sub.-central European; subatl.-subatlantic; subatl.-subm.-sub-Atlantic-sub-Mediterranean; subpont.-ca.-subpontian-central-Asian-submediterranean; Pont.-pan.-Pontic-Pannonian; subpont.-subpontic; pont.-subm.-Pontic-submediterranean; subpont.-subm.-subpontic-submediterranean; subpont.-ca.-subm.-subpontian-central Asian-submediterranean; pont.-subm.-pan-pontic-submediterranean-pannonian; adv.-adjunctive plant.

Results and discussion

Forests are some of the most biodiverse habitats on Earth. Ensuring strong and sustained protection of World Heritage forests and surrounding landscapes is crucial for maximizing their value as solutions to climate change mitigation, climate change adaptation and biodiversity conservation (Carvalho Resende et al, 2021). It can be concluded that Croatia is well covered with forests because the total area of forests and forest lands in Croatia is 2,759,039 hectares, which makes up 49.3 percent of the country's land area (Sitaš and al., 2022). However, the continuous reduction of forest fund areas and forest degradation is present in the world and here in Croatia. The Republic of Croatia is making efforts to prevent the drying out of oak forests. According to the Ministry of Environmental Protection and Green Transition of the Republic of Croatia (<https://www.haop.hr/hr/tematska-podrucja/>), pedunculate oak forms ecologically very important forest communities that are habitats for numerous endangered and/or protected plant, animal and fungal species, and the causes of the drying out are: the extinction of the lowland elm (due to Dutch disease), and repeated attacks of pests and diseases (lepidoptera, goldenrod, cuckoo's eye, etc.) in the first half of the 20th century. The decline of pedunculate oak has been recorded in Croatia since 1900, and anthropization reached its peak during the era

of industrialization and urbanization in the 19th century (Župan and Skenderović, 2017). Comparing maps from the first military survey of the Austrian monarchy from the 18th century (<https://maps.arcanum.com>) with the current state of forest areas in the same area from Osijek to Čepin, one can see the devastation of the forest area and the draining of the swamp for agricultural purposes, and this particularly applies to the large swam Palača and Kolođvarska bara, which had an area of more than 23,000 acres, and which began to dry out in the 19th century (Živaković Kerže, 2008). Prpić (1989) points out that in the lowland forests of Croatia, certain species of forest trees are endangered, which make up the majority of them in terms of wood mass and surface area, which indicates that the structure of lowland forests has been disrupted. The problem that appears as its significant consequence is significant in forestry and economic terms, and especially in nature conservation. Today, the drying of pedunculate oak is a consequence of inappropriate water engineering interventions (construction of canals, embankments, etc.), and the reasons for the threat include the inappropriate use of mechanization in forest exploitation, neglect of drainage when building forest roads, climate change, polluted floodwater, immission acidification (acid rain and dry deposition), automobile traffic, intensive agriculture, the use of pesticides in forests, landfills, etc. Baričević (1999) points out that research confirms that more than 700 ha of the most valuable pedunculate oak forests, at the time of drying about thirty years ago, suffered very intense and sudden external and internal changes because they suffered the drying of the main tree species, changes in habitat conditions and forest communities, and negative succession of forest vegetation. He also emphasizes that the greatest negative changes occurred (and are occurring) in the community of pedunculate oak and large yellow sedge with quaking sedge. Matić (2012), studying the effect of forests on grapevine cultivation on the slopes of Krndija, comes to the conclusion that, due to all of the above, we can claim that the impact of forests on agricultural production, and especially on viticulture and winemaking, is significant and large. The Ministry of Agriculture, Fisheries and Forestry, through the European Agricultural Fund for Rural Development, encourages special measures and interventions (Intervention 73.05. Investments – Reconstruction (conversion) of degraded forests, restoration of degraded forest areas in the agricultural zone (<https://ruralnirazvoj.hr/intervencije/73-05>) and emphasizes their contribution to biodiversity and soil protection. Oak forests greatly affect the level of groundwater. The level of temporary groundwater strongly depended on the seasonal rhythm of water absorption by pedunculate oak trees and on the behavior of soil shrinkage and swelling (Vincke and Delvaux, 2005). Field hedges delimit fields and plots and usually run along the edges of canals and field paths. They also separate agricultural areas from main roads. They have the function of marking the boundaries of individual plots, the function of protecting against dust and the passage of wildlife, the function of protecting against wind, the function of preserving plant and animal species by providing them with a safe habitat, and ultimately serve to preserve biodiversity. Plant species within field hedges were examined at ten locations: five locations were selected between Cret Bizovački, Topoline and Normanci, and the other 5 locations represent hedges within agricultural areas within the willage of Antunovac and Ernestinovo. A list of plant species recording in the field hedges of the study area, their life form and floral element, are presented in Table 1.

Table1. List of plant species, family affiliation, life form, floral element

Latin name of the plant species	Family	Life form	Floral element
<i>Acer campestre</i> L.	Aceraceae	P	Subse.
<i>Acer negundo</i> L.	Aceraceae	P	Adv
<i>Acer platanoides</i> L.	Aceraceae	P	Subse
<i>Carpinus betulus</i> L.	Betulaceae	P	Se
<i>Corylus avellana</i> L.	Betulaceae	N	Subse
<i>Humulus lupulus</i> L.	Cannabaceae	H	Eur

<i>Sambucus ebulus</i> L.	Caprifoliaceae	G	Subpont.-subm
<i>Sambucus nigra</i> L.	Caprifoliaceae	N	Subse
<i>Euonymus europea</i> L.	Celastraceae	N	Subse
<i>Euonymus verrucosa</i> Scop.	Celastraceae	N	Subpont
<i>Cornus mas</i> L.	Cornaceae	P	Pont.- subm
<i>Cornus sanguinea</i> L.	Cornaceae	N	Subse
<i>Amorpha fruticosa</i> L.	Fabaceae	N	Adv
<i>Robinia pseudoacacia</i> L.	Fabaceae	P	Adv
<i>Juglans regia</i> L.	Juglandaceae	P	Subiran.-is-subm
<i>Morus alba</i> L.	Moraceae	P	Adv
<i>Morus nigra</i> L.	Moraceae	P	Adv
<i>Ligustrum vulgare</i> L.	Oleaceae	N	Subse
<i>Reynoutria japonica</i> Houtt	Polygonaceae	G	Subeur.-subeurasian
<i>Clematis vitalba</i> L.	Ranunculaceae	P	Subatl.-subm
<i>Rhamnus catharticus</i> L	Rhamnaceae	N	Subpont.-ca
<i>Crataegus laevigata</i> (Po'r.) DC.	Rosaceae	P	Subse
<i>Crataegus monogyna</i> Jacq,	Rosaceae	P	Subse
<i>Crataegus pentagyna</i>	Rosaceae	P	Pont.-pan
<i>Malus sylvestris</i> Mill.	Rosaceae	P	Subse
<i>Prunus avium</i> L.	Rosaceae	P	Subse
<i>Prunus cerasifera</i> (Ehrh)	Rosaceae	P	Circ
<i>Prunus cerasus</i> L.	Rosaceae	P	Subse
<i>Prunus spinosa</i> L.	Rosaceae	P	Subpont.
<i>Pyrus pyraister</i> (L.) Burgsd.	Rosaceae	P	Cirkeur.- m
<i>Rosa canina</i> L.	Rosaceae	N	Subse
<i>Rosa rugosa</i> Thunb.	Rosaceae	N	Subcirk
<i>Rubus fruticosus</i> L	Rosaceae	N	Subeur
<i>Populus alba</i> L.	Salicaceae	P	Subj-sib
<i>Salix alba</i> L.	Salicaceae	P	Subeur
<i>Salix caprea</i> L.	Salicaceae	P	Eur
<i>Salix purpurea</i> L.	Salicaceae	P	Subeur
<i>Ailanthus altissima</i> (Mill.) Swingle	Simaroubaceae	P	Circ.-circumpolar

Table 1 gives the basic taxonomic analysis, the recorded flora contains 38 species distributed in 24 genera and 27 families of vascular plants. The analyses of plant life forms showed the dominance of phanerophytes (63%), followed by nanophanerophytes (29%), geophytes (5%), and hemicryptophytes.

Conclusions

Pedunculate oak forests in Slavonia represent patches and are determinants of the agricultural landscape. They preserve a large biodiversity that is becoming endangered with the decline of pedunculate oak. Field hedges are also determinants of the landscape and represent the conservation of biodiversity along field corridors, roads and canals. Greater efforts are needed to preserve them.

References

- Baričević, D. (1999). Ecological and vegetative changes in pedunculate oak forests in the management unit "žutica" Šumarski list br. I 217-2X: 17-28
- Carvalho Resende, T., Gibbs, D., Harris, N., Osipova, E. (2021). World Heritage forests Carbon sinks under pressure, the United Nations Educational, Scientific and Cultural Organization (UNESCO), 7, place de Fontenoy, Gland, Switzerland
- Čavlović, J. (2010) Prva nacionalna inventarizacija šuma Republike Hrvatske, Ministarstvo regionalnog razvoja šumarstva i vodnoga gospodarstva; Šumarski fakultet Sveučilišta u Zagrebu
- Domac, R. (1994). Flora Hrvatske, Školska knjiga, Zagreb.

- Gajić, M. (1980). Pregled vrsta SR Srbije sa biljnogeografskim oznakama. Glasnik Šumarskog Fakulteta, Beograd:54,
- Kershaw, K. A. (1973). Quantitative and dynamic plant ecology. Edward Arnold. London.
- Knežević, M. (2006). Atlas korovne, ruderalne i travnjačke flore, Sveučilište Josipa Jurja Strossmayera, Osijek, Poljoprivredni fakultet u Osijeku
- Matić, S. (2012). Značenje šuma za poljoprivrednu proizvodnju, Radovi Zavoda za znanstvenoistraživački i umjetnički rad u Bjelovaru, Sv. 6: 47-68
- Ministry of Environmental Protection and Green Transition of the Republic of Croatia (2017). Hrast lužnjak (*Quercus robur* L.). Available from: <https://www.haop.hr/hr/tematska-podrucja/prirodne-vrijednosti-stanje-i-ocuvanje/bioraznolikost/flora/hrast-luznjak-quercus>
- Narodne Novine 27/21, 101/22. Regulation on the List of Habitat Types and Habitat Map
- Nikolić, T. (2022). Flora Croatica Database-Vascular Plants Taxonomy & Bibliography of Croatian Flora Sastavnica Informacijskog sustava zaštite prirode. Available from <https://hirc.botanic.hr/fcd/>
- Plišo Vusić, I., Šapić, I., Vukelić, J.(2019). Prepoznavanje i kartiranje šumskih staništa Natura 2000 u Hrvatskoj (II)– 91F0, Poplavne šume s vrstama *Quercus robur*, *Ulmus laevis*, *Ulmus minor* i *Fraxinus angustifolia*; 91L0, Hrastovo grabove šume Ilirskoga područja, Šumarski list, 9–10, CXLIII (2019): 462–465
- Prpić, B. 1989: Sušenje hrasta lužnjaka (*Quercus robur* L.) u Hrvatskoj u svijetlu ekološke konstitucije vrste. Glas. šum. pokuse 25: 1—21.
- Raunkiaer, C. 1934. The Life Forms of Plants and Statistical Plant Geography. Oxford University Press, London
- Rauš, Đ., N. Šegulja, 1983: Flora Slavonije i Baranje. Glasnik za šum. pokuse. 21, 179—211.
- Sitaš, B. Milković, I., Žagar, K., Juričić Musa, A., Stankić, M., Kratofil, L., Devčić, I., Stojić, M., Vrebčević, M., Vargović, L., Vincenc, G. (2022), Hrvatske šume, d.o.o. Godišnje izvješće, Zagreb
- Vincke and Delvaux, (2005) Porosity and available water of temporarily waterlogged soils in a *Quercus robur* (L.) declining stand, Plant and Soil, Vol. 271, Springer:189–203
- Živković Kerže, Z. (2008). van Nepomuk Spannbauer i njegovo značenje za odvodnju osječškoga kraja (Poseban osvrt na isušivanje močvare Palača). Godišnjak Njemačke narodnosne zajednice Vol. 15. No 1: 117-126
- Župan D., Skenderović R. (2017). Slavonske šume kroz povijest, Zbornik radova znanstvenog skupa s međunarodnim sudjelovanjem, Slavonskom Brodu

PRELIMINARY COMMUNICATION

The impact of trap design on the efficiency of blue crab fishing

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Summary

Sustainable fishing practices, the conservation of biodiversity and the control of invasive and non-native species require new tools in Mediterranean marine, brackish and freshwater habitats. Non-selective fishing traps are life-threatening tools for lung-breathing protected species bycatch, such as turtles. The aim of this research was to examine how two modifications of the entrance to prevent turtle bycatch on three different trap designs affect the catch of blue crab (*Callinectes sapidus*). Average catch was compared per trap type using nonparametric tests. Preliminary results show the metal cage traps are more efficient than cylindrical traps for catching blue crab.

Key words: biodiversity conservation, non-native species control, freshwater turtles

Introduction

Blue crab (*Callinectes sapidus* Rathbun, 1896) is a non-native species in the Mediterranean originating from the western Atlantic, where as an economically important species supports fishing industry in the USA fisheries (Kevrekidis et al., 2023). Since the first records in Europe in 1901, it was observed in the Mediterranean in 1947 (Mancinelli et al., 2021) and established populations in southern Italy and Croatia and northern coasts of Albania (Lipej and Rogelja, 2021). The first records in Croatia in 2004 place it within the Neretva delta and Ston area (Glamuzina et al., 2023), and it colonized the lagoons and deltas from the northern Adriatic coasts in Istria over the coasts of Dalmatia including the islands (e.g. Brač, Hvar, Korčula) (Tutman et al., 2017). Recent research considers it to be one of the most invasive non-native species in the Mediterranean as a highly prolific, opportunistic predator with tolerance to a wide range of temperature and salinity (Castriota et al., 2024). Despite the above, it is not included in the list of invasive species (EU Regulation 1143/2014/100), but it is recognized as having promising economic potential (Jug-Dujaković et al., 2023). However, blue crab affects biodiversity of coastal marine and freshwater habitats such as the Adriatic deltas that are especially vulnerable to non-native and invasive species. For this reason, commercial fishing presents the only way to control its population and preserve biodiversity (Marchessaux et al., 2023).

Neretva delta is the largest and therefore important fish spawning area for eastern Adriatic and is protected as a part of the Natura 2000 ecological network and ornithology and ichthyology reserve. The presence of endangered species of turtles, European pond turtle (*Emys orbicularis*) and Balkan striped-necked terrapin (*Mauremys rivulata*), has been reported in this area, which, due to their high resistance to salinity can be found in freshwater and brackish habitats (Jelić et al., 2015). Trap fishing, which is often practiced in the area, can easily kill these lung-breathing species because once they enter the trap, they can no longer surface to breathe air. Selective fishing gear that reduces sea turtle bycatch has been developed for North American turtle species and is available for Canadian commercial fisheries (Larocque et al., 2012), while in the Mediterranean Sea only sea turtle bycatch reduction was applied until recently (Lucchetti et al., 2019). To provide the recommended selective fishing tool needed to reduce mortality from

bycatch of endangered fauna and at the same time to successfully catch non-native and invasive Mediterranean crab species (Castriota et al., 2024), two fishing trap types with the entrance modifications were designed and registered as intellectual property (Šalamon and Gavrilović, 2025a; Šalamon and Gavrilović, 2025b). The aim of this research was to examine how these two modifications of the entrance to prevent turtle bycatch on three different trap types affect the catch of blue crab (*Callinectes sapidus*).

Material and methods

Trap efficiency was evaluated during 19 days in three periods: March and July 2024 and February 2025, at two locations in Neretva delta Natura 2000 area where marine fishing recently started to target the blue crab. Three types of fishing traps, each with two different entrance modifications and control, were tested: metal cage trap and two types of cylindrical traps (cylindrical 1 with one entrance and cylindrical 2 with two entrances). Table 1 presents the dimensions and specifications of fishing traps, including entrance details, modifications tested, the number of traps used, and the total fishing time per operation. Modification 1 tested for each trap type was entrance size reduction to form solid ring of 6 to 10 cm in diameter, depending on the trap type. Modification 2, included elements of modification 1 with the additions of a transparent net of 60 mm mesh size applied to the funnel trap entrance proximal to the reduced ring entrance.

Caught specimens were counted in regular intervals of 8 to 12 hours to minimize erroneous records due to possible exit from the trap or predation in the trap. The recorded catch was standardized to daily average catch per one trap of each trap type per hour. After testing if the data belonged to normal distribution using the Shapiro-Wilk normality test, the effect of trap type and fishing occasion on the standardized catch were evaluated using nonparametric Kruskal-Wallis tests and post hoc pairwise Wilcoxon test using the Benjamini and Hochberg (1995) P-value adjustments for multiple comparisons. Statistical testing and graphics were run using the R software.

Table 1. Nine different fishing traps with modification specifications used in the three fishing periods in Neretva delta Natura 2000 area.

periods in Neretva delta Natura 2000 area.									
Fishing trap design	Modification 1			Modification 2			Control		
	03/24	07/24	03/25	03/24	07/24	03/25	03/24	07/24	03/25
Metal cage									
Number used	4	4	4	3	3	3	3	3	3
Fishing time	104	121	126	104	121	126	104	121	126
Specification	1 entrance: fixed ring 10 cm diameter at the end of the funnel, with 25 cm fixed curtain preventing the catch exit			Modification 1 with addition of transparent 6cm net protection of the square funnel entrance 17 x 40 cm (Šalamon and Gavrilović, 2025a)			Metal net (32 mm mesh size) cage trap 80cm x 90cm x 40cm, 1 funnel exit: fixed rhombus shaped 35 x 6 cm at the end of the funnel		
Cylindrical 1									
Number used	4	0	3	3	0	3	3	0	3
Fishing time	104	0	126	104	0	126	104	0	126
Specification	1 entrance: fixed ring 6 cm diameter at the end of the funnel			Modification 1 with addition of transparent 6cm net protection of the ring funnel entrance (30 cm diameter)			Cylindrical collapsible ring supported trap, 30 cm diameter, 150 cm length (32 mm mesh size) with 1 flexible funnel exit of 18 cm diameter and additional internal funnel		

Cylindrical 2									
Number used	4	0	3	3	0	3	3	0	3
Fishing time	104	0	126	104	0	126	104	0	126
Specification	2 entrances: fixed rings 8 cm diameter at the end of the funnels			Modification 1 with addition of transparent 6cm net protection of the ring funnels entrances (30 cm diameter) (Šalamon and Gavrilović, 2025b)			Cylindrical collapsible spiral coil supported trap, 30 cm diameter, 100 cm length (32 mm mesh size) with 2 flexible funnel exits of 20 cm diameter followed by 15 cm curtain preventing the catch exit		

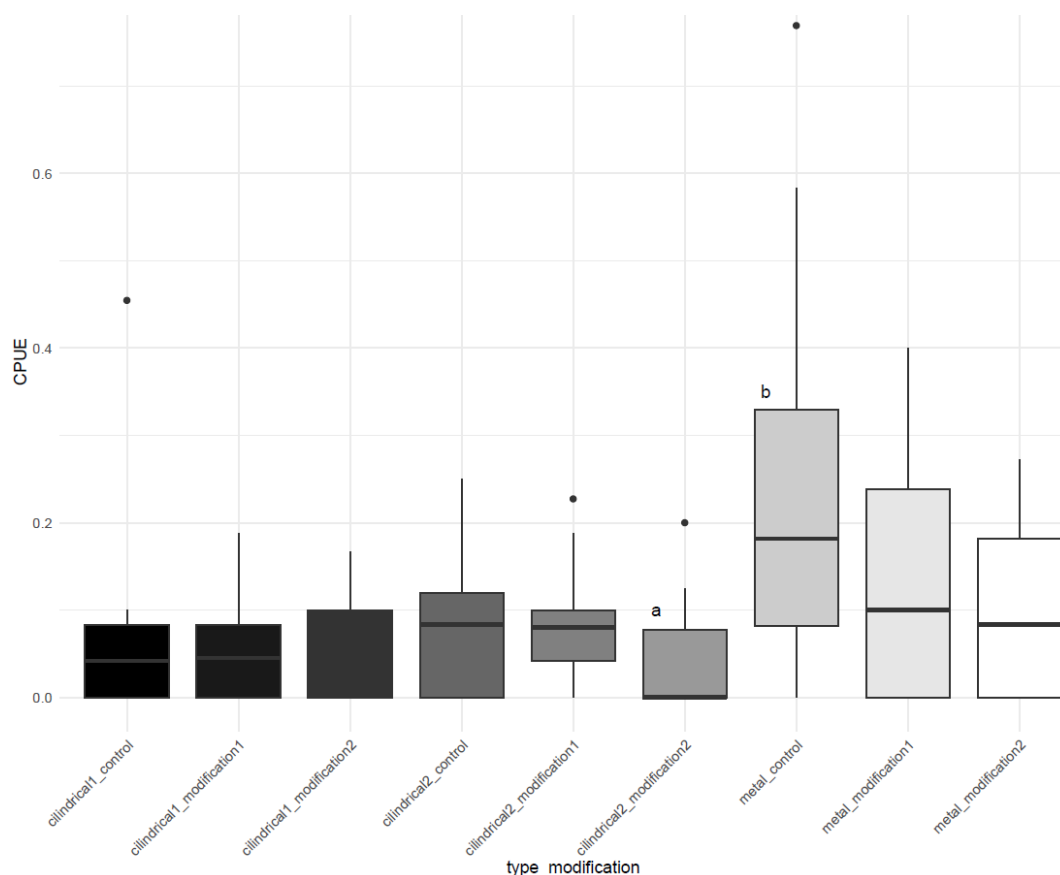
Results and discussion

The average daily crab catch ranged from 0 to 0.77 crabs per trap per hour with an average catch of 0.1 ± 0.13 . Basic statistics on crab catch by trap type and modification are shown in Table 2.

Table 2. Averaged daily catch for all trap types. CPUE – catch per unit effort [crab trap⁻¹ hour⁻¹]

Trap type	Trap modification	CPUE maximum	CPUE median	CPUE mean	CPUE standard deviation
Metal cage:	Control	0.77	0.18 ^a	0.23	0.22
	Modification 1	0.40	0.1	0.13	0.13
	Modification 2	0.27	0.08	0.11	0.09
Cilindrical 1	Control	0.45	0.04	0.07	0.12
	Modification 1	0.19	0.05	0.06	0.06
	Modification 2	0.17	0.04 ^b	0.05	0.06
Cilindrical 2	Control	0.25	0.08	0.08	0.08
	Modification 1	0.23	0.08	0.08	0.07
	Modification 2	0.20	0	0.04	0.06

The null-hypothesis was rejected in Shapiro-Wilk normality test for the catch quantity variable, therefore the non-parametric tests for discerning the differences of the groups were used. Kruskal-Wallis test showed a significant difference in the crab catch between different trap groups ($\chi^2 = 18.12$, $df=8$, $p=0.02$). Post hoc pairwise Wilcoxon test showed the significant difference between the crab catch of the control metal trap and the cylindrical two entrance trap with modification 2 ($p=0.04$). Although differences were observed between other groups of traps tested, they were not statistically significant (Graph 1). Similar to our results for blue crab catch, Larocque et al. (2012) reduce fisheries impacts on turtles using bycatch reduction devices with bars across the net opening with no effect on targeted fish catch rates. Atar et al. (2002) compared the capture efficiency and catch rates of three different traps (collapsible ellipsoid trap, collapsible box trap and hoop nets-traditional traps) for the blue crab fisheries in Beymelek Lagoon, Antalia, Turkey. Although all traps and nets targeted the blue crab, the simple design, operation and efficiency of hoop nets made them most effective, what is somehow similar to our results where cage traps were most effective.



Graph 1. Daily average catch per one trap per hour for each of the nine trap modifications. Groups marked with a and b indexes differ.

Conclusions

Cage traps are more effective than cylindrical traps for harvesting blue crabs in terms of catch rate. Slight differences were observed between other groups of traps tested, but modifications to reduce turtle bycatch did not appear to significantly affect blue crab catch. However, further research is needed to assess the differences between the two cage trap modifications in terms of blue crab catch efficiency.

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References

- Atar, H. H., Ölmez, M., Beka, S., Secer, S. (2002) Comparison of three different traps for catching blue crab (*Callinectes sapidus* Rathbun 1896) in Beymelek Lagoon. Turkish Journal of Veterinary & Animal Sciences, 26 (5), 1145-1150.
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B, 57, 289–300.
- Castriota, L., Falautano, M., Perzia, P. (2024). When nature requires a resource to be used – The case of *Callinectes sapidus* distribution, aggregation patterns, and spatial structure in Northwest Europe, the Mediterranean Sea, and adjacent waters. Biology, 13(4), 279.
- EU Regulation 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive non-native species. Official Journal of the European Union, L 317, 4–20.

- Glamuzina, L., Pešić, A., Marković, O., Tomanić, J., Pećarević, M., Dobrosravić, T., Brailo, M., Conides, A., Grđan, S. (2023). Population structure of the invasive Atlantic blue crab, *Callinectes sapidus* on the Eastern Adriatic coast (Croatia, Montenegro). *Naše more*, 70, 153–159.
- Jelić, D., Kuljerić, M., Koren, T., Treer, D., Šalamon, D., Lončar, M., Podnar-Lešić, M., Janev-Hutinec, B., Bogdanović, T., Mekinić, S., Jelić, K. (2015). *Crvena knjiga vodozemaca i gmazova Hrvatske*. Zagreb, Ministarstvo zaštite okoliša i prirode, Državni zavod za zaštitu prirode, Hrvatsko herpetološko društvo HYLA.
- Jug-Dujaković, J., Radočaj, T., Iveša, N., Špelić, I., Gavrilović, A. (2023). The possibility of commercialization of the catch of the invasive Atlantic blue crab, *Callinectes sapidus* (Rathbun, 1896). 58th Croatian & 18th International Symposium on Agriculture. Book of Abstracts: 155. (Carović-Stanko, K. & Širić, I., eds). Dubrovnik, 11. - 17. February, 2023.
- Kevrekidis, K., Kevrekidis, T., Mogias, A., Boubonari, T., Kantaridou, F., Kaisari, N., Malea, P., Dounas, C., Thessalou-Legaki, M. (2023). Fisheries biology and basic life-cycle characteristics of the invasive blue crab *Callinectes sapidus* Rathbun in the Estuarine Area of the Evros River (Northeast Aegean Sea, Eastern Mediterranean). *Journal of Marine Science and Engineering*, 11(3), 462.
- Larocque, S. M., Cooke, S. J., Blouin-Demers, G. (2012). Mitigating bycatch of freshwater turtles in passively fished fyke nets through the use of exclusion and escape modifications. *Fisheries Research*, 125, 149-155.
- Lipej, L., Rogelja, M. (2021). Status of the invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) in Slovenia. *Acta Biologica Slovenica*, 64(2), 24–33.
- Lucchetti, A., Bargione, G., Petetta, A., Vasapollo, C., Virgili, M. (2019). Reducing sea turtle bycatch in the Mediterranean mixed demersal fisheries. *Frontiers in marine science*, Section Marine fisheries, 6, <https://doi.org/10.3389/fmars.2019.00387>
- Mancinelli, G., Bardelli, R., Zenetos, A. (2021). A global occurrence database of the Atlantic blue crab *Callinectes sapidus*. *Scientific data*, 8(1), 111.
- Marchessaux, G., Mangano, M.C., Bizzarri, S., M'Rabet, C., Principato, E., Lago, N., Veyssiere, D., Garrido, M., Scyphers, S.B., Sarà, G. (2023). Invasive blue crabs and small-scale fisheries in the Mediterranean sea: Local ecological knowledge, impacts and future management, *Marine Policy*, 148, 105461, <https://doi.org/10.1016/j.marpol.2022.105461>.
- Šalamon, D., Gavrilović, A. (2025.a) Registration of industrial design of the fishing trap, Design No. 015091663-0001. European Union Intellectual Property Office (EUIPO). *Community Designs Bulletin* 2025/032.
- Šalamon, D., Gavrilović, A. (2025.b) Registration of industrial design of the fishing trap, Design No. 015091663-0002. European Union Intellectual Property Office (EUIPO). *Community Designs Bulletin*Int, 2025/032.
- Tutman, P., Dulčić, J., Dragičević, B. (2017). The invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea: Portunidae) across the eastern Adriatic Sea, Croatia: permanent resident or just provisional visitor? // 7th ESENIAS Workshop with Scientific Conference Networking and Regional Cooperation Towards Invasive Non-native Species Prevention and Management in Europe. Sofija: Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER–BAS); East and South European Network for Invasive Non-native Species, 129.

ORIGINAL SCIENTIFIC PAPER

Ecological analysis of invasive plant species of the Požega Valley

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Summary

Research on invasive plant species in the Požega Valley has intensified over the last ten years. Monitoring of the spread of invasive species was carried out in 2011 and again in 2023 and 2024. The aim of the work is to determine the habitats where invasive taxa occur based on the values of ecological indices. According to the studies carried out, the analysis of the ecological indices shows the dominance of mesophytes, plants from slightly acidic to neutral habitats as well as mesotrophic and eutrophic habitats. The ecological indicators for invasive plant species indicate that the taxa most prefer moderately and well aerated habitats with medium organic matter content, semi-shaded species dominate and spread most in extremely thermophilic or thermophilic habitats.

Key words: invasive plants, Požega Valley, ecological factors, ecological analysis

Introduction

Invasive species are plant, animal and fungal species that originate from other floristic-zoogeographical areas. They suppress the autochthonous gene pool in the course of competition by invading available ecological niches. This types are usually spread as an accidental by-product of various human activities. The danger of these plant species is that they have a high reproductive capacity and aggressiveness, which suppresses the autochthonous flora of an area. In addition, these species usually have no natural enemies. In the Požega Valley, the flora has been very well researched, as it is a very interesting research area due to the geological, geomorphological and climatic conditions. This is one of the reasons why 1,654 plant species have been recorded in this area so far (Tomašević, 2016). Research on invasive plant species in the Požega Valley has been intensified in the last ten years through the work of Zima (2012), Samardžić and Galić (2019), Zima and Štefanić (2021). The aim of the work is to determine the habitats in which invasive species have spread.

Material and methods

The research area covered the Požega Valley, including the slopes of the surrounding mountains. Monitoring of the distribution of invasive species was carried out in 2011 and again in the summer months of 2023 and 2024. The identification of plant species was carried out using the following floristic "keys": Domac (1994) and Javorka and Csapody (1975), while the nomenclature was harmonized according to Tutin et al. (1964 - 1980). Ecological indices were also separated for each species according to Landolt (1977). The ecological analysis of the invasive flora of the Požega Valley were carried out on the basis of ecological indices for moisture (F), chemical reaction (R), amount of nutrients (N), amount of organic material (H), transparency (D), salinity (S), light (L), warmth (T) and habitat continentality (K).

Results and discussion

A more complete picture of the ecological characteristics of the habitat in which the invasive flora of the Požega Valley has developed was obtained by analyzing ecological indices for basic ecological factors (humidity, chemical reaction, nitrogenous substance content, organic substance content, airiness, light, temperature and continentality) (Table 1). The ecological analysis of invasive, urban and weedy flora was studied by Rakić et al. (2007), Knežević et al. (2009), Ilić and Nikolić (2015), Petrović et al. (2016), Nikolić et al. (2017).

Table 1 List of invasive plant species in the Požega Valley and their ecological indices

Taxa	F	R	N	H	D	L	T	K
<i>Abutilon theophrasti</i> Medik.	2	3	4	3	4	4	5	3
<i>Acer negundo</i> L.	3	3	4	3	4	3	5	2
<i>Ailanthus altissima</i> (Mill.) Swingle	1	3	4	3	3	3	5	2
<i>Amaranthus retroflexus</i> L.	2	3	4	3	3	4	4	3
<i>Ambrosia artemisiifolia</i> L.	3	3	4	2	2	4	5	3
<i>Amorpha fruticosa</i> L.	4	3	3	3	3	3	5	2
<i>Angelica archangelica</i> L.	x	x	x	x	x	x	x	x
<i>Artemisia annua</i> L.	3	3	5	3	4	4	5	4
<i>Asclepias syriaca</i> L.	3	3	3	3	4	4	5	2
<i>Bidens frondosa</i> L.	5	3	4	4	4	4	5	2
<i>Chamomilla suaveolens</i> (Pursh) Rydb.	3	3	5	3	5	4	4	3
<i>Conyza canadensis</i> (L.) Cronquist	2	3	3	3	4	4	4	3
<i>Datura stramonium</i> L.	3	3	4	4	4	4	5	2
<i>Duchesnea indica</i> (Andrews) Focke	3	3	4	3	4	3	5	2
<i>Echinocystis lobata</i> (Michx.) Torr. et Gray	4	3	4	2	4	3	5	2
<i>Eleusine indica</i> (L.) Gaertn.	2	2	4	3	4	4	5	3
<i>Elodea canadensis</i> Michx.	5	4	4	3	x	3	4	3
<i>Erigeron annuus</i> (L.) Pers.	2	3	4	3	4	4	4	3
<i>Erigeron annuus</i> (L.) Pers. ssp. <i>strigosus</i> (Muhlenb. ex Willd.) Wagenitz	2	3	4	3	3	4	4	3
<i>Galinsoga parviflora</i> Cav.	3	2	4	3	4	4	4	2
<i>Helianthus tuberosus</i> L.	3	3	4	3	3	4	5	3
<i>Juncus tenuis</i> Willd.	3	3	3	3	5	4	3	2
<i>Lepidium virginicum</i> L.	2	3	4	2	3	4	4	3
<i>Oenothera biennis</i> L.	2	3	3	3	4	4	4	3
<i>Panicum capillare</i> L.	2	3	4	3	3	4	5	3
<i>Parthenocissus quinquefolia</i> (L.) Pl.	3	3	3	3	4	3	4	3
<i>Phytolacca americana</i> L.	3	3	4	3	3	4	5	2
<i>Reynoutria japonica</i> Houtt.	3	3	4	3	x	3	4	2
<i>Robinia pseudoacacia</i> L.	2	3	4	3	3	3	4	2
<i>Rudbeckia laciniata</i> L.	4	3	4	4	4	3	5	2
<i>Solidago canadensis</i> L.	3	3	3	x	x	3	4	3
<i>Solidago gigantea</i> Aiton	4	3	3	x	x	3	4	3
<i>Sorghum halepense</i> (L.) Pers.	1	2	3	3	3	4	5	3
<i>Xanthium strumarium</i> L. ssp. <i>italicum</i> (Moretti) D. Löve.	3	3	5	3	2	4	5	3

Table 2. shows the ecological analysis of invasive plants in the Požega Valley. The analysis of ecological indices for moisture shows the dominance of mesophytes (medium and variable habitat moisture), as 15 taxa (44.1%) have an ecological moisture factor of 3. The least represented plants of ecological factor 1, which characterizes plants of dry habitats, are *Ailanthus altissima* (Mill.) Swingle and *Sorghum halepense* (L.) Pers. and factor 5, where *Bidens frondosa* L. and *Elodea canadensis* Michx stand out. The mean value of the index is 2.818. As far as the chemical response of the environment is concerned, the absence of plants with ecological indices 1 and 5 is striking. 29 plant taxa (85.3%) have an ecological index of 3, which indicates a slightly acidic to neutral habitat. Among these species *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Conyza canadensis* (L.) Cronquist, *Erigeron annuus* (L.) Pers., *Phytolacca americana* L., *Robinia pseudoacacia* L. are very common and some others stand

out. The mean value is 2.939. The values of the ecological indices for the content of nitrogenous substances range from 3 to 5. Most of the plant taxa, namely 21 (61.8%), are characterized by a score of 4, which means that the plants are associated with nitrogen-rich habitats. It is significant that a total of 30 taxa (88.3%) indicate types of mesotrophic and eutrophic habitats. Here, *Ailanthus altissima* (Mill.) Swingle, *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Lepidium virginicum* L. and *Reynoutria japonica* Houtt stand out due to their frequency. The calculated mean value of the nitrogenous substance content index is 3.818. The analysis of the organic substance content shows the dominance of indicators of medium organic substance content, i.e. plants characterized by a value of 3. There are 25 such taxa (73.5%). The absence of species with an organic matter content eco-factor of 1 and 5 is striking, indicating soils with extremely high and extremely low organic matter content. The average value of this ecological factor is 3.000. The analysis of the indicator values of plants for habitat aeration shows that 15 taxa (44.1%) have an eco- factor of 4, which represents the indicators of moderately permeable soils, and 10 taxa (29.4%) have an eco- factor of 3, which represents well-aerated habitats. Species of poorly aerated habitats are *Chamomilla suaveolens* (Pursh) Rydb. and *Juncus tenuis* Willd. while the plants of well-aerated habitats are *Ambrosia artemisiifolia* L. and *Xanthium strumarium* L. ssp. *italicum* (Moretti) D. Löve. The mean value of the ecological index for soil permeability is 3.586. The values of the ecological indices for habitat lighting show that semi-shaded species predominate and those that grow in full daylight and can tolerate some shade. Indicator value 4 was found for 21 taxa (61.8%) and indicator value 3 for 12 (35.3%) taxa. The mean value of the environmental index for light is 3.636. Among the invasive plant taxa studied, those that thrive in extremely thermophilic (18 - 52.9%) or thermophilic habitats (14 - 40.0%) are most strongly represented. Of the species that live in extremely thermophilic habitats, particularly numerous are: *Abutilon theophrasti* Medik., *Ailanthus altissima* (Mill.) Swingle, *Ambrosia artemisiifolia* L., *Amorpha fruticosa* L., *Artemisia annua* L., *Asclepias syriaca* L., *Datura stramonium* L., *Phytolacca americana* L., *Sorghum halepense* (L.) Pers. and others. Plants with an index of 1 and 2 are not represented. The mean value of 4.515 indicates the dominance of plants from thermophilic habitats. The ecological index continentality indicates the degree of adaptation of the species to the climatic conditions. The plants of the studied flora are mostly adapted to subcontinental climatic conditions. 18 taxa (52.9 %) are described with an indicator value of 3, while 14 taxa (40.0 %) have a significant proportion of plants described with indicator 2, which is characteristic of plants of suboceanic climates. The average value of the ecological factors for continentality is 2.606.

Table 2. Ecological analysis of invasive plants in the Požega Valley

Ecological index	Number of taxa (%)	Number of taxa (%)	Number of taxa (%)	Number of taxa (%)	Number of taxa (%)	Number of taxa (%)	Mean
F Moisture	2 (5.9)	10 (29.4)	15 (44.1)	4 (11.8)	2 (5.9)	1 (2.9)	2.818
R Chemical reaction (pH value)	-	3 (8.8)	29 (85.3)	1 (2.9)	-	1 (2.9)	2.939
N Amount of nitrogen substances	-	-	9 (26.5)	21 (61.8)	3 (8.8)	1 (2.9)	3.818
H Amount of organic matter	-	3 (8.8)	25 (73.5)	3 (8.8)	-	3 (8.8)	3.000
D Airiness	-	2 (5.9)	10 (29.4)	15 (44.1)	2 (5.9)	5 (14.7)	3.586
L Light	-	-	12 (35.3)	21 (61.8)	-	1 (2.9)	3.636
T Temperature	-	-	1 (2.9)	14 (40.0)	18 (52.9)	1 (2.9)	4.515
K Continentalty of the habitat	-	14 (40.0)	18 (52.9)	1 (2.9)	-	1 (2.9)	2.606

Conclusion

According to the research carried out, the analysis of environmental indices for humidity indicate the dominance of mesophytes. The chemical reaction of the environment indicates a dominant representation of plants of ecological index 3, which indicates a weakly acidic to neutral habitat. 88.3% of taxa belong to mesotrophic and eutrophic habitats. The analysis of the content of organic substances indicate the dominance of the indicator of the medium content of organic substances. The analysis of plant indicator values for habitat ventilation indicates that taxa most prefer moderately and well-ventilated habitats. The mean value of the ecological index for light indicates that half-shade species dominate. The most abundant taxa are those that thrive in extremely thermophilic or thermophilic habitats. Invasive flora is to the greatest extent adapted to subcontinental climatic conditions.

References

- Domac R. (1994). Flora Hrvatske. Školska knjiga. Zagreb
- Ilić O., Nikolić Lj. (2015). Floristička i ekološka analiza korovske flore u organskom usevu lucerke. *Acta herbologica*, Vol 24 (No 1): 25-38.
- Javorka S., Csapody V. (1975). *Iconographia florum partis austro – orientalis Europae Centralis*. Akadémiai Kiadó, Budapest
- Knežević A., Džigurski D., Milošev D., Ljevnaić-Mašić B., Šeremešić S., Belić M. (2009). Ekološka analiza flore prirodnog pašnjaka na solonjcu u okolini naselja Kumane. *Acta herbologica*. Vol 18. (No 2): 77-86.
- Landolt E. (1977). *Ökologische Zeigerwerte zur Schweizer Flora*. Veröff. Geobot. Inst.ETH Stiftung Rübel Zürich 64, 1-208
- Nikolić Lj., Milošev D., Šeremešić S. (2017). Ekološka analiza korovske flore pšenice u uslovima plodoreda. *Letopis naučnih radova / Annals of agronomy*. Vol 41. (No 2): 95-103
- Petrović J., Čurčić S., Stavretović N. (2016). Invazivne biljne vrste i ekološki čimbenici koji utječu na njihovo širenje na području spomenika prirode „Obrenovački zabran“ (Središnja Srbija). *Šumarski list*. Vol CXXXX. (No 1-2): 45-52.
- Rakić S., Randelović V., Zlatković B. (2007). Fitogeografska i ekološka analiza urbane flore grada Poževe. *9th Symposium of Flora of Southeastern Serbia and Neighbouring Regions*. Proceeding. 101-107
- Samardžić I., Galić I. (2019). Invazivne biljne vrste Požeške kotline i Slavenskog gorja. Javna ustanova za upravljanje zaštićenim područjem Požeško-slavonske županije. Požega
- Tomašević M. (2016). Flora Požeške kotline i okolnog gorja. Zagreb-Požega. str. 9.
- Tutin I. G. et al. (1964.-1980.). *Flora Europaea*. Vol 1-5. Cambridge University Press. Cambridge.
- Zima D. (2012): Invazivne biljne vrste Požeške kotline. Doktorska disertacija, Sveučilište J.J. Strossmayera u Osijeku. Poljoprivredni fakultet.
- Zima D., Štefanić E. (2021). Invazivna vaskularna flora Požeške kotline, Republika Hrvatska: raznolikost i procjena rizika. *Zbornik Veleučilišta u Rijeci*. Vol 9 (No 1): 441-451

Session 5

Agribusiness and Smart Agriculture

PRELIMINARY COMMUNICATION

Noise exposure assessment for agricultural tractor operators during varied agrotechnical operations

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Summary

The aim of the research was to determine the noise exposure level for the operator during sowing operations using different seed drills attached to the same tractor. The study was conducted at Poljoprivreda Knežević, where noise levels affecting the tractor operator during various agricultural operations were measured over a two-day period. The investigation involved monitoring noise inside a John Deere 7290R tractor cabin equipped with two distinct seed drills. Data were collected and processed over the two-day study.

The results indicated that the Kuhn Espro 6000 R seed drill generated slightly higher noise levels on the operator's left side, whereas the Väderstad Tempo F8 seed drill produced higher noise levels on the right side. Additionally, the study revealed that noise levels during certain operations exceeded the permissible exposure limit of 87 dB(A).

Key words: agricultural tractor, health, operator, safety, noise.

Introduction

Noise is an increasing concern due to its harmful impact on operator health and contribution to environmental pollution. Agricultural machinery is a major source of such noise. Defined as unwanted or harmful sound, noise can cause cumulative hearing damage, with frequency and intensity being key factors (Murphy and Harshman, 2022). Beyond hearing loss, noise affects the neurovegetative and endocrine systems, leading to nervous tension, circulatory issues, and digestive disturbances. It also impairs concentration, causes irritability, fatigue, and drowsiness (Administrator, 2012).

Modern tractor cabs are designed to protect operators from environmental hazards such as noise, weather, and exhaust fumes (Karakulak and Yetkin, 2020), and also function as safety structures against accidents like rollovers (Kumar et al., 2015). Poor maintenance and inadequate soundproofing can raise noise levels, making it essential to keep doors and windows closed. Sound-absorbing materials—such as foams, porous composites, and acoustic wool—are installed around seats, walls, and roofs to reduce resonance. Zhipeng et al. (2022) found that perforated panels and membrane insulation lowered peak noise by 1.3 dB(A). When noise remains excessive, personal protective equipment like earplugs or earmuffs is recommended (Senfer, 2020). Ensuring operator safety, particularly from noise, remains a key priority for agricultural machinery manufacturers (Jurić et al., 2025).

Yamin et al. (2021) conducted a study across Pakistan's Faisalabad and Narowal districts to evaluate tractor noise prevalence and its psychological impact on operators using tractors without enclosed cabs or noise-reduction measures. The researchers focused on four common agricultural operations: soil scraping, cultivation, disc harrowing, and sowing. Noise levels were measured at operational speeds of 5.3 kmh⁻¹ and 10.6 kmh⁻¹. Among these tasks, sowing generated the lowest noise level at 81.9 dB(A), while soil scraping and cultivation produced the highest levels at 86.9 dB(A) and 84.9 dB(A), respectively. As these values surpassed the upper

warning threshold, the authors emphasized the necessity of protective equipment for operators under such conditions. Dewangan and Patel (2019) expanded on this by analyzing noise exposure for operators of six agricultural tractors equipped with five tillage implements: a board plough, disc harrow, rotavator, duck-foot cultivator, and a secondary disc harrow. Measurements taken at three operational speeds revealed noise levels ranging from 91.7 dB(A) to 97.5 dB(A), significantly exceeding the 87 dB(A) permissible limit. The authors underscored the urgent need for enhanced cabin sound insulation and mandatory use of personal protective equipment (PPE) to mitigate health risks.

According to the regulation on the protection of workers RH (OF, 148/2023) from exposure to noise at work, exposure limit values and warning values for exposure during an 8-hour workday and sound pressure levels have been adopted: exposure limit: $L(EX,8h) = 87 \text{ dB(A)}$ and $p(\text{peak}) = 200 \text{ Pa}$ (140 dB(C) relative to $20 \mu\text{Pa}$); upper warning limit: $L(EX,8h) = 85 \text{ dB(A)}$ and $p(\text{peak}) = 140 \text{ Pa}$ (137 dB(C) relative to $20 \mu\text{Pa}$); lower warning limit: $L(EX,8h) = 80 \text{ dB(A)}$ and $p(\text{peak}) = 112 \text{ Pa}$ (135 dB(C) relative to $20 \mu\text{Pa}$).

The study aimed to quantify operator noise exposure during sowing using different seed drills attached to the same tractor.

Material and methods

The study was conducted at the Knežević farm in Čačinaci, Croatia (June 2024). Noise measurements were performed using a John Deere 7290R tractor (213 kW power, equipped with cab suspension, seat suspension, and front axle suspension) attached to two distinct seed drills: a Kuhn Espro 6000 R (6-meter working width) and a Väderstad Tempo F8 (8-row working width), during soybean sowing operations. All measurements were conducted under uniform operator control. Soybean sowing covered a total area of 95 hectares, divided into two approximately equal plots of 47.5 hectares each. Noise data were collected using a Kimo DS 300 noise dosimeter paired with a Class A microphone sensor (Figure 1). The device recorded measurements at one-second intervals over an eight-hour work shift.



Figure 1. Kimo DS 300 dosimeter with Class A microphone sensor.

Technical specification

Features for Lq and Lp channels are:

Dynamic range V1 only (40-140 dB); Frequency weightings (A and C); Filters (8 filters by octave bands from 63 Hz to 8 kHz); Values (LAF, LAS, max, min, LAeq, LCeq, LXeq, max, min, Lex, d, EAT, DOSE, exposure points); Simultaneous measurement mode (LAeq – LCeq - LXeq (X : from 63 Hz to 8 kHz)); Duration of Leq integration (From 1s to 60 s (step of 1s)); Sound pressure sampling (48 kHz); Microphone Type electret – diameter (9.5 mm (3/8") – 15 mV/Pa).

The study adhered to national noise regulations (Official Gazette 46/2008), which define noise descriptors and measurement protocols. Methods aligned with the following standards: HRN ISO 1996-1, -2, -3: Acoustics – Description, measurement, and assessment of environmental noise, HRN ISO 9612: Acoustics – Guidelines for measuring occupational noise exposure, HRN EN 60804: Integrating-averaging sound level meters. In accordance with these standards,

the microphone sensors were positioned on both the left and right sides of the operator's head during measurements. The following parameters were analyzed: a) $LC_{pk\max}$ (green indicator) – maximum sound pressure level (Pa), or noise level; b) LC_{eq} – equivalent continuous sound pressure level (Pa), or noise level; c) LA_{eq} (blue indicator) – continuous sound level measured in both sound channels, the most important and most commonly used value, representing the average noise exposure over the measurement period in dB(A); d) L_{ex8} – daily noise exposure level in dB(A) and e) EA.T – sound exposure level in dB(A).

Results and discussion

Processed noise measurement data from the operator's left and right sides, corresponding to the two seed drills, are summarized in the tables and graphs below.

Table 1. Noise levels during soybean sowing using a John Deere 7290R tractor paired with a Väderstad Tempo F8 seed drill

John Deere+Vaderestad Tempo F8	LA_{eq} dB(A)	LC_{eq} dB(A)	$LC_{pk\max}$ dB(A)	EA.T Pa ² h	L_{ex8} dB(A)
Left side	79.9	88.7	135.1	0.494	82.0
Right side	81.9	90.4	137.2	0.768	84.0

Analysis of the collected data (Table 1) revealed consistently higher noise levels on the operator's right side during identical operations. Specifically, the peak noise level on the right side measured 2.1 dB(A) higher than on the left. While the LA_{eq} (equivalent continuous A-weighted sound level) did not surpass the upper warning limit of 85 dB(A) or the permissible exposure limit of 87 dB(A) on either side, it exceeded the lower warning threshold of 80 dB(A) (Table 1). These findings align with prior research on operator noise exposure (Baráč et al., 2024; Duracz and Rybacki, 2023; Han et al., 2022).

Figure 2 illustrates a direct correlation between rising noise levels (blue trace) and increasing sound pressure (green trace). Notably, the right-side measurements exhibited both higher average noise levels and a pronounced peak of 137.2 dB(A).

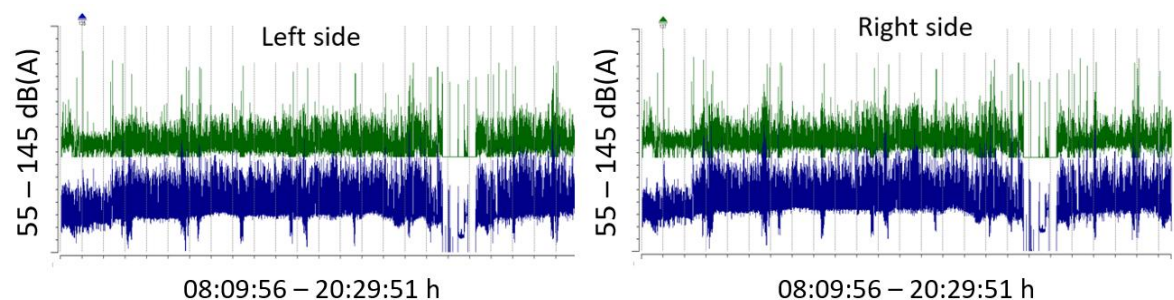


Figure 2. Relationship between sound pressure and noise levels on the operator's left and right sides during operation of the Väderstad seed drill.

Table 2. Measured noise levels during soybean sowing using a John Deere tractor paired with a Väderstad seed drill

John Deere+Kuhn Espro 6000 R	LA_{eq} dB(A)	LC_{eq} dB(A)	$LC_{pk\max}$ dB(A)	EA.T Pa ² h	L_{ex8} dB(A)
Left side	81.5	89.4	134.4	0.494	82.0
Right side	80.06	90.0	132.7	0.400	81.0

Analysis of the data in Table 2 revealed that the daily noise exposure level (L_{ex8}) remained near the lower warning limit of 80 dB(A), with values of 82 dB(A) on the operator's left side and 81 dB(A) on the right. Notably, the left side exhibited a peak noise level approximately 2 dB(A) higher than the right. Contrary to previous tractor-implement configurations, the equivalent continuous A-weighted sound level (L_{Aeq}) was higher on the left side in this setup. None of the measured values exceeded the permissible exposure limit of 87 dB(A), consistent with findings from recent studies (Barač et al., 2025; Zhu et al., 2022; Wang et al., 2024). The observed discrepancies in noise levels between the left and right sides may stem from variations in the structural design and dimensions of the seed drills. This underscores the need for future research to include multiple seed drills of identical design to isolate the impact of construction differences on operator noise exposure.

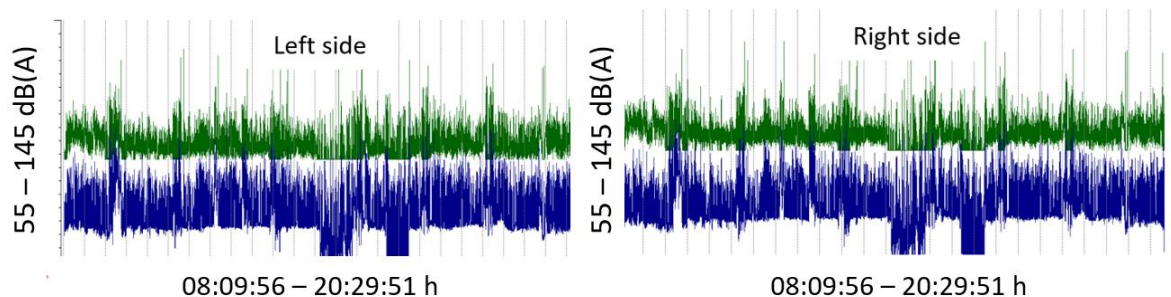


Figure 3. Relationship between sound pressure and noise levels on the operator's left and right sides during operation of the Kuhn seed drill

Figure 3 illustrates a direct correlation between increasing noise levels (blue trace) and rising sound pressure (green trace). Notably, the left-side measurements exhibited higher average noise levels, with a pronounced peak of 134.4 dB(A).

Conclusions

The study yielded the following conclusions:

- The Kuhn Espro 6000 R seed drill generated the highest noise levels on the operator's left side, whereas the Väderstad Tempo F8 seed drill produced peak noise levels on the right side. This asymmetry is likely attributable to differences in the seed drills' structural designs, despite both being attached to the same tractor. Future studies should incorporate multiple seed drills of identical design to isolate the influence of construction variations on noise distribution.
- While none of the average noise levels exceeded the upper warning limit (85 dB(A)) or permissible exposure limit (87 dB(A)), all measurements surpassed the lower warning threshold (80 dB(A)), except for left-side readings from the Väderstad seed drill. To mitigate risks, regular inspection and replacement of deteriorated door/window seals, coupled with enhanced cabin acoustic insulation, are recommended.
- Future research should expand to include diverse machinery models, operational conditions, and longitudinal assessments to isolate noise determinants, while integrating health impact studies and advanced mitigation strategies like active noise cancellation. Concurrently, policies should prioritize ergonomic cabin design, operator training, and cost-effective retrofitting to align agricultural practices with noise exposure regulations. These steps will bridge gaps between laboratory findings and sustainable, operator-centric farming solutions.

References

- Administrator (2012). Noise and occupational safety. Occupational safety. Available from: <https://zastitanaradu.com.hr/buka-i-zastita-na-radu/>.
- Barač Ž., Radočaj D., Plaščak I., Jurišić M., Marković, M. (2024). Prediction of Noise Levels According to Some Exploitation Parameters of an Agricultural Tractor: A Machine Learning Approach. *AgriEngineering*. Vol 6(2): 995-1007. <https://doi.org/10.3390/agriengineering6020057>.
- Barač Ž., Plaščak I., Radočaj D., Jurišić M. (2025). The Impact of Noise on Agricultural Tractor Operator in Relation to Certain Operational Parameters: An Analytical Hierarchy Process (AHP) Approach. *Agriculture*. Vol 15(5): 466. <https://doi.org/10.3390/agriculture15050466>.
- Dewangan L.K.N., Patel T. (2019). Noise Exposure To Tractor Drivers In Field Operations. *International Journal of Vehicle Performance*. Vol 5(4): 430-442. <https://doi.org/10.1504/IJVP.2019.104085>
- Duracki K, Rybacki P., (2023). Noise Emission in the Cabs of Modern Farm Tractors. *Technical Gazette*. Vol 30(2): 669-675. <https://doi.org/10.17559/TV-20220921083039>
- Han H.W., Im W.H., Choi H.J. (2022). Effect Of Sound Insulation On Noise Reduction In An Agricultural Tractor Cab. *Scientific Reports* Vol 12: 22038. <https://doi.org/10.1038/s41598-022-26408-3>.
- Jurić T., Jurić M., Vidaković I., Barač Ž. (2025). Safety Work as a Factor in Reducing the Number of Injuries of Tractor Operators. *Technical Journal*. Vol 19(1): 119-126. <https://doi.org/10.31803/tg-20231220092909>
- Karakulak S., Yetkin E. (2020). Agricultural Tractor Cabin Safety Analysis and Test Correlation. *International Journal of Automotive Science And Technology*. Vol 4 (1.): 1-9. DOI:10.30939/ijastech..641569
- Kumar A., Mahajan A., Prasanth S., Darekar S., Chellan J., Kumar A.K., Kumar R., Kumar J. (2015). Agricultural Tractor Cabin Structure Design for Durability and Rollover Protective Structure Test. *SAE Interantional Technical Paper*. 26: 0163. <https://doi.org/10.4271/2015-26-0163>.
- Murphy D., Harshman W. (2022). Noise induced hearing loss in agriculture. PennState Exstension, The Pennsylvania State University. Available from: <https://extension.psu.edu/noise-induced-hearing-loss-in-agriculture>
- Official Gazette (148/2023). Rules On The Protection Of Workers From Exposure To Noise At Work. Ministry of Labour, Pension System, Family and Social Policy. Republic of Croatia.
- Senfer D. (2020). Noise and vibration protection. Final work. Karlovac Polytechnic (Department of Security and Protection) Karlovac.
- Wang S., Ma X., He M. (2024). Research On Control Of Low Frequency Noise In Tractor Cab. *Journal of Chinese Agricultural Mechanization*. Vol 45(5): 134-139. DOI: 10.13733/j.jcam.issn.2095-5553.2024.05.021
- Yamin M., Yousaf Z., Bhatti K.M., Ibrahim M., Akbar F.N., Shamshiri R., Mahmood A., Tauni R.A. (2021). Noise Exposure And Its Impact On Psychological Health Of Agricultural Tractor Operators. *Noise Control Engineering Journal*. Vol 69(6): 500-506 DOI: <https://doi.org/10.3397/1/376947>.
- Zhipeng W., Yanyan Z., Liming S., Xiaohe Z. (2022). Noise Reduction in Tractor Cabs Using Coupled Resonance Acoustic Materials. **IEEE Access**. Vol 10: 32689-32695. doi: 10.1109/ACCESS.2022.3160734.
- Zhu K., Ma X., Xu W. (2022). Research On Improvement Of Structural Noise In Tractor Cabs. *Journal of Chinese Agricultural Mechanization*. Vol 43(2): 98-103. DOI: 10.13733/j.jcam.issn.20955553.2022.02.014

EXPERT PAPER

Combatting food fraud: Challenges, consequences, and the need for advanced analytical tools

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Summary

Food fraud is a growing global threat that jeopardises food safety, threatens public health and causes economic losses of \$40–50 billion annually. Traditional traceability systems cannot prevent fraud due to data vulnerabilities and weak enforcement. Advanced technologies such as blockchain, Internet of Things (IoT) and DNA authentication improve transparency but face obstacles such as high costs. Combatting food fraud requires stricter regulations, technological integration and greater consumer awareness of the challenge.

Key words: food fraud, food safety, analytical tools, supply chain integrity

Introduction

The world's population is expected to reach 9.8 billion by 2050 and 11.2 billion by 2100, making food security one of the most pressing challenges of the 21st century. As the population continues to grow, so does the demand for safe, nutritious and sustainably produced food. In recent decades, the food market has undergone significant globalisation, with international trade networks increasing in both scale and complexity. However, this rapid development has also exposed the food sector to systemic vulnerabilities, among which food fraud has emerged as a particularly critical concern. Food fraud is far from a new phenomenon. Historical evidence suggests that it has existed since the earliest days of the food and beverage trade (Chukwugozie et al., 2024). Nevertheless, concerns about fraudulent practises within the food supply chain have increased significantly in recent years. There has been a significant increase in reported cases of food fraud in the global market, emphasising the significant damage it causes not only to consumers but also to the integrity of the food industry as a whole. With an estimated annual economic cost of USD 40-50 billion (Platonova, 2024), it is primarily a financial crime, but also poses serious public health risks due to the variety and severity of fraudulent practises. Some experts argue that the more frequently food changes hands, the higher the risk of fraudulent activity. A striking example of the scale of this problem was observed during EUROPOL's Operation OPSON VII between 2018 and 2019, which targeted counterfeit and substandard foodstuffs and beverages. The most frequently adulterated products included olive oil, fish, organic food, milk, cereals and honey. The authorities confiscated 3,950 tonnes of fraudulent food and 9.7 million litres of counterfeit beverages. The consequences of food fraud extend along the entire supply chain and affect distributors, retailers and, above all, consumers. The impact of such fraud can lead to significant economic losses, erosion of consumer confidence and expose consumers to increased health and safety risks through the consumption of adulterated or counterfeit products. Furthermore, food fraud has a profound social and environmental implications, as it is often associated with unethical practises that undermine sustainability and responsible sourcing. It usually involves the deliberate counterfeiting or adulteration of food products, often achieved by replacing genuine ingredients with inferior or even harmful alternatives. Such fraudulent activities compromise various aspects of food

quality, including food safety, nutritional value, geographical authenticity, traditional production methods, ethical standards and environmental sustainability. Given the multifaceted and global nature of this problem, there is an urgent need for advanced analytical tools and robust management systems to effectively detect, prevent and combat food fraud in all its forms. This paper examines the different types and causes of food fraud and explores current food traceability systems and their role in mitigating fraudulent activities. It also analyses new innovative food authentication and fraud prevention technologies and highlights their potential to improve the safety and integrity of the global food supply chain.

Food fraud - what and why

There are different types of food fraud that are widely discussed in both academic literature and professional practise. One of the most well-known definitions of food fraud comes from Spink and Moyer (2011), who describe it as "a collective term for the deliberate and intentional substitution, addition, manipulation or misrepresentation of food, food ingredients or food packaging, or for false or misleading statements about a product for the purpose of economic gain". According to an extensive literature review by Bastian (2023), the most common forms of food fraud include mislabelling (20.7%), artificial enhancement (17.2%) and substitution (16.4%). The highest number of reported cases relates to beverages with over 21% of incidents, followed by dairy and meat products, which each account for around 14% of reported cases. These forms of food fraud represent only a fraction of the cases known in the industry and in the scientific community. Mislabelling occurs when the ingredients, quality or origin of a product are falsely or incorrectly labeled. Examples of this are the sale of diluted orange juice as 100% pure orange juice or the false marketing of non-organic products as certified organic products. Artificial enhancement, while related to substitution, involves techniques aimed at improving the appearance or perceived quality of food. A well-documented example is the addition of melamine to dairy products to artificially increase the protein content. Substitution, on the other hand, involves replacing a high-quality ingredient with an inferior or cheaper alternative, e.g. by selling horse meat as beef. Misrepresentation in the broader sense includes all misleading or false information about a food product. An example of this is the marketing of farmed salmon as "wild-caught" salmon. False or misleading statements specifically aimed at economic gain, such as labelling conventionally produced food as organic, also fall into this category. To complete the types of food fraud, the FAO (2021) has adapted a classification system from Spink and Moyer (2011), as shown in Chukwugozie et al. (2024) in Table 1.

Table 1. Types of food fraud

Type of Fraud	Definition	Examples
Simulation	An illegal product is made to resemble a lawful product but not to be a replica of it.	Duplicates of well-known products are made without the same food safety assurances/measures. Goods that were stolen are combined with real items. For example, in Italy, more than 266,000 liters of fraudulent mineral water were discovered, packed to resemble a famous water brand.
Food Theft	The theft of genuine goods is done under the guise of genuine purchases.	Goods that were stolen are combined with real items.
Tampering & Mislabelling	The use of legitimate goods and packaging for fraudulent purposes.	Altered expiration dates; false manufacturing methods or origin statements.
Over-run	Overproduction agreements are broken to produce the legal product.	Production underreporting.

Counterfeit	The counterfeit goods and packaging are identical in every way.	A product copy, usually produced without regard to food safety controls.
Diversion	The marketing or distribution of legal goods outside of their intended markets.	Food intended as relief-aid food is diverted away from where it is required. Or waste food intended for pet food is diverted into processed foods.

As an example of a simulation, in 2023 more than 260,000 litres of olive oil were confiscated in Spain and Italy alone due to being unsafe for consumption. As a result, a criminal network involved in the distribution of such products was uncovered. Another notable case of simulation involved over 266,000 litres of fraudulent mineral water discovered in Italy packaged to resemble a well-known brand of water. The counterfeiting of meat products remains one of the most widespread forms of food fraud in the world. EUROPOL's Operation OPSON VII, conducted in 67 countries to combat food fraud, found meat fraud to be the most common category of seized goods (estimated value: over EUR 27.5 million). Alcohol was the most frequently seized product in the operation. Food theft is the illegal removal of food from the supply chain, which is then sold without modification. Meanwhile, overrun food products are genuine but are produced using unauthorised processes. A recent case from 2025 is an example of tampering and mislabelling: coffee was falsely labelled as decaffeinated by the FDA. Although the health consequences were temporary and medically reversible, the incident has raised significant regulatory concerns. Finally, the diversion relates to the unauthorised sale of food intended for donation, preventing delivery to the intended recipients.

Food traceability

Food traceability plays a crucial role in overcoming these challenges and improving the sustainability of the food supply chain. As Zhou and Xu (2022) stated, traceability practises help to standardise agricultural operations, improve management methods, reduce pesticide use and promote environmentally sustainable practises. To fully understand the importance of food traceability, it is important to know its definition and function within food systems. Traceability refers to the ability to track the history, distribution, location and use of products, parts, materials or services (Olsen and Borit, 2013; ISO, 2017). While there are different types of traceability, in the context of food, the term "food traceability" refers specifically to the monitoring of the journey of food from its origin through each stage of processing, packaging, distribution and transport to the final consumer - a process often described as "from farm to table". Food traceability plays a fundamental role in ensuring food safety and security, including agri-food risk management and regulatory compliance. Transparent traceability within the supply chain serves to protect consumers by enabling the identification of food safety issues and facilitating rapid responses, such as targeted food recalls. In the agri-food sector, various approaches and technologies support food traceability and food origin certification to ensure food safety and provide valuable information to consumers (Bandinelli et al., 2023). These mechanisms help reinforce consumer confidence while promoting responsible sourcing and regulatory compliance in the food industry.

Traditional food traceability methods

Food distributed on the market must be labelled with the mandatory and essential information, including the country of origin, the specific geographical area or place of production and the type of production - such as organic certification. For example, Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI), for example, are quality certification labels that fall under traditional food labelling schemes. These quality labels are awarded by state organisations and independent certification bodies in order to guarantee the authenticity

and regional specificity of the products. While traditional food traceability methods provide valuable information, they often fail to ensure full transparency and safety within the supply chain for a variety of reasons. The complexity of the global food trade and the potential for data manipulation in food labelling - especially when multiple regulatory authorities and independent certification organisations are involved - pose a major challenge. Recent literature (Reitano et al., 2024) indicates that consumers are increasingly expressing concerns about the quality, authenticity and safety of food, which may be related to the limitations of traditional traceability methods in ensuring full transparency.

Advanced traceability methods

In order to effectively combat food fraud in all its complexity, new and advanced technologies for labelling and tracing food have been developed at a rapid pace in recent years. For example, DNA analysis and stable isotope techniques enable the determination of a food product's geographical origin, while hyperspectral imaging has proven its worth in identifying the origin of foods such as fruits, vegetables and cereals (Bandinelli et al., 2023). A relatively recent advance in agri-food safety is the integration of the Internet of Things (IoT), which is primarily used to track food and monitor food safety and quality (Bouzembrak et al., 2019). Examples of technological applications within these systems include barcodes, QR codes and RFID (Radio Frequency Identification). In addition, IoT-enabled communication technologies such as Wi-Fi, Ethernet, NFC, 4G and Bluetooth improve real-time data collection and traceability. Among the emerging technologies, blockchain has shown immense potential and a critical role in improving transparency, safety and trust within the food supply chain. Its decentralised and tamper-proof nature provides a robust framework to ensure the integrity of food traceability systems.

Blockchain technology

Blockchain, a technology originally developed for cryptocurrencies, is a decentralised digital ledger capable of tracking transactions across a network of high complexity. It consists of a chain of blocks that store information, each of which can be verified with a digital signature (Di Pierro, 2017). Each block in the chain contains a timestamp, transaction details and a unique encrypted code known as a hash - an irreversible encrypted version of the original string - which ensures the integrity and security of the data. According to Di Pierro (2017), the blockchain is organised into three main components: Blocks, nodes and a consensus protocol. This decentralised system allows data to be securely recorded and validated without the need for a central authority. Originally developed as an infrastructure for cryptocurrencies, the scope of blockchain technology has expanded considerably since its inception. Today, it is increasingly used in various industries where it improves traceability, transparency and security in supply chain management.

Blockchain-based food traceability

Blockchain has become a significant tool in the agricultural and food sector. By enabling full traceability and transparency throughout the supply chain, it helps to create a reliable and secure food traceability system. One of the key features of this technology is its immutability, i.e. once data has been entered, it cannot be altered or manipulated. In addition, every participant in the network has equal access to the recorded information, which promotes trust and accountability within the system. This level of security suggests that products labelled and tracked using blockchain could be less susceptible to food fraud, contamination and associated risks. Above all, blockchain is characterised by its scalability and wide range of applications, making it a promising tool to protect consumers from fraudulent practises while improving food safety (Bandinelli et al., 2023). Examples include BC-based platforms such as Provenance Tags (2025), where the details, history and ownership of wines can be tracked and traced. Despite its

advantages, blockchain also has certain limitations, such as high implementation costs or challenges related to its relatively recent adoption in the food sector. Further research and exploration is needed to better understand and optimise the role of blockchain in food traceability.

Conclusions

Food supply chains are expanding and becoming more complex, with a tendency to become even more intricate in the future. One consequence of this expansion is food fraud, a global challenge that has multiple impacts, including significant economic losses, health risks and social and environmental concerns. Conventional methods of food traceability can provide some transparency, but are often no longer sufficient. This is partly because the data is vulnerable to manipulation and many traceability systems are generally fragile. Modern food traceability systems therefore offer more effective solutions. Technologies such as the Internet of Things (IoT), DNA analyses and blockchain have proven to be important tools for improving food traceability. Blockchain offers an innovative approach for the agri-food sector. As a decentralised technology, it ensures that data cannot be altered, providing an unprecedented level of transparency. This makes it a valuable resource in the fight against food fraud and should boost consumer confidence while enabling wider improvements in the food supply chain. Although these systems have significant potential, there are still some challenges, including the high cost of implementation and the need to adapt regulations. Future developments should focus on enhancing the accessibility of these technologies, raising consumer awareness and establishing stricter regulations. Improved consumer vigilance will also be crucial. In the long term, these efforts can lead to a more transparent, safer and ultimately stronger global food supply chain and food market.

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References

- Bandinelli R., Scozzafava G., Bindi B., Fani V. (2023). Blockchain and consumer behaviour: Results of a Technology Acceptance Model in the ancient wheat sector. *Cleaner Logistics and Supply Chain*. 8: 100117.
- Bastian A. (2023). A bibliometric analysis of food fraud. *West Science Interdisciplinary Studies*. 1(9): 702-713.
- Bouzembrak Y., Klüche M., Gavai A., Marvin H.J. (2019). Internet of Things in food safety: Literature review and a bibliometric analysis. *Trends in Food Science & Technology*. 94: 54-64.
- Chukwugozie D.C., Njoagwuani E.I., David K., Okonji B.A., Milovanova N., Akinsemolu A.A.,..., Ghosh S. (2024). Combatting food fraud in Sub-Saharan Africa: Strategies for Strengthened safety and security. *Trends in Food Science & Technology*. 104575.
- Di Pierro M. (2017). What is the blockchain? *Computing in Science & Engineering*. 19 (5): 92-95.
- FAO (2021). Food fraud – Intention, detection and management: Food safety technical toolkit for Asia and the Pacific (No. 5). FAO. Available from: <https://openknowledge.fao.org/items/753d4030-466a-4681-a120-2e469ebddeb6>

- ISO (2017). ISO 10007:2017. Quality management - Guidelines for configuration management. International Organization for Standardization.
- Reitano M., Pappalardo G., Selvaggi R., Zarbà C., Chinnici G. (2024). Factors influencing consumer perceptions of food tracked with blockchain technology. A systematic literature review. *Applied Food Research*. 100455.
- Qian J., Ruiz-Garcia L., Fan B., Villalba J.I.R., McCarthy U., Zhang B., ..., Wu W. (2020). Food traceability system from governmental, corporate, and consumer perspectives in the European Union and China: A comparative review. *Trends in Food Science & Technology*. 99: 402-412.
- Platonova M. (2024). What is food fraud, and how can nuclear science detect it? International Atomic Energy Agency (IAEA). Available from: <https://www.iaea.org/newscenter/news/what-is-food-fraud-and-how-can-nuclear-science-detect-it>
- Provenance Tags (2025). Available from: <https://ptagchain.io/cases/fine-wines-and-exquisite-spirits>
- Spink J., Moyer D.C. (2011). Defining the public health threat of food fraud. *Journal of food science*. 76(9): 157-163.
- Zhou X., Xu Z. (2022). Traceability in food supply chains: a systematic literature review and future research directions. *International Food and Agribusiness Management Review*. 25(2): 173-196.

PRELIMINARY COMMUNICATION

The influence of consumer attitudes towards animal welfare on purchasing behavior

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Summary

The European population feeds on 58% animal protein, which is made possible by raising animals in a space where they are completely dependent on the care, will and skills of humans. The survey aimed to understand consumer attitudes towards animal welfare and their influence on purchasing and was conducted using a questionnaire distributed via social networks. More than half of the respondents believe that awareness of animal welfare in Croatia has increased in the last ten years and express a positive attitude. A large number of respondents (79.6%) consider animal welfare to be important, and more than half of respondents consider information about how animals are kept on the farm to be important. The results also show that 82.5% of respondents believe that animal welfare affects the quality of meat and eggs, but 60.2% of respondents believe that there is not enough information about the treatment of animals in food production.

Key words: animal welfare, consumer attitudes, purchasing

Introduction

In the early 1960s, 52% of the protein in the European population's diet came from plant-based foods (Bonnet et al., 2020). As incomes increased, the consumption of foods with a higher proportion of animal proteins, fats and sugars increased, and proteins from animal foods became more available over time (Alonso et al., 2020). Today, animal products are the source of 58% of protein, made possible by intensive livestock farming in an environment where animals are entirely dependent on the care, will and ability of humans (Bonnet et al., 2020). At the beginning of the 21st century, the sustainability of such farming systems has become questionable and consumers are increasingly interested in the origin of food and the way it is produced (Pavičić and Ostović, 2013). In the past, scientists have used animals as experimental models in biomedical research. Animals were used to test the effectiveness of preparations and procedures so that they could be transferred to humans for treatment purposes. Recently, however, there have been increasing efforts to ban the use of animals as experimental organisms (Gregurić Gračner et al., 2019). Animal welfare can be described as the absence of stress or the presence of a sense of satisfaction (Gregurić Gračner et al., 2019). Given the variety of definitions of welfare, it can be emphasised that animal welfare refers to the individual, its physical and mental state, and can vary greatly depending on husbandry conditions and treatment (Pavičić and Ostović, 2013). Animal welfare has an indirect impact on the safety and quality of food. The meat of a stressed or injured animal can be of poorer quality because it loses colour and tenderness of the tissue (Očić, 2021). The inefficient use of natural resources, the negative impact on human health, the negative impact on the environment and the disregard for animal welfare could lead to intensive livestock systems being unsustainable and considered unacceptable by the public (Alonso et al., 2020). The aim of the research was to understand

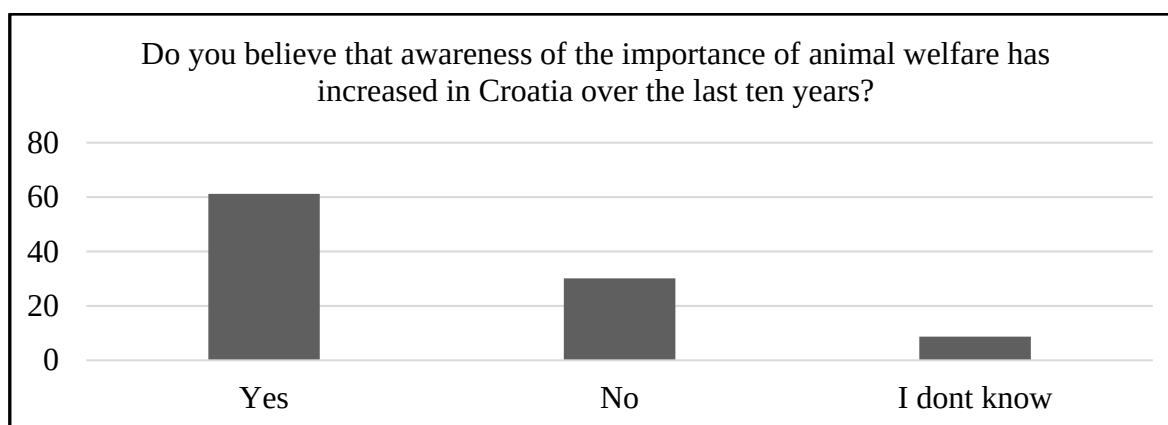
consumer attitudes and their intention to buy products produced according to the following animal welfare principles.

Material and methods

The study was conducted with a questionnaire distributed between 15 June and 15 August 2023 via the social networks Facebook, Instagram, WhatsApp and Viber. The sample was based on the availability of respondents interested in answering the survey. The questionnaire contained closed questions on socio-demographic characteristics, personal attitudes towards animal welfare, preferences when buying meat and eggs, the importance of animal welfare principles in the business of retail chains and the willingness to pay a higher price for products from species-appropriate animal husbandry. The data processing and creation of the diagrams were carried out in Microsoft Office Excel.

Results and discussion

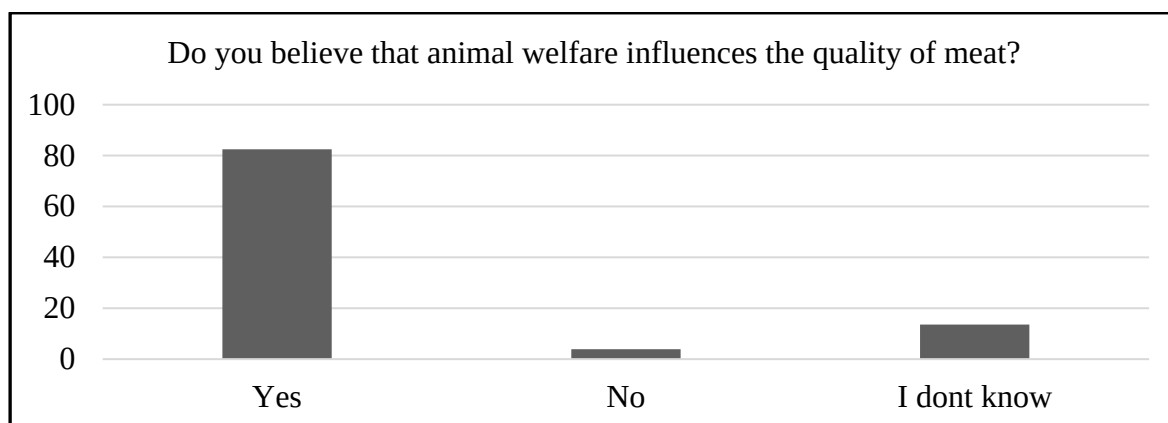
103 people took part in the study, 79.6% of whom were female and 20.4% male. Most respondents were in the 18-25 age group (49.5%), followed by the 26-35 age group (38.8%), the 36-50 age group (6.8%) and the smallest group was over 59 years old (4.9%). 65% of respondents have a high or higher level of education, 34% have completed secondary school and 1% have completed elementary school. As Graph 1 shows, more than half of the respondents (61.2%) believe that awareness of the importance of animal welfare in Croatia has increased over the last ten years.



Graph 1. The importance of animal welfare in Croatia over the last ten years

In a survey conducted by the European Commission in 2007, consumers were asked to rate the importance of protecting pets on a scale of 0 to 10. The average score was 7.8 (Napolitano et al., 2008). According to a 2015 survey of 27,000 citizens from 28 EU Member States, 94% of respondents considered the protection of pets to be important (Temple and Manteca, 2020). The results of such surveys are important because consumers participate in the process of shaping public opinion and influence government legislation and policy decisions. Consumers have influence on the market because they can refuse to buy products from production systems they do not like (Alonso et al., 2020). Similar to previous studies, the majority of respondents in this study (79.6%) believed that animal welfare is important. A proportion of respondents (14.6%) associated animal welfare with a particular species, and the smallest proportion (5.8%) did not think about animal welfare at all. In a study conducted by Cornish et al. (2016) among veterinary students, it was found that students thought the same procedures were humane towards certain animal species, but not humane enough towards cats and dogs, to which they attributed greater cognitive abilities. When assessing and treating pain, they pay more attention to horses than cows and pigs. The majority of respondents are in favour of frequent meat

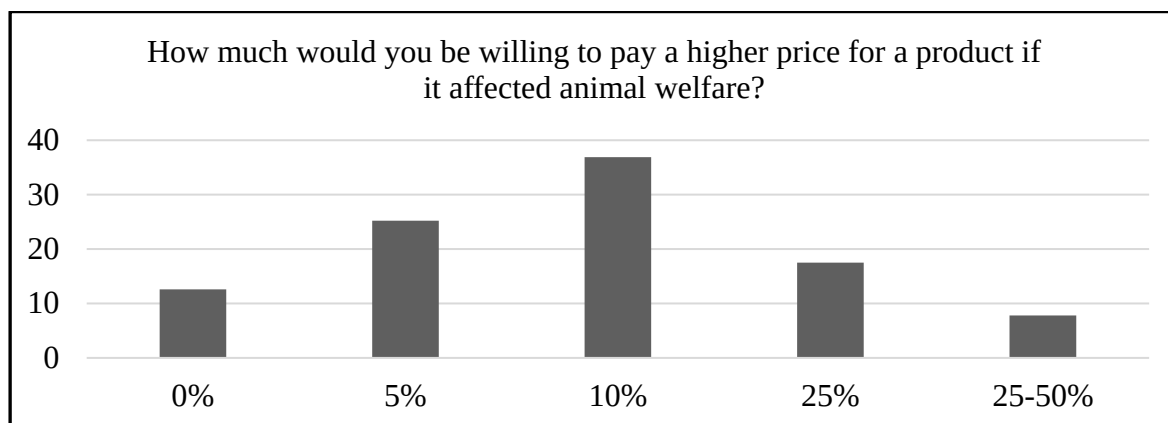
consumption: 42.7% consume it two to three times a week and 35.9% three to five times a week. A smaller proportion (6.8%) consume meat once a week, while 4.9% do not eat meat and do not consider it necessary. Intensive livestock farming contributes to water, soil and air pollution as well as global threats such as antibiotic resistance and zoonoses. The public is increasingly concerned about animal suffering, which is probably related to the increasing number of vegetarians and vegans in industrialised countries (Bonnet et al., 2020). For 66% of respondents, the price of meat is important but not decisive compared to other preferences. Several studies have confirmed that today's consumers are willing to pay a higher price for meat from farms that guarantee animal welfare standards (Lagerkvist 2013, Miranda-de la Lama et al., 2017). According to Dransfield et al. (2005), consumers are willing to pay an average of 5% more for meat from free-range pigs, with a fifth of consumers stating that they would be willing to pay 20% more. Information on how the animals are kept on the farm, if available, would be considered important by more than half of respondents (52.4%), while for 33% of respondents it would form the basis of a decision on whether to buy meat. A smaller proportion (9.7%) considered this information to be unimportant. Similarly, respondents considered information about the conditions under which animals are transported and the way they are killed to be important. A positive attitude towards organic livestock farming was expressed by 87% of respondents. Consumers often believe that organic farming systems have higher standards of animal welfare compared to conventional farming systems and are better for human health due to the absence of agrochemicals (Alonso et al., 2020). The results presented in Graph 2 show that 82.5% of respondents believe that animal welfare also influences the quality of the meat.



Graph 2. The impact of animal welfare on meat quality

Just as many (82.5%) of respondents believe that the way laying hens are kept influences the quality of the eggs. Improving animal welfare can help to reduce the use of antimicrobials, which has a positive long-term impact on human health (Temple and Manteca, 2020). According to Miranda-De La Lama et al. (2017), the majority of respondents stated that such products are of higher quality and better for human health. A 2005 survey of consumers from 25 European Union Member States found that 57% of respondents were willing to pay a higher price for eggs from animal-friendly production systems, with 11% of respondents willing to pay an additional cost of 25% or more (Napolitano et al., 2008). More than half of respondents (60.2%) believe that there is not enough information about the treatment of animals in food production, which is consistent with similar studies (Cornish et al., 2016). A smaller proportion (26.2%) of respondents answered that there is only enough information for some products, 7.8% of respondents are not interested in such information and 5.8% believe that there is enough information available. One of the best ways to get more information is to visit farms, but some consumers do not like to think about the connection between live animals and the food they

buy. Consumers' need and willingness to be better informed could be met by labelling with clear, science-based and understandable information that increases transparency and trust between stakeholders in the food supply chain. Labelled products can encourage consumers to exercise their political responsibility as free market participants and make purchasing decisions as “ethically competent consumers” (Alonso et al., 2020). Respondents were also asked how important it is for retail chains to promote animal welfare as one of their business principles. For 71.9% of respondents, this is important, although not a deciding factor in their choice of where to shop. 20% are unaware of this and do not consider it important and 9% shop exclusively at such retail chains. Due to the growing interest in production practises and the associated animal welfare standards, consumers in industrialised countries no longer consider price to be the sole determinant of food purchases. The attributes they value first and foremost include quality, flavour, freshness and animal welfare (Ammann et al., 2024). Attitudes and willingness to pay more for products produced in compliance with animal welfare standards differed in relation to socio-demographic characteristics. Women, younger participants, pet owners, people with higher levels of education and higher incomes showed higher levels of concern and were willing to pay more for products that comply with animal welfare principles (Alonso et al., 2020). How much more the respondents in this study would be willing to pay is shown in Graph 3.



Graph 3. Willingness to accept higher product prices

Although the majority of participants are convinced that animal welfare standards have an impact on product quality, the largest number (36.9%) are prepared to pay a 10% higher price. A much smaller number (7.8%) would pay a 25-50% higher price and some respondents (12.6%) are not willing to pay a higher price. The intentions expressed do not necessarily correspond to actual purchasing behaviour. Consumers may state certain preferences in surveys, but may be subject to financial constraints when purchasing (Napolitano et al., 2008). The limitations of the study lie in the disproportionate demographic factors of the sample, particularly the higher representation of women and respondents aged 18-25 compared to other groups. A larger sample should be included in future studies.

Conclusions

More than half of respondents (61.2%) believe that awareness of animal welfare in Croatia has increased in the last ten years, and more than two thirds (79.6%) believe that animal welfare is important. More than half of respondents also believe that information about animal welfare on the farm, during transport and at slaughter is important. The majority of respondents (87%) believe that organic farming ensures higher animal welfare standards compared to conventional farming and that animal welfare influences the quality of meat and eggs (82.5%). However, 60.2% of respondents believe that there is not enough information about how animals are

treated in food production. Although expressed intentions do not necessarily reflect actual purchasing behaviour, consumer attitudes are important as they can help shape public opinion and influence the market. The results show that further education and awareness raising as well as more transparent consumer information is needed.

References

- Alonso M.E., Gonzalez-Montana J.R., Lomillos J.M. (2020). Consumers' Concerns and Perceptions of Farm Animal Welfare. *Animals*. 10 (3): 385.
- Ammann J., Mack G., El Benni N., Jin S., Newell-Price P., Tindale S., Hunter E., Vicario-Modrono V., Gallardo-Cobos R., Sánchez-Zamora P., Miškolci S., Frewe, L.J. (2024). Consumers across five European countries prioritise animal welfare above environmental sustainability when buying meat and dairy products. *Food Quality and Preference*. 117: 105179.
- Bonnet C., Boumra-Mechemache Z., Requillart V., Treich N. (2020). Viewpoint: Regulating meat consumption to improve health, the environment and animal welfare. *Food policy*. 97: 101847.
- Cornish A., Raubenheimer D., Mc Greevy P. (2016). What We Know about the Public's Level of Concern for Farm Animal Welfare in Food Production in Developed Countries. *Animals*. 6(11): 74.
- Dransfield E., Ngapo T.M., Nielsen N.A., Bredahl L.P., Sjöden O., Magnusson M., Camp M.M., Nute G.R. (2005). Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production. *Meat Science*. 69(1): 61-70.
- Gregurić Gračner G., Lončarić N., Bubić Špoljar J., Dovč A., Fuš K., Gračner D., Pavičić Ž. (2019). 3R načelo o zaštiti dobrobiti životinja koje će se koristiti u znanstvene svrhe-jučer, danas, sutra. *Veterinarska stanica*. 50(1): 2019.
- Lagerkvist C.J. (2013). Consumer preferences for food labelling attributes: comparing direct ranking and best-worst scaling for measurement of attribute importance, preference intensity and attribute dominance. *Food Qual Prefer*. 29(2): 77-88.
- Miranda-de la Lama G. C., Estevez-Moreno L.X., Sepulveda W.S., Estrada-Chavero M. C., Rayas-Amor A.A., Villarroel M., Maria G.A. (2017). Mexican consumers' perceptions and attitudes towards farm animal welfare and willingness to pay for welfare friendly meat products. *Meat Science*. 125:106-113.
- Napolitano F., Pacelli C., Girolami A., Braghieri A. (2008). Effect of Information About Animal Welfare on Consumer Willingness to Pay for Yogurt. *Journal of Dairy Science*. 91(3): 910-917.
- Očić V. (2021). Veća dobrobit životinja donosi dobit gospodarstvu. *Gospodarski list*. 22.
- Pavičić Ž., Ostović M. (2013). Dobrobit farmskih životinja. *Hrvatski veterinarski vjesnik*. 7-8.
- Temple D., Manteca X. (2020). Animal Welfare in Extensive Production Systems Is Still an Area Concern. *Frontiers in Sustainable Food Systems*. 4: 545902.

Challenges in building a career for young people in the agribusiness industry

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Summary

Global data shows that fewer and fewer young people are opting for a career in the agribusiness industry today. The main objective of the research is to identify the main challenges that young people face when choosing a career in this field. A sample of 120 high school graduates was used to analyse attitudes towards career development in the agribusiness industry. The results of the study show that young people believe that there are low wages and poor job opportunities in the agribusiness industry. These results can be useful for faculties and scientific institutes when working with young people.

Key words: career, agro-industry, youth, challenges, obstacles

Introduction

In today's fast-paced and ever-changing times, the lives of young people are very different from those of the past. The basic characteristics of today's youth lie a priori in the use of modern technologies, mobile phones and computers, in all areas of their activities, including the business world. Therefore, there are fewer and fewer young people who choose to build their future in agribusiness industry. According to Consentino et al. (2023), the global historical trend of a lack of young people opting for a career in agribusiness industry and staying in rural areas is confirmed. There are also fewer and fewer people who decide to start a business in this field or study agribusiness. According to the research results of Mulema et al. (2020), the main obstacles for young people to start a business in the agribusiness sector are: lack of start-up capital, low profitability of the business and personal ambitions. Based on a sample of 132 respondents, the author Tau (2024) argues that government-sponsored measures would increase the presence of young people in the sector and that they are one of the basic recommendations for greater involvement of young people in the world of agribusiness. Similar results were found in the research of Magadla et al. (2024). According to Chinsinga and Chasukwa (2012), young people in agribusiness industry are often marginalised by the government and not considered important enough. The results of the study by Ma et al. (2017) indicate that young people have little interest in agribusiness industry because this industry does not offer great opportunities for advancement, nor is there enough additional training offered in this field as in other industries, which makes them more attractive. In addition, the Canadian Agricultural Human Resource Council (2021) survey of a sample of 66 respondents found that young people do not see jobs in agribusiness as long-term, i.e. they do not see this industry as a field in which they could work their entire working lives. The author Ose (2021) used a sample of 19 respondents in Uganda to find out why young people in the country have little interest in agribusiness. In the conclusion of his research, the author recommends the use of innovative business models in agribusiness that could increase young people's interest in the sector. The research results of the authors Ahaibwe et al. (2013) show that young people working in the agribusiness sector

do not yet utilise technological progress as much as their peers in other sectors. They see this sector as a traditional industry and therefore do not utilise technological progress to any significant extent, despite the possibility of improving work in this area with modern technology. Particularly interesting are the findings of OECD research (2018), according to which young people who do not work in the agribusiness industry believe that young farmers are not sufficiently integrated into society because they work predominantly in rural areas. This is of course a widespread misconception that needs to be mentioned and definitely refuted. And young people from the agribusiness industry are just as involved in social processes as their peers from other sectors. The GIZ Foundation (2020) in its publication on youth involvement in agribusiness identifies four key strategies to increase the number of young people in the sector, namely: explaining to young people how to increase productivity in the sector; giving young people the opportunity to use information and communication technologies in the sector; giving young people adequate education and developing their skills for work in the sector; and making appropriate policies on youth employment issues. Research by Bogiri (2024) has shown that some of the ways to increase the number of young people at agronomic faculties and in agribusiness industry in general are as follows: Creating appropriate and sufficiently attractive curricula in the field, there is a need to understand that formal education in agribusiness industry is inadequate, and to provide more informal forms of education to enable young people to receive agricultural counselling at their request. According to Haruna et al. (2019), it is recommended that professors in agribusiness faculties influence the establishment and maintenance of school farms by students to acquire practical skills; that the government increase funding, provide scholarships for students to maintain interest and offer a wider range of different programmes in this field. According to Mwaura and Glove (2021), young people's interest in working in agribusiness industry will increase with the transition to a green economy, i.e. a green transformation. The results of the study by Girdziuta et al. (2022) on a sample of 430 young people in Latvia show that young people most often choose a career in agribusiness industry for the following reasons: their parents' positive experiences in this field, love for animals and nature, and the possibility of narrow specialisation and advancement in this field.

Material and methods

The data for the empirical study was collected from a sample of 120 respondents. The sample consisted of young people with a high school diploma in Croatia who provided information on their assessment of studying and future employment opportunities in the agro-industry. The sample included two high schools from the Split area and two high schools from the Zagreb area. The research sought to identify the biggest challenges for young people when it comes to studying and pursuing a career in the agribusiness industry. The questions asked to the respondents were related to their gender (socio-demographic data), but also to their commitment to their profession, i.e. to the faculty they will study. In addition, respondents were asked questions about the attractiveness of studying in this field and what they thought were the fundamental points that make agribusiness unattractive. For the last two questions, respondents were offered a multiple-choice procedure and had the opportunity to choose one of the options. The data was collected via the Google Forms platform and processed using SPSS statistical software. The respondents' attitudes are illustrated by the presentation of the statistical data.

Results and discussion

The study involved 120 respondents. The respondents were high school graduates from the 2024/2025 school year who are studying in Croatia. The most important sampling criterion was that the sample should consist of 120 high school graduates, which was fulfilled. The sample included 87 females (72.5%) and 33 males (27.5%). Of the total number of respondents, 78

(65%) had already decided on the faculty they would like to study at, while the remaining 42 (35%) had not yet made a decision regarding their choice of faculty. Agribusiness as a field of study was considered attractive by 36 respondents (30%), while the remaining 84 respondents (70%) did not consider agribusiness attractive. Respondents were asked why they did not consider an agribusiness degree programme attractive and the responses are shown in the table below (Table 1).

Table 1. The main reasons why studying agribusiness is not attractive

Answers	Frequency	Percent
Poor employment opportunities after college	69	57.5
College is very difficult	12	10
Insufficient use of technology at college is a disadvantage	30	25
I have no ambition to work in agriculture	9	7.5
Total	120	100

The results of the research show that one of the main obstacles preventing young people from choosing a career in agribusiness is the fear of unemployment. Similar results were found in the studies by Jjuuko (2022) and Roy (2023). Table 2 below shows why young people do not find working in agribusiness attractive.

Table 2. Why is it unattractive to work and build a career in agribusiness industry?

Answers	Frequency	Percent
Low perception of agriculture as a profession	15	12.5
Poor opportunity for advancement	10	8.33
Low salaries in this field	11	9.16
Lack of education	13	10.83
Profit depends on weather conditions	15	12.5
Hard and physically demanding work	37	30.83
Insufficient use of technology	19	15.85
Total	120	100

The results of the research show that young people are reluctant to pursue a career in agribusiness industry, mainly because they perceive the work as physically demanding and difficult. They also believe that advanced technologies are not used in the field and that both profits and wages are largely dependent on weather conditions. As far as physically hard labour is concerned, the research by Yeboah et al. (2020) also found that young people are not only afraid of hard work in the agribusiness industry, but also of possible injuries. Regarding the lack of use of technology in the agribusiness industry, it is worth mentioning that a similar finding was also noted in the research by Prasyeto et al. (2012), which states that young people see the agricultural industry as a sector that is resistant to innovation in terms of technology. The research also aimed to gather evidence that could provide recommendations for faculty and research institutions regarding careers in agribusiness industry. These recommendations are detailed in the conclusion.

Conclusions

Working in the agribusiness industry brings with it numerous challenges. These challenges include physically demanding labour, dependence on weather conditions and concerns about inconsistent profitability. The aim of the research was to assess the attitudes of young people in Croatia towards careers in agribusiness industry and to identify the obstacles and challenges

they face when studying and pursuing a career in this field. The results of the research show that respondents believe that working in this field is difficult and unpredictable and that technology in this area is insufficiently applied. It is important to change the paradigm and present work in agribusiness industry as a modern and technologically advanced field. Young people value technology as an integral part of their lives and it is important to show them that choosing agribusiness industry does not exclude this aspect of their future. There is also a need to develop informal agribusiness education programmes, modernise curricula and create greater awareness of the importance of this sector. Ultimately, life without food is not possible, and today's youth will help ensure a sustainable future for generations to come by pursuing a career in agribusiness.

References

- Ahaibwe G., Mbowa S., Lwanga M.M. (2013). Youth engagement in agriculture in Uganda: Challenges and prospects (Research Series No. 106). Economic Policy Research Centre (EPRC).
- Bogiri R. (2024). Developing the agriculture sector through youth entrepreneurship: The case of Vanuatu and lessons from Fiji (Policy Brief). Griffith University.
- Canadian Agricultural Human Resource Council (CAHRC) (2021). Ontario agriculture and food processing skills and occupation gaps. Canadian Agricultural Human Resource Council.
- Chinsinga B., Chasukwa M. (2012). Youth, agriculture and land grabs in Malawi. Paper presented at the Young People, Farming and Food Conference, Accra, Ghana.
- Consentino F., Vindigni G., Spina D., Monaco C., Peri I. (2023). An agricultural career through the lens of young people. *Sustainability*. 15(14): 11148. Available from: <https://doi.org/10.3390/su151411148>
- Girdziute L., Besuspariene E., Nausedienė A., Novikova A., Leppala J., Jakob M. (2022). Youth's (un)willingness to work in the agriculture sector. **Frontiers in Public Health**. 10. Available from: <https://doi.org/10.3389/fpubh.2022.937657>
- GIZ Fondation. (2020). Job creation for Africa's youth: Are there pathways in agricultural value chains? GIZ.
- Haruna O.I., Asogwa V.C., Ezhim I.A. (2019). Challenges and enhancement of youth participation in agricultural education for sustainable food security. *African Educational Research Journal*. 7(4): 174-182. Available from: <https://doi.org/10.30918/AERJ.74.19.028>
- Jjuuko R. (2022). Youth transition, agricultural education and employment in Uganda: Freeing individual agency. *Journal of Vocational Education and Training*. 74 (4): 705-706. Available from: <https://doi.org/10.1080/13636820.2022.2118939>
- Ma S., Eom J., Kim K. (2017). Job creation potential for the youth and challenges in the agricultural industry. *Agricultural Policy Focus*. 148.
- Magadla A., Darko M.T., Enow S. (2024). The role of small-scale agricultural business on youth employment at a selected district in the Eastern Cape, South Africa. *Open Journal of Business and Management*. 12(3): 1123-1139. Available from: <https://doi.org/10.4236/ojbm.2024.123085>
- Mulema J., Mugambi I., Kansiime M., Chan H.T., Chimalizeni M., Pham T.X., Oduo, G. (2020). Barriers and opportunities for youth engagement in agribusiness: Empirical evidence from Zambia and Vietnam. **Development in Practice**. 30(8): 1043-1058. Available from: <https://doi.org/10.1080/09614524.2021.1911949>
- Mwaura G., Glover D. (2021). Green jobs for young people in Africa: Work in progress Evidence synthesis paper series. 7.

- OECD (2018). The future of rural youth in developing countries: Tapping the potential of local value chains (Development Centre Studies). OECD Publishing. Available from: <https://doi.org/10.1787/9789264298521-en>
- Ose Y. (2021). Solutions to youth agripreneurship challenges – Lessons learned from youth agripreneur champions in Uganda. Rome, FAO.
- Prasetyo Y.E., Apriliyadi E.K., Hidajat E.W., Noviant F. (2012). Resistance to innovation: Case of appropriate technology implementation in rural agriculture communities. SSRN Electronic Journal. Available from: <https://doi.org/10.2139/ssrn.2101656>
- Roy S.K. (2023). Influencing factors for pursuing agriculture as a career for agriculture undergraduates: A two-stage approach. Research Square. Available from: <https://doi.org/10.21203/rs.3.rs-2974745/v1>
- Tau T.N. (2024). Challenges hindering youth participation in agriculture in Botswana. International Journal of Business Management and Economic Review. 7(1): 82-90. Available from: <https://doi.org/10.35409/IJBMER.2024.3551>
- Yeboah T., Chigumira E., John I., Anyidoho N.A., Manyong V., Flynn J., Sumberg J. (2020). Hard work and hazard: Young people and agricultural commercialisation in Africa. Journal of Rural Studies. 76: 142-151.

REVIEW ARTICLE

Consumer preference for biofortified food: systematic review

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Summary

The aim of this review is to investigate consumer preferences for biofortified foods. The review analyses consumer acceptance of biofortified foods, focusing on foods biofortified with vitamin A, zinc, iron, selenium and iodine in different regions. The report outlines the opportunities and challenges in the acceptance of biofortified foods and shows that consumer education, affordability and sensory appeal are crucial for market success. To improve uptake, policy support, subsidy programmes and improved marketing strategies should be implemented to make biofortified foods more accessible and increase their acceptance.

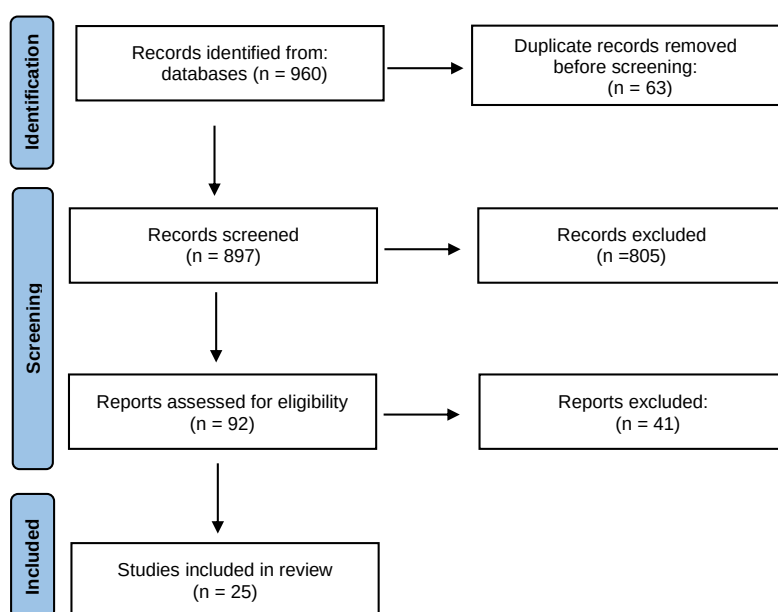
Key words: consumers, preferences, biofortified food, willingness to pay

Introduction

Biofortified foods are not explicitly regulated by specific legislation but correspond to the concept of functional foods as described in the Scientific concepts of functional foods in Europe consensus document (1999). Functional foods provide health benefits that go beyond basic nutrition by promoting well-being and reducing the risk of disease. They can take various forms, including natural foods, foods with added or removed ingredients and those with increased bioavailability (Europe consensus document, 1999). The document emphasises that functional foods must be consumed in the form of normal foods (not pills or capsules) and must be effective with normal dietary intake. While fortified foods (to which nutrients are added post-harvest) are recognised for their enhanced functionality, biofortification the process of increasing the nutrient content in crops through agricultural practises, conventional breeding or biotechnology is not directly addressed. However, the document recognises that technologically or biotechnologically modified foods can be considered functional or fortified if their health claims are scientifically sound, clear and unambiguous. This view is in line with Foti et al. (2021) and Regulation (EC) No 1925/2006 on food safety, which monitors the addition of substances to food to ensure their safety. Biofortification plays an important role in combating hidden hunger, especially among low-income populations with limited dietary diversity (Talsma et al., 2017; Otey et al., 2020). Since biofortification of foods such as cassava, rice, beans, wheat, maize and sweet potatoes can significantly improve the nutrition of households that lack access to a more diverse diet in several countries (Otey et al., 2020; Welk et al., 2021). The review aims to analyse the market and consumer preferences for biofortified foods by presenting the increasing trends of biofortified foods in the market and examining consumer perceptions of biofortified foods in low- and high-income countries. In the main part of this review, the authors have attempted to systematise the findings of the selected papers on this topic and identify new research directions that could help focus the growing interest in biofortified foods.

Material and methods

The review followed the PRISMA (2020) guidelines, including the 27 items checklist and the flow diagram to guide identification, screening and inclusion of studies that are considered essential and minimum components of a protocol for a systematic review or meta-analysis (Page et al., 2021). Initially, a preliminary database of articles was created by collecting searches from several major databases, including Scopus, Web of Science, Elsevier's ScienceDirect, AgEcon Search, EconPapers and CAB Abstracts. The selection of articles related to consumer preferences for biofortified foods was based on keywords: 'consumer', 'preferences', 'sensory attributes', 'biofortification', 'food', 'purchase intention' and 'willingness to pay'. The studies considered for this review were analysed for the period from 2015 to 2025. Duplicates were excluded and only articles from academic journals, conference proceedings and books published in English were selected for the next screening. Studies were conducted in Europe, Africa, Asia and Latin America which are mainly affected by micronutrient deficiencies. Qualitative and quantitative methods were used in the studies to assess consumer preferences for biofortified foods. 92 abstracts were screened for eligibility. Of these, 41 articles were excluded because they did not fulfil the above criteria. Of these 51 articles, the texts were analysed and only 25 articles fully met the criteria for this systematic review (Graph 1).



Graph 1. Preferences for biofortified foods PRISMA flow diagram

Results and discussion

Micronutrient deficiencies, often referred to as hidden hunger, remain a pervasive global health crisis affecting an estimated two billion people, particularly in regions such as Asia, Africa and Latin America (Marques et al., 2021). Deficiencies of iron, iodine, zinc, selenium and vitamin A are among the most widespread and pressing nutritional problems. Biofortification, an agricultural strategy to increase the nutritional content of staple crops through biotechnology, conventional breeding or agronomic practises, has proven to be a cost-effective and sustainable solution (Aparo et al., 2023). By enhancing a plant's genetic ability to accumulate or synthesise essential micronutrients, biofortification improves the quality of nutrition and offers significant benefits to rural populations who may not have access to supplements or fortified foods. Despite its promising potential, biofortification faces several obstacles, including genetic and agronomic limitations, concerns about nutrient bioavailability, complicated regulatory

requirements and varying levels of consumer acceptance. However, research points to growing opportunities enabled by advanced breeding technologies, integration of biofortified crops into public nutrition initiatives, and targeted commercialisation supported by inclusive policy frameworks (Zuma et al., 2018; Marques et al., 2021; Ashoka et al., 2023). In economic terms, biofortification has shown a high return on investment. Studies demonstrate its potential to increase agricultural yields and farmer profitability, thereby promoting economic development in general (Olum et al., 2021; Olson et al., 2021). Socially, it promotes the right to adequate food and serves as a strategic tool in times of crisis, such as the COVID-19 pandemic, when food insecurity increases. Biofortified crops can be effectively included in school feeding programmes and food aid programmes to improve nutrition while educating the population about healthier eating habits. Thus, biofortification is not only a nutritional intervention, but also a catalyst for economic resilience and social well-being (Olson et al., 2021). In this broader context, several studies have investigated how consumers perceive and react to biofortified foods. Rizwan et al. (2021) investigated consumer acceptance of zinc biofortified wheat in Pakistani Punjab and emphasised the importance of zinc in combating malnutrition. Their results show that while consumers still prefer traditional wheat, educated households and households with young children are more willing to switch to biofortified varieties, especially if sensory attributes such as appearance and flavour are retained. Mahboob et al. (2022) came to similar conclusions, finding that while consumers perceive zinc-enriched flour to be of high quality, price sensitivity is a barrier to widespread adoption. Outside of South Asia, Birol et al. (2015) conducted a cross-national study in Africa, Asia and Latin America and concluded that consumer acceptance of biofortified foods particularly those fortified with vitamin A, iron and zinc is strongly influenced by sensory attributes and the availability of health-related information. For example, when consumers were informed about the benefits of vitamin A-rich orange maize in Zambia or iron-fortified beans in Guatemala, acceptance increased significantly. In these cases, consumer engagement increased significantly when nutritional information was delivered through trusted sources like community leaders or radio. A growing body of research on willingness to pay (WTP) shows that consumers are generally willing to pay a premium for biofortified foods. However, the extent of this premium varies according to crop type, regional context and the effectiveness of the communication strategy. For biofortified products with distinct visual characteristics such as the colour change in vitamin A-enriched maize or cassava premiums ranged from 8 to 50% depending on the context and level of awareness (Birol et al., 2015; Ayetigbo et al., 2018; Hama-Ba et al., 2019; Jada and van den Berg, 2024). A meta-analysis by De Steur et al. (2017) found an average WTP premium of 21.3 % for various biofortified crops worldwide. However, these premiums depend on maintaining desirable sensory attributes, as consumers are not willing to accept higher prices if they perceive that flavour or cooking quality decreases. In addition, regional dynamics influence consumer perceptions, with certain markets placing more emphasis on international certification, branding and traceability. Consumer segmentation studies offer additional insights. In Rwanda, Murekezi et al. (2017) identified three distinct groups that responded to iron biofortified beans: those who preferred them, those with low interest and those who disliked them. Socioeconomic characteristics such as income level and nutritional awareness were strong predictors of group affiliation. Notably, 82% of marginal likers had received nutrition education, emphasising the influence of information on consumer decisions. A comprehensive review by Talsma et al. (2017) of 72 studies in low and middle-income countries found generally positive sensory responses to biofortified crops, particularly orange-fleshed sweet potatoes and maize. The most influential factors for consumer preference were product availability and awareness of nutritional benefits, with informed consumers willing to pay up to 25% more. Further support comes from Kolapo (2023), who investigated consumer behaviour in Nigeria and found strong correlations between socio-demographic factors and the acceptance of vitamin A-fortified

foods. In the study, consumers were categorised into three groups: advocates, opponents and indifferent. Those in favour (advocates) generally older, urban and educated women were willing to pay a 35% premium, while those against preferred traditional alternatives and were more price sensitive. Urban consumers showed greater interest, probably due to the fact that they receive more information about nutrition, which emphasises the importance of improved distribution systems and public education in expanding market potential. The role of biofortification of selenium and iodine has also been investigated. D'Amato et al. (2020) investigated strategies to increase the selenium content in crops such as rice and tomatoes and found that both the antioxidant properties and the shelf life and sensory quality, especially of meat products and tomatoes, improved. In Germany, Wortmann et al. (2018) investigated consumer acceptance of selenium biofortified apples and found moderate interest, especially when labelled with health claims such as 'rich in selenium' in contrast, the term 'biofortified' generated less enthusiasm, suggesting that more consumer-friendly terminology could improve acceptance. Similarly, Welk et al. (2021) investigated German consumers' attitudes towards iodine-fortified fruit and vegetables and found that 85% favoured these foods over iodine supplements. Demand was particularly strong among vegetarians and vegans, who are more susceptible to iodine deficiencies. However, the study also revealed a significant knowledge gap, as almost a third of adults and almost half of children do not consume enough iodine. To address this, the authors recommend national health campaigns and targeted marketing to raise awareness and promote an iodine-rich diet. Communication and labelling remain key challenges in high-income countries Foti et al. (2021) found that Italian consumers often confuse 'biofortified' with 'organic', leading to misunderstandings about the nature of such products. Mitra-Ganguli et al. (2022) emphasised that marketing strategies should avoid the term 'biofortified' altogether and instead focus on the natural nutritional benefits. Despite this confusion, consumer interest remains high. Foti et al. reported that 64% of Italians surveyed are willing to pay €2.6–3.0 per kilogramme for biofortified tomatoes, and 67% of them are willing to pay €2.0–3.0 for a pack of cherry tomato sauce, although availability and clear labelling were identified as the main barriers. These findings were corroborated by Timpanaro et al. (2020), who pointed to the need for standardised definitions, transparent labelling and clear pricing to promote consumer confidence and wider market acceptance. All the studies analysed found that consumers' purchasing decisions are influenced by lifestyle, socio-demographic factors, nutritional knowledge and dietary habits. The results emphasise the importance of effective communication strategies and clear legislation to bridge the gap between consumer knowledge and product attributes and ultimately increase consumer acceptance and expand the market potential for biofortified foods. Before conclusion some limitations should be mentioned. Whilst an attempt was made to comprehensively search the main databases, non-English sources were excluded, which may have overlooked relevant studies. Future studies should aim to fill the gaps identified by using larger and more diverse samples, utilising longitudinal methods and focusing on under-represented regions and populations.

Conclusions

This review summarises the current literature on the status of biofortified foods. Consumer acceptance of biofortified foods is influenced by a combination of consumer socio-demographic characteristics, sensory and nutritional perceptions. While studies indicate a high willingness to pay for biofortified varieties, especially when the nutritional benefits are effectively communicated to consumers, price sensitivity and availability remain significant barriers. Future efforts should focus on improving consumer education through targeted communication strategies, improving market accessibility and affordability, and standardising labelling to avoid confusion and improve consumer purchasing decisions. By overcoming these challenges,

biofortification can become more widely accepted and a sustainable solution for reducing micronutrient deficiencies worldwide.

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References

- Aparo N.O., Olum S., Atimango A.O., Odongo W., Aloka B., Ongeng D., Gellynck X., Steur H.D. (2023). Farmers’ Intention to Adopt Agronomic Biofortification: The Case of Iodine Biofortified Vegetables in Uganda. *Horticulturae*. 9: 401.
- Ashoka P., Sangeeta Spandana B., Saikanth D.R.K., Kesarwani A., Nain M., Pandey S.K., Singh B.V., Maurya C.L. (2023). Bio-fortification and Its Impact on Global Health. *Journal of Experimental Agriculture International*. 45(10): 106–115.
- Ayetigbo O., Latif S., Abass A., Müller J. (2018). Comparing characteristics of Root, Flour and Starch of biofortified Yellow-Flesh and White-Flesh Cassava Variants, and Sustainability Considerations: A Review. *Sustainability*. 10(9): 3089.
- Birol E., Meenakshi J., Oparinde A., Pérez S., Tomlins, K. (2015). Developing country consumers’ acceptance of biofortified foods: a synthesis. *Food Security*. 7(3): 555-568.
- D’Amato R., Regni L., Falcinelli B., Mattioli S., Benincasa P., Bosco A.D., Pacheco P., Proietti P., Troni E., Santi C., Businelli D. (2020). Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. *Journal of Agricultural and Food Chemistry*. 68(14): 4075-4097.
- Foti V.T., Scuderi A., Bellia C., Timpanaro G. (2021). Biofortification of tomatoes in Italy: Status and level of knowledge. *Agricultural Economics*. 67(6): 227-235.
- Hama-Ba F., Mouquet-Rivier C., Diawara B., Weltzien E., Icard-Vernière C. (2019). Traditional African Dishes Prepared From Local Biofortified Varieties of Pearl Millet: Acceptability and Potential Contribution to Iron And Zinc Intakes of Burkinabe Young Children. *Frontiers in Nutrition*. 6: 115.
- Jada K. S., Van den Berg M. (2024). Social norms, nutrition messaging, and demand for biofortified staple crops: Evidence from a discrete choice experiment in Ethiopia. *Agricultural Economics*. 55(5): 695-713.
- Kolapo A. (2023). Heterogeneous preferences and market potentials for biofortified foods in sub-Saharan Africa: Evidence from Nigeria. *Future Foods*. 8: 100278.
- Mahboob U., Ceballos-Rasgado M., Moran V H., Joy E.J.M., Ohly H., Zaman M., Lowe N.M. (2022). Community Perceptions of Zinc Biofortified Flour during an Intervention Study in Pakistan. *Nutrients*. 14(4): 817.
- Marques E., Darby H., Kraft J. (2021). Benefits and limitations of non-transgenic micronutrient biofortification approaches. *Agronomy*. 11(3): 464.
- Mitra-Ganguli T., Pfeiffer W., Walton J. (2022). The global regulatory framework for the commercialization of nutrient enriched biofortified foods. *Annals of the New York Academy of Sciences*. 1517: 154-166.
- Murekezi A., Oparinde A., Birol E. (2017). Consumer market segments for biofortified iron beans in Rwanda: Evidence from a hedonic testing study. *Food Policy*. 66:35-49.
- Olson R., Gavin-Smith B., Ferraboschi C., Kraemer K. (2021). Food Fortification: The Advantages, Disadvantages and Lessons From Sight And Life Programs. *Nutrients*. 13: 1118.

- Olum S., Gellynck X., Wesana J., Odongo W., Aparo N.O., Aloka B., Ongeng D., De Steur H. (2021). Economic Feasibility of Iodine Agronomic Biofortification: A Projective Analysis with Ugandan Vegetable Farmers. *Sustainability*. 13(19): 10608.
- Oteh O.U., Hefferon K., Agwu N.M. (2020). Moving biofortified cassava products closer to market in Nigeria. *Frontiers in Sustainable Food Systems*. 4:589424
- Page M.J., McKenzie J.E., Bossuyt P.M., Boutron I., Hoffmann T.C., Mulrow C.D. ... (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 372: n71.
- Regulation (EC) No 1925/2006 (2006). Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. Available from: <https://eur-lex.europa.eu/eli/reg/2006/1925/oj>
- Rizwan M., Zhu Y., Qing P., Zhang D., Ahmed U.I., Xu H., Iqbal M.A., Saboor A., Malik A.M., Nazir A., Wu X., He P., Tariq A. (2021). Factors Determining Consumer Acceptance of Biofortified Food: Case of Zinc-Fortified Wheat in Pakistan's Punjab Province. *Frontiers in Nutrition*. 8:647823.
- Scientific concepts of functional foods in Europe. Consensus document (1999). *British Journal of Nutrition*. 81: S1-S27.
- Steur H.D., Wesana J., Blancquaert D., Van der Straeten D., Gellynck X. (2017). Methods matter: a meta-regression on the determinants of willingness-to-pay studies on biofortified foods. *Annals of the New York Academy of Sciences*. 1390(1): 34-46.
- Talsma E.F., Melse-Boonstra A., Brouwer I.D. (2017). Acceptance and adoption of biofortified crops in low- and middle-income countries: a systematic review. *Nutrition Reviews*. 75(10): 798-829.
- Timpanaro G., Bellia C., Foti V.T., Scuderi A. (2020). Consumer behaviour of purchasing biofortified food products. *Sustainability*. 12(16): 6297.
- Welk A.K., Kleine-Kalmer R., Daum D., Enneking U. (2021). Consumer Acceptance and Market Potential of Iodine-Biofortified Fruit and Vegetables in Germany. *Nutrients*. 13(12): 4198.
- Wortmann L., Enneking U., Daum D. (2018). German consumers' attitude towards selenium-biofortified apples and acceptance of related nutrition and health claims. *Nutrients*. 10(2): 190.
- Zuma M.K., Kolanisi U., Modi A.T. (2018). The Potential of Integrating Provitamin A-Biofortified Maize in Smallholder Farming Systems to Reduce Malnourishment in South Africa. *International Journal of Environmental Research and Public Health*. 15: 805.

PRELIMINARY COMMUNICATION

Food habits of foreign workers from the Philippines, Nepal, and India in Croatia: Experiences and adaptationJosip Juračak¹, Bruno Vlajsović¹, Damir Kovačić¹¹University of Zagreb Faculty of Agriculture,
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Summary

Due to labour shortages, Croatia is increasingly employing workers from the Philippines, Nepal and India, who are confronted with changing diets. In this study, we used focus groups to examine the differences in the diet of these workers after their arrival in Croatia. The data collected shows that Filipinos reduced the number of meals, consumed more bread and gained weight after arriving in Croatia. Nepalese workers eat hotel food, less traditional dishes and have lost weight. Indian workers eat less than before, often consuming bakery products, and have also lost weight. The biggest challenges for all groups of workers are the high cost of food and limited access to traditional ingredients, which affects their eating habits and body weight.

Key words: workforce, migration, adaptation, dietary habits

Introduction

Croatia is experiencing a long-term trend of decreasing total population and working-age population and is forced to import foreign labour (Akrap, 2015). According to the Ministry of the Interior (MUP), 206,500 work and residence permits were issued to foreigners in Croatia in 2024, most of them in construction (75,071), tourism and hospitality (56,228), industry (28,486), transport and communication (16,149), and trade (7,925). The largest number of permits were issued to citizens from Bosnia and Herzegovina, Nepal, Serbia, India, the Philippines and North Macedonia (MUP, 2025). Assuming stable economic growth and continued negative demographic trends, the Croatian Employers' Association estimates that Croatia will be short of between 400,000 and 500,000 foreign workers by 2030 (Novi list, 2024).

Croatia has extensive experience with foreign workers, especially in the construction, tourism and hospitality sectors. In the past, most foreign workers came from Southeastern Europe, which made their integration relatively easy. In recent years, in addition to the influx of foreign workers from the region, the number of workers from Southeast and South Asia (Nepal, the Philippines, India and Bangladesh) has also increased significantly. This labour force is culturally different from the local population, which makes it necessary to create and implement an appropriate migration policy. While scientific publications and the media are increasingly discussing the need to integrate foreign workers into Croatian society, with a focus on learning the Croatian language and labour and social rights, issues of nutrition have, to our knowledge, not yet been addressed.

Upon arrival in Croatia, foreign workers from South and Southeast Asia are confronted with a different nutritional situation. Croatian food consumption is characterised by a high consumption of red and processed meat, ultra-processed foods rich in fats, sugar and other refined carbohydrates, as well as sugary drinks, while fruit, vegetables and whole grain products are consumed relatively rarely. Combined with a more or less sedentary lifestyle, this leads to a high number of obese people who are predisposed to various diseases, especially cardiovascular disease (CVD) and type 2 diabetes (T2D).

There are numerous countries that have problems with the diet of foreign workers, especially those from a different cultural background, and many studies have been conducted on this topic. Lee et al. (2017) conducted a study on the dietary habits of workers from Southeast Asia (Vietnamese, Thai, Cambodian and Myanmar) living in South Korea. The study analysed the diets of foreign workers before and after immigration and revealed significant changes, including a lower frequency of consumption of fresh fruit, cooked vegetables, rice noodles, green tea and sticky rice, and increased consumption of kimchi, seaweed, milk, coffee and pizza. These changes were attributed to the rapid acculturation of their diet. The frequency of consumption of their traditional foods decreased significantly after their arrival in Korea, except for the Thai workers. As many as 28.2% of the participants stated that their health had deteriorated after their arrival in South Korea due to difficulties in adapting to the diet, increased consumption of instant food, and lack of exercise.

A study conducted in France, Mali and Morocco examined the eating habits of immigrants and their children and their evolution across space and time (Calandre and Ribert, 2019). The study showed that, depending on the context, children reproduce certain norms, ideas and practises of their parents, as well as some that prevail in the country of origin, which also change at a local and global level. The different eating habits of immigrants and their children emerge and change according to the evolution of the family situation, socio-cultural characteristics and economic resources. Individuals adapt to different social situations and move from one culture to another.

Njambi (2023) investigated changes in the diet of foreign students after immigration to the United Kingdom. The results of the study showed that the consumption of foods that are not considered Western (mainly nuts, fruit and vegetables) and protein-rich foods (beans, meat soup, fish and sausages) decreased, while the consumption of highly processed, high-fat foods such as chips, cheese, bacon, sausages and ham, which are considered Western, increased.

Vahambi and Damba (2013) examined the perceived barriers to obtaining food by Latino immigrants in Toronto. Four main categories of barriers were identified: limited financial resources, language difficulties, cultural food preferences, and lack of knowledge about available food sources and services in the community.

Holmboe-Ottesen and Wandel (2012) conducted a meta-study on changes in migrants' dietary habits and the consequences of these changes for health, focussing on South Asian migrants in Europe. The majority of migrants consumed significantly more energy and fats after migration and at the same time reduced their intake of carbohydrates and fibre. The consumption of vegetables, whole grains, and legumes decreased, while the consumption of refined carbohydrate sources increased. The consumption of meat and dairy products also increased. The results suggest that these changes in diet contribute to a higher risk of obesity, T2D and cardiovascular disease.

The aim of this study is to determine the dietary behaviour, dietary restrictions and satisfaction with available food among foreign workers from South and Southeast Asia in Croatia. As far as we know, this is the first study of its kind to contribute to a better understanding of the dietary habits of foreign workers from South and Southeast Asia in Croatia. The results of the study provide a basis for further research on this topic.

Material and methods

Foreign workers from Nepal, India and the Philippines took part in the study. The selection of participants was limited to individuals who had sufficient command of the English language to take part in the interview without the assistance of a translator. Contact with the participants was established via a domestic employment agency for foreign workers. Participants were offered a reward of 15 euros for their participation.

The study was conducted in July 2024 using the focus group method. Two focus groups were conducted. The first focus group took place in Čakovec with a sample of 10 workers from the Philippines, consisting of 5 females and 5 males. The age of the Filipino workers ranged from 25 to 43 years, with an average age of 34 years. They had come to work in Croatia between 2021 and 2024. The second focus group took place in Kutina with a sample of 9 participants, 5 of whom were from India and 4 from Nepal. The second focus group included only male participants, whose ages ranged from 23 to 45, with an average age of 35. They came to Croatia in 2023.

The focus group recall consisted of open-ended questions divided into the following topics: Diet and food before migration, current dietary patterns, attitudes towards Croatian food, satisfaction with food in Croatia, and views on health and good appearance. The focus groups were led by a trained moderator. The sessions were audio recorded and the recordings were later listened to and transcribed. One focus group lasted 90 minutes, the other 95 minutes. The qualitative analysis was carried out using content analysis.

The Ethics Committee of the Faculty of Agriculture University of Zagreb approved the conduct of this research.

Results and discussion

Before emigrating to Croatia, Filipino workers had three meals a day and one or two snacks in between, with lunch being the most important. Breakfast usually consisted of dishes such as fried rice, noodles with rice, soups, dried salted fish, wheat bread, butter with jam and coffee. Lunch was usually eaten within the family. In the Philippines, lunch is served all at once and without courses. The usual lunch includes rice with chicken, lamb, beef and occasionally frogs, with the addition of sauces and vegetables. Bananas and ice cream are often eaten for dessert. A simpler lunch consists only of rice with coffee or salted water. Beverages include water or juice. Dinner consists of similar ingredients to lunch. Wealthier families consume grilled chicken or fish once or twice a week, followed by beer or whiskey after dinner.

After arriving in Croatia, workers have changed their eating patterns. They usually eat two meals: breakfast and lunch, with an occasional snack. Breakfast is early and usually consists of a meal of rice. For a snack, which they rarely eat, they usually eat sandwiches. Lunch remains their most important meal and consists of rice with meat, chicken, turkey and, more rarely, beef and fish. As far as salads are concerned, they eat tomatoes most often. The fruits they eat most often are apples, blueberries, bananas, avocados and cherries. They mostly drink water and on weekends wine, beer, "gemischt" (wine spritzer), or rakija (a type of brandy).

The foods they enjoy most in Croatia include zucchini, green vegetables, croissants, cheese. Their favourite foods in Croatia include zucchini, green vegetables, croissants, cheese burek, doughnuts and kebabs. They have not yet tried to prepare traditional Croatian dishes. They prepare their own food and only eat fast food in restaurants.

They usually buy the ingredients from supermarket chains. They rarely shop at local markets, mostly just eggs and chicken. Their biggest problem is finding traditional Filipino ingredients such as chilli, soy sauce, certain vegetables and special chicken parts (e.g. chicken feet). Although there is a specialized store for East Asian ingredients in Čakovec, they don't shop there because of the high prices. Instead, they order most of the traditional ingredients online from Slovenia.

They believe that the food sold in Croatia is of good quality. What bothers them is the excessive amount of salt in the food, especially in fast food products. Since arriving in Croatia, they have gained between one and five kilogrammes, which they attribute to the high consumption of rice and fast food. They find the food in Croatia very expensive and feel that they are eating more unhealthily than before they arrived in Croatia.

Before coming to Croatia, the workers from Nepal ate three meals a day: breakfast, lunch, and dinner. Dinner was the most important meal, with rice, chicken, and fish as the main ingredients. They did not consume beef. In addition to water, they usually drank rakija (a homemade liquor, "raksi") and fruit wine. In Nepal, food is usually bought in supermarkets and alcoholic drinks in specialized shops. Most of them did not consume fast food like McDonald's because it was too expensive.

After arriving in Croatia, they eat three meals a day, with dinner still being the most important. The workers involved in the study work and live in a hotel, so they mostly eat hotel food that they like. Unlike in Nepal, in Croatia they often eat bread, and especially like pizza, ćevapi (grilled minced meat) and bakery products. They often eat sweets after dinner. Nepalese shops in Croatia are too expensive, so they only buy food in supermarkets.

They believe that the food in Croatia is tasty and of good quality. Due to the high prices, they cannot afford to prepare traditional Nepalese food. After arriving in Croatia, they lost several kilogrammes, which they attribute to hard work and eating less traditional Nepalese food.

Before coming to Croatia, Indian workers usually had three main meals and one snack between lunch and dinner, which typically consisted of snacks and tea. For Indian workers, breakfast was the most important meal, which usually consisted of rice and meat (fish, chicken, beef). Vegetables were also an important part of the meal and were consumed in large quantities. Indian food is prepared with many spices and is very spicy. The influence of Arabic and Italian cuisine is increasingly noticeable in Indian cuisine. For drinks, they mostly drank water, tea and lemonade, while alcoholic drinks included rakija, often illegal "wart" rakija, and beer. They often dined in traditional restaurants with family or friends. Ingredients and spices were usually bought in supermarkets and alcohol in specialized shops. Due to the high prices, they did not consume international fast food such as McDonald's.

In Croatia, they work a lot and eat less than in India. Dinner is still the most important meal and consists of rice, meat, fish and sausages. They often eat bakery products, and among the local dishes they particularly like pizza, ćevapi, burek and kebab. They mostly drink water and, at weekends, beer and rakija. Food in Croatia is expensive and they buy it in supermarkets and discount stores. They report that they have lost a few kilogrammes since arriving in Croatia.

Conclusions

Workers from the Philippines, Nepal and India have adjusted their diets in Croatia, but with challenges. Filipino workers have reduced the number of meals, eat more bread and bakery products and have generally put on weight, which they attribute to the hard work. Nepalese workers eat mainly hotel food, rarely prepare traditional dishes and have lost weight. Indian workers eat less than at home, often consume bakery products and have also lost weight. They all point to the high food prices and the difficult access to traditional ingredients. Despite the quality of the food, adaptation was hindered by financial and cultural factors. The results of this study represent a starting point for further analyses of the situation and needs for a more successful adaptation of foreign workers from Far Eastern countries to food and dietary conditions in Croatia.

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References

- Akrap A. (2015). Demografski slom Hrvatske: Hrvatska do 2051. *Bogoslovska smotra*. 85(3): 855-868.
- Calandre, N., Ribert E. (2019). Sharing norms and adapting habits. The eating practices of immigrants and immigrants' children from Malian and Moroccan origins in France. *Social Science Information*. 58(1): 141-192.
- Dragojević Mijatović A. (2024). Od zemlje iseljenika, postali smo - zemlja useljenika. Hrvatskoj u ovom trenutku nedostaje najmanje 200.000 radnika. *Novi list*. 26/02/2024. Available from: <https://shorturl.at/yY9RJ>.
- Holmboe-Ottesen G., Wandel M. (2012). Changes in dietary habits after migration and consequences for health: a focus on South Asians in Europe. *Food & nutrition research*. 56(1): 18891.
- Lee E.J., Lee K.R., Lee S.J. (2017). Study on the change and acculturation of dietary pattern of Southeast Asian workers living in South Korea. *Appetite*. 117: 203-213.
- Ministry of the Interior (MUP) (2025). Statistike izdanih dozvola za boravak i rad 2024. Available from: <https://shorturl.at/M3HDy>.
- Njambi W.E. (2023). Dietary Habits Fluctuations after Immigration: A Focus on University Students Immigrants in the United Kingdom. *International Journal of Scientific Research and Management (IJSRM)*. 11(6): 82-86.
- Vahabi M., Damba C. (2013). Perceived barriers in accessing food among recent Latin American immigrants in Toronto. *International journal for equity in health*. 12: 1-11.

Outlook on the Croatian dairy sector – Status and perspectives

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Summary

The Croatian dairy sector has experienced a significant structural decline, characterised by a sharp reduction in the number of dairy cows and milk suppliers, resulting in a 16% drop in raw milk production since 2018. Although milk yield per cow and deliveries per supplier have increased, these productivity gains have not been sufficient to counterbalance the overall production decrease. Rising production costs, particularly for feed, energy and labour, alongside supply constraints from a shrinking dairy herd have further exacerbated the challenges facing the sector. At the same time, increasing dependence on imports has led to a decline in the level of self-sufficiency, which fell from 52.2% in 2018 to just 29% in 2024. Despite a notable increase in exports, domestic supply is still insufficient to meet demand, indicating structural imbalances.

Key words: Croatian dairy sector, structural changes, self-sufficiency, dairy market trends

Introduction

The Croatian agricultural sector plays an indispensable role in securing the country's food sovereignty and self-sufficiency, even though Croatian agricultural production accounts for less than 1% of the European Union's (EU) total agricultural production (Grgić et al., 2019). This is particularly important given the current geopolitical challenges. However, the sector is struggling to achieve growth in line with overall economic expansion. Agricultural production declined by 7.4% in 2023, along with a decrease in gross value added 9% and in income by 16.1% (CBS, 2024). In 2024, agricultural production is expected to decline by 0.35%, while income is expected to fall by 6.29% (CBS, 2024).

These negative trends are particularly pronounced in key livestock markets, including the dairy sector. Livestock production accounted for only 34.5% of total agricultural gross output in 2023, with expectations for 2024 indicating a further decline to approximately 31% of total agricultural gross output (CBS, 2024). Within the livestock sector, the highest value share is accounted for by cattle 27.2%, followed by cow's milk 24.6%, pigs 22.9% and poultry 14.4% (CBS, 2025). These figures emphasise the central role of dairy production within the Croatian livestock sector, although its share of total agricultural production is declining.

Given these trends, the aim of this paper is to analyse the key indicators of the Croatian dairy sector from 2018 to 2024 and assess the self-sufficiency of the sector. By analysing production trends, market dynamics and structural changes, this paper provides insights into the challenges and opportunities that dairy farming in Croatia is facing.

Material and methods

The analysis is based on secondary data sources, primarily statistical reports from the Croatian Bureau of Statistics (CBS), Eurostat and other relevant institutional databases. The dataset includes key indicators of the Croatian dairy sector from 2018 to 2024. Data processing

included trend analyses and percentage change calculations to assess sectoral dynamics. The results provide insights into structural shifts in milk production and productivity within the Croatian dairy market.

The level of self-sufficiency of the dairy sector was assessed using the standard formula:

$$\text{Self-Sufficiency Ratio (SSR)} = \left(\frac{\text{Milk deliveries (C)}}{\text{Milk deliveries (C)} + \text{Import in Milk Equivalent (I)} - (\text{Export in Milk Equivalent (E)})} \right)$$

Domestic use in milk equivalent (ME) includes stock changes and is calculated as:

$$\text{Domestic Use (ME)} = \text{Milk Deliveries} + \text{Import in ME} - \text{Export in ME}$$

Milk equivalent (ME) is used to standardize different dairy products into a comparable unit. Conversion coefficients used to calculate ME are:

- 1 kg of liquid milk = 1.00 kg of ME
- 1 kg of yogurt = 1.10 kg of ME
- 1 kg of condensed milk = 2.00 kg of ME
- 1 kg of cheese = 8.50 kg of ME
- 1 kg of skim milk powder (SMP) = 10.72 kg of ME
- 1 kg of whole milk powder (WMP) = 7.81 kg of ME

The yield of butter and cream is excluded from ME calculations, as their milk equivalent is already accounted for in SMP.

Results and discussion

The Croatian dairy sector has undergone significant structural changes during the analysed period (2018–2024). The most striking trend is the decline in the number of dairy cows, which has decreased by 48% compared to 2018 and is 33% below the 2018-2023 average (Table 1). This reduction in the dairy herd is one of the main factors behind the overall decline in raw milk production. Despite the lower number of cows, milk yield per cow has increased by 35% compared to 2018, indicating an improvement in productivity per animal, likely due to better genetics, feeding, technology and management practises. However, these productivity gains have not been enough to offset the overall decline in milk production, which is down 16% compared to 2018 and 10% below the 2018-2023 average. These findings are in line with Zrakić et al. (2015), who identified structural challenges in the Croatian dairy sector and emphasised that the shrinking dairy herd and increased productivity have not been enough to counteract the overall decline in milk production.

Table 1. Croatian dairy market Outlook (2018-2024)

	2018	2019	2020	2021	2022	2023	2024	Avg 2018- 2023	2024 vs Avg. 2018- 2023	2024 vs 2018
Dairy cows	135851	130025	109807	102333	79042	71423	70063	104747	-33%	-48%
Raw milk production (t)	453459	435606	433980	428937	405425	376982	378681	422398	-10%	-16%
Milk/cow (l)	4548	4608	5432	5449	6610	5867	6157	5419	14%	35%
Drinking milk	217539	224081	230587	215322	231982	207675	210746	221198	-5%	-3%

production (t)										
Cows cheese (t)	34226	33417	31330	31881	32737	32494	32455	32681	-1%	-5%
Cream (t)	29668	31803	31678	30441	32029	31882	31752	31250	2%	7%
Butter (t)	4331	4569	4175	4516	4726	3731	3908	4341	-10%	-10%
Fermented milk products (t)	92890	92539	88817	89238	86901	89245	93218	89938	4%	0,4%
Average farm-gate raw milk price €/kg	0.33	0.33	0.33	0.34	0.43	0.50	0.48	0.38	28%	46.5%

Source: CBS, Eurostat 2018-2024

The production of drinking milk has remained relatively stable, with a slight decline of 3% compared to 2018 and 5% below the 2018-2023 average. On the other hand, the production of key dairy products such as cheese and butter has experienced mixed trends. Cheese production fell slightly by 5% compared to 2018 and remained relatively stable over this period. However, butter production fell by 10% compared to both 2018 and the long-term average. Cream production increased by 7% compared to 2018, indicating a possible shift in processing towards high-quality dairy products. Fermented dairy products have shown a slight growth (0.4% compared to 2018), indicating a relatively stable demand.

A notable trend in the sector is the increase in the farm-gate price for raw milk, which has risen by 46.5% compared to 2018 and is 28% above the average for 2018-2023. This price increase is due to the recent volatility in the agricultural sector, leading to higher production costs (feed, energy, labour) and supply shortages due to the declining dairy herd.

The number of milk suppliers has steadily declined over the period observed, falling by 56% from 2018 to 2024 and by 40% below the 2018-2023 average (Table 2). This trend reflects the ongoing structural change in the sector, with small-scale dairy farms exiting production due to economic pressure and unfavourable market conditions.

Table 2. Number of milk suppliers and milk deliveries per supplier

	2018	2019	2020	2021	2022	2023	2024	Avg 2018-2023	2024 vs Avg. 2018-2023	2024 vs 2018
Number of suppliers	6193	5473	4750	4115	3480	2923	2705	4489	-40%	-56%
Delivery by supplier (kg)	80107	80426	100141	113777	127733	136992	139993	106529	31%	75%

Source: CBS, Eurostat 2018-2024

Despite the decline in the number of suppliers, the average milk delivery per supplier has increased significantly, by 75% compared to 2018 and 31% above the average for 2018-2023. This indicates a consolidation trend where the remaining dairy farms have expanded their production capacity. These results are consistent with the broader patterns observed in the Croatian agricultural sector, where small-scale producers are struggling to remain competitive while larger farms are increasing their productivity and efficiency to maintain their operations.

This next set of values represents the balance sheet of the dairy sector and illustrates the relationship between domestic production, trade flows, and overall consumption (Table 3).

Table 3. Croatain dairy market balance sheet (2018-2024)

	2018	2019	2020	2021	2022	2023	2024	Avg 2018- 2023	2024 vs Avg. 2018- 2023	2024 vs 2018
Milk deliveries (t)	453459	435606	433980	428937	405425	376982	378681	422398	-10%	-16%
Import - ME (t)	542858	576768	531177	589416	621803	667096	1162071	588186	98%	114%
Export - ME (t)	127398	117873	121122	119668	123872	117439	234670	121229	94%	84%
Domestic use -milk equivalent (t)	868930	894485	844055	898688	903362	926687	1306092	889368	47%	50%
Self-sufficiency (%)	52.2%	48.7%	51.4%	47.7%	44.9%	40.7%	29.0%	48%	-39%	-44%

Source: Authors calculations based on CBS and Eurostat data (2018-2024)

Imports of milk equivalents (ME) have more than doubled (+114% compared to 2018), with a strong increase of 98% compared to the 2018-2023 average, indicating a growing reliance on foreign dairy products to meet domestic demand, further highlighting the impact of declining domestic production. On the other hand, exports have also increased significantly, by 84% compared to 2018 and almost double (94% increase) compared to the 2018-2023 average.

Total domestic use of milk equivalents has increased by 50% compared to 2018, which is an increase of 47% compared to the 2018-2023 average. However, this higher demand was primarily met by imports rather than domestic production, resulting in a substantial decline in self-sufficiency. The self-sufficiency rate has dropped from 52.2% in 2018 to just 29% in 2024.

Conclusions

The Croatian dairy sector has undergone significant structural changes in recent years, characterised by a continuous decline in the number of dairy cows, the number of milk suppliers and overall milk production. Despite increases in productivity per supplier, these improvements have not been sufficient to compensate for the decline in overall production. Rising production costs, supply constraints due to a shrinking dairy herd and market volatility have contributed to this negative trend. At the same time, the increasing dependence on imports has led to a sharp decline in self-sufficiency, which fell from 52.2% in 2018 to just 29% in 2024. The sharp rise in imports and exports indicates a growing reliance on international markets to balance domestic supply and demand.

Future perspectives depend on targeted policy interventions to support farm consolidation, improve efficiency and stabilise production. Greater investment in technological innovation and sustainability measures could improve the resilience and competitiveness of the sector in the long term.

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References

- Croatian Bureau of Statistics. (2024). EKONOMSKI RAČUNI ZA POLJOPRIVREDU U 2023. Available from: <https://podaci.dzs.hr/2024/r/77170>
- Croatian Bureau of Statistics. (2024). PRVA PROCJENA REALNOG DOHOTKA U POLJOPRIVREDI U 2024. Available from: <https://podaci.dzs.hr/2024/hr/77172>
- Croatian Bureau of Statistics. (2024). INDEKSI POLJOPRIVREDNE PROIZVODNJE U 2023. Available from: <https://podaci.dzs.hr/2024/hr/76724>
- Croatian Bureau of Statistics. (2025). GODIŠNJI OTKUP I PRODAJA POLJOPRIVREDNIH PROIZVODA U 2024. Available from: <https://podaci.dzs.hr/2024/hr/77152>
- Eurostat. (2018-2024). Milk and milk product statistics. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics
- Grgić I., Krznar S., Bratić V. (2019). Poljoprivredna proizvodnja Republike Hrvatske prije i nakon pristupanja EU. In: *Aktualni zadaci mehanizacije poljoprivrede*, Bilandžija N., Kovačev I. (eds.), 487-496. Zagreb, Croatia: University of Zagreb Faculty of Agriculture.
- Zrakić, M., Salputra, G., Levak, V. (2015). Potential impact of EU Common Agriculture Policy on Croatian dairy sector - modelling results. *Mljekarstvo*. 65(3): 195- 202.

PRELIMINARY COMMUNICATION

The effects analysis of the smart technologies on income and costs of agricultural enterprisesLjubomir Madunić¹, Mate Budimir², Zrinka Tolušić Sić³¹*Acht d.o.o., Domovinskog rata 15, Cista Provo, Croatia (ljubomir.madunic1@gmail.com)*²*Split Dalmatia County Hunting association, Kralja Zvonimira 75, Split, Croatia*³*Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia***Summary**

The transition from traditional agribusiness management to smart management or the arrival of smart agriculture in Croatia must have a specific purpose. One of the reasons for the transition to smart management of agribusinesses is higher profits or economic and financial sustainability. Smart agriculture, which includes the use of computers, drones, modern applications or artificial intelligence, makes the work of agribusinesses easier, but also brings higher profitability, because it is expected that they will make fewer mistakes and do more in less time and with fewer resources, i.e. be more productive and efficient. The main objective of this research is to determine whether smart agriculture always contributes to the stabilisation of agribusinesses and economic sustainability or whether the transition to smart management brings certain losses for enterprises. The financial results were measured in a sample of 15 agribusinesses in Croatia before and after the transition to smart agriculture. Based on this analysis, it was determined how much the smart management of agricultural enterprises actually affects revenues and costs. The research results indicate that smart management of agribusinesses and the use of all the benefits that modern technology brings significantly improves the work of agribusinesses and contributes to higher income, but not always and not in every case. However, there are exceptions, i.e. cases where the introduction of smart agribusiness management has led to a decrease in income despite expectations to the contrary.

Key words: agribusiness, smart management, profitability, technology**Introduction**

In the age of smart technologies, all industries are transforming and making technological changes to increase their competitiveness, profitability and sustainability. Agriculture is no exception and is introducing numerous technological innovations that are also being implemented in agricultural enterprises in Croatia. Smart technologies in agriculture are an essential part of the fourth industrial revolution (UNIDO, 2021). According to Li (2022), smart technology in agriculture is a combination of knowledge about this field, intelligence and technology. Smart technologies in this sector include various digital and automated processes such as big data (Big Data), artificial intelligence (AI), robots, the Internet of Things (IoT), and virtual and augmented reality, which have applications in both business and everyday life (Javaid et al., 2022). According to a research by Ahmed et al (2024), smart agriculture contributes to greater efficiency and economic benefits for companies in the agricultural sector. Agriculture is a branch that brings prosperity to any region, and technological innovations implemented in this sector increase productivity and profitability, according to Perez-Pons et al. (2020). In their research, the authors Harniati et al. (2023) analysed the impact of one of the agronomic innovations, smart greenhouses, on the company's business success and food quality. The results of the research show that the application of this innovation contributes to increased efficiency and greater economic benefits for companies in the agricultural sector. It

is also important to mention smart farms as a particular form of innovation in agribusiness. According to the research results of Musa and Basir (2021), smart farms contribute to a more profitable, resilient and green management of the agricultural system. Author Li (2022) believes that artificial intelligence is already deeply integrated into agriculture and that it is already playing a major role in increasing the efficiency, profitability and productivity of this sector. According to Ignaciuk et al. (2021), the initial cost of implementing a smart agriculture system is high, but the long-term return on this investment is substantial. Furthermore, the benefits of using modern technology in agriculture far outweigh the initial costs. Similar findings were made in the research by Sain et al. (2017) on a sample of companies in Ghana. According to the results published in FAO (2015), the implementation of smart technologies in agriculture should be part of every country's national plan. According to Neufeldt et al. (2015), the implementation of smart technologies in agriculture not only contributes to increasing the efficiency and effectiveness of agricultural enterprises, but also to expanding their production. It is worth mentioning the research by Saadu et al. (2024), which used a sample of 377 households and small farms to investigate whether there was an increase in income after the implementation of one of the smart agriculture systems. The results of the research show that the income of the respondents increased by 21.9% on average, i.e. that the implementation of the smart agriculture system led to an improvement in crop yields, income and food security for small agricultural producers. Similar results were found in a sample of 360 food producers in a study conducted by Midjangninou et al. (2024), in which respondents reported an increase in income after adopting modern weather and climate change forecasting technologies in the agricultural industry.

In light of the results presented above, the future of the use of smart technologies in agriculture should definitely be in focus. The authors Casey et al. (2021) suggest using special business models for implementing modern technologies (which are expensive) on small farms. They suggest that investors actually invest money in modern technologies on small farms and at the same time share in their profits. This is an interesting topic for future research.

Material and methods

In the research process, data collected from 15 agricultural companies in Croatia was analyzed. All companies in the sample use some form of smart technology in their work (smart greenhouses, smart farms, drones, climate change prediction software, etc.), and the research sought to determine whether there was a change in revenue and costs in these companies after the introduction of the new form of work. The data was collected using a questionnaire (Google Forms) and a personal analysis of the companies' financial statements. The questionnaire also collected data on potential savings (if any) due to the use of smart technologies, as well as data on how the use of smart technologies contributes to increased revenue.

The reference years for the data analysis were 2016 (the year in which none of the companies in the sample had access to smart technologies in this area) and 2024 (the year in which all companies in the sample had access to smart technologies). Ethical principles were respected during the data analysis process, and the confidentiality of the data was guaranteed to the research participants. The main objective of such comparative and statistical analysis was to show the change in revenues and costs due to the impact of smart technologies. The programmes used for data processing were SPSS and MS Excel.

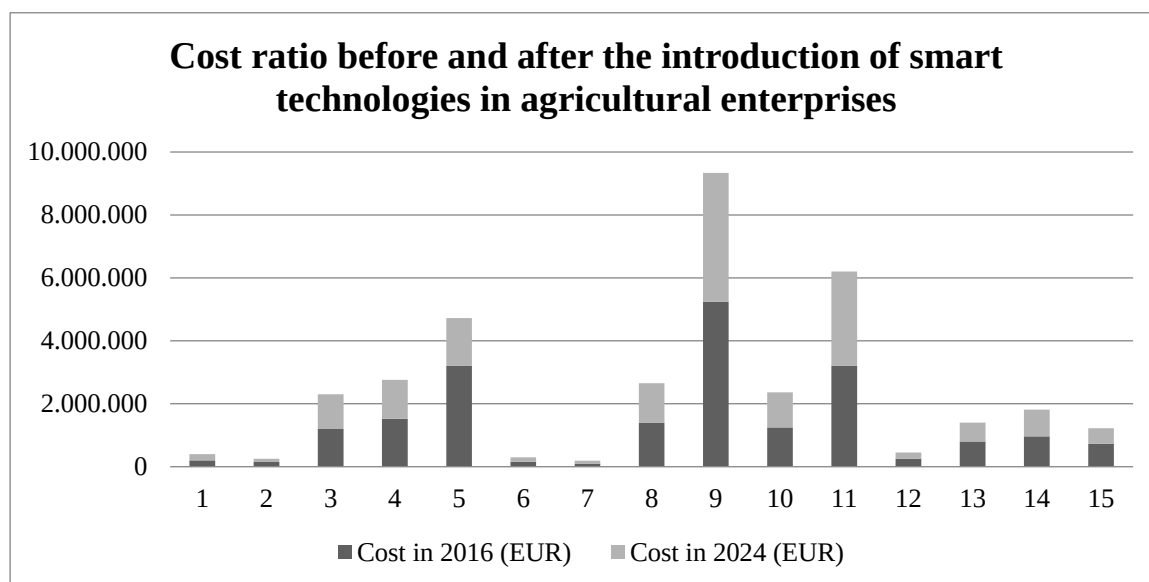
Results and discussion

The study included 15 agribusinesses. All companies (100%) in the sample use some form of smart agribusiness management. Furthermore, all companies (100%) belong to the small and medium-sized enterprise category. The forms of advanced technologies they use are listed in the Table 1.

Table 1. Forms of advanced technologies

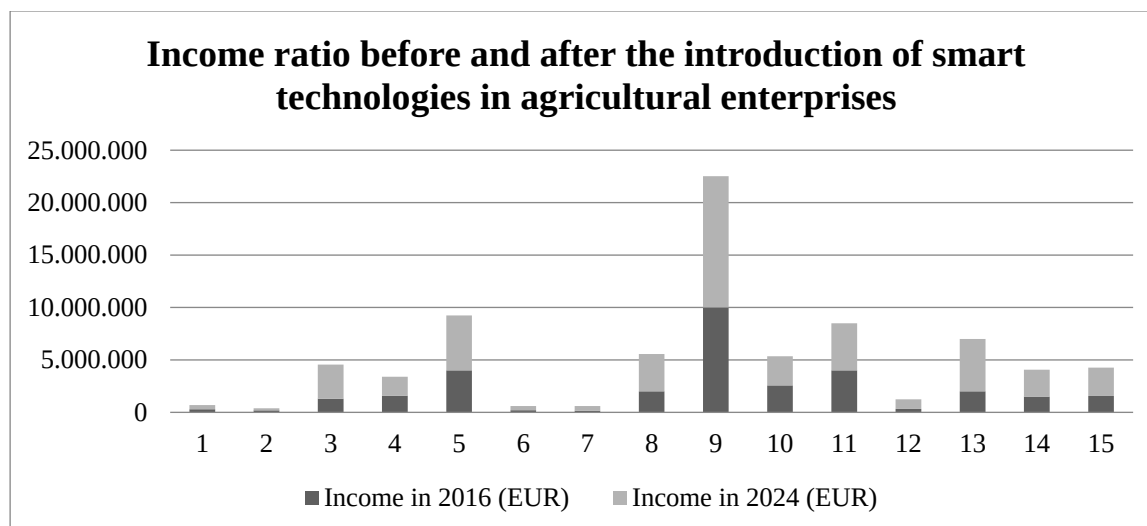
Answers	Frequency	Percent
Smart greenhouse	2	13.33
Smart farm	1	6.67
A specific form of AI	5	33.33
Drones	1	6.67
Weather forecasting software	3	20
Mobile applications specialized in agriculture	3	20
Total	15	100

Therefore, the most commonly used form of advanced technology in the agribusinesses in the sample is AI, but other forms of smart management in agribusinesses have also been identified. Below, Graph 1 illustrates the relationship between cost changes in the agribusinesses from the sample before (2016) and after (2024) the introduction of advanced technologies. The data are drawn from the income statements of the companies in the sample.



Graph 1. Cost ratio before and after the introduction of smart technologies in agricultural enterprises

As can be seen, the costs of the companies in the sample ranged from 100,000 euros to over 5,000,000 euros. It is clear that after the introduction of smart technologies, costs have fallen in at least 14 companies because the companies have become more efficient and effective or more productive. The introduction of smart technologies contributed to a reduction in costs in all cases except one, where costs actually increased. It is very important to mention here that the main contribution of smart technologies was reflected in labour savings. The companies in the sample managed to realise savings in the form of wages for employees through the introduction of smart technologies. In addition, by using artificial intelligence and smart software, the companies saved time and thus increased their productivity. The extra time was used for additional production (and sales). The study by Shyni et al. (2019) also came to similar conclusions, showing that the use of smart technologies in agriculture significantly reduced the costs of agribusinesses. Graph 2 shows the income comparison before and after the introduction of smart management (2016 vs. 2024). The data were taken from the income statements of the analysed companies.



Graph 2. Income ratio before and after the introduction of smart technologies in agricultural enterprises

As shown in Graph 2, the revenues of all companies in the sample increased after the introduction of smart technologies because they enabled companies to operate more efficiently and cost-effectively. The growth in revenue is due to the additional production made possible by the use of smart technologies, smarter weather forecasts, additional information gained through the use of smart intelligence, etc. The same was found in the research by Saadu et al (2024). This clearly shows that smart technologies have a significant impact on increasing income in agribusinesses.

Conclusions

Smart technologies are part of everyday life and have already found their way into all areas of society, including agriculture. The research provided important insights into the efficiency of the use of smart technologies on agricultural companies in Croatia. In a sample of 15 agricultural companies in Croatia that use smart technologies, 14 of them recorded a reduction in operating costs from 2016 to 2024. On the other hand, all companies in the sample experienced revenue growth due to the use of smart technologies. Such results can be encouraging for companies in the agricultural industry that have yet to transition from the traditional management approach to management with modern technologies. The results of this research can serve as a valuable reference for small and medium-sized enterprises in Croatia and encourage them to take steps towards higher productivity and efficiency.

References

- Ahmed M.M., Sharif U., Raza A., Safdar M. (2024). Climate-smart agriculture for resilience and profitability. In *Sustainable practices for agriculture and marketing convergence* (pp. 324). IGI Global. <https://doi.org/10.4018/979-8-3693-2011-2.ch002>
- Casey J., Bisaro A., Valverde A., Martinez M., Rokitzki M. (n.d.). *Private finance investment opportunities in climate-smart agriculture technologies*. CASA.
- FAO (2015). *Climate-smart agriculture: A call for action. Synthesis of the Asia-Pacific Regional Workshop, Bangkok, Thailand, 18 to 20 June 2015*. Food and Agriculture Organization of the United Nations.
- Harniati T., Trisnasari W., Saridewi T.R. (2023). Smart Greenhouse Technology for Hydroponic Farming: Is it Viable and Profitable Business? *International Journal on*

- Advanced Science, Engineering & Information Technology. 13(4): 1333. <https://doi.org/10.18517/ijaseit.13.4.17916>
- Ignaciuk A., Maggio G., Sitko N.J. (2021). Assessing the profitability and feasibility of climate-smart agriculture investment in Southern Malawi: Understanding the costs and benefits in a volatile and changing climate. FAO Agricultural Development Economics Working Paper 21-07. Rome, FAO. Available from: <https://doi.org/10.4060/cb5381en>
- Javaid M., Haleem A., Singh R.P., Suman R. (2022). Enhancing smart farming through the applications of Agriculture 4.0 technologies. International Journal of Intelligent Networks. 3: 150-164. <https://doi.org/10.1016/j.ijin.2022.09.004>
- Li Y. (2022). The impact of smart agriculture on regional economy. In *Proceedings of the 2022 2nd International Conference on Economic Development and Business Culture (ICEDBC 2022)*, 1853-1858. Available from: https://doi.org/10.2991/978-94-6463-036-7_276
- Midjangninou S.L.A., Bonou A., Chogou S.K. (2024). Impact of climate-smart agriculture on the income and food security of cassava-producing households in the Savalou commune in Benin. Agriculture and Food Security 5: 345.
- Musa S.F.P.D., Basir K.H. (2021). Smart farming: towards a sustainable agri-food system. British Food Journal. 123(4): 1122-1136. <https://doi.org/10.1108/BFJ-03-2021-0325>
- Neufeldt H., Negra C., Hancock J., Foster K., Nayak D., Singh P. (2015). *Scaling up climate-smart agriculture: Lessons learned from South Asia and pathways for success*. ICRAF Working Paper No. 209. World Agroforestry Centre. Available from: <https://doi.org/10.5716/WP15720.PDF>
- Pérez-Pons M.E., Parra-Dominguez J., Plaza M., Chamoso P., Alonso R.S. (2020). Efficiency, profitability and productivity: Technological applications in the agricultural sector. ADCAIJ: Advances in Distributed Computing and Artificial Intelligence Journal 9(4): 47-54. <https://doi.org/10.14201/ADCAIJ2020944754>
- Saadu B., Ibrahim H.Y., Nazifi B., Mudashiru A. (2024). Adoption of climate-smart agricultural practices and its impact on smallholder farming households in some rural areas of North-Western Nigeria. Agricultura Tropica et Subtropica. 57(OV): 23-34. <https://doi.org/10.2478/ats-2024-0003>
- Sain G., Loboguerrero A.M., Corner-Dolloff C., Lizarazo M., Nowak A., Martínez-Barón D., Andrieu N. (2017). Costs and benefits of climate-smart agriculture: The case of the Dry Corridor in Guatemala. Agricultural Systems. 151: 163-173. Available from: <https://doi.org/10.1016/j.agsy.2016.05.004>
- Shyni M., Sunitha T., Shopa P., Thangaraj S.J.J. (2019). Smart farming solutions for cost effective agriculture using IoT. International Journal of Advanced Science and Technology. 28(11): 364-369.
- UNIDO (2021). *Smart Agribusiness Issue Paper*. Vienna, Austria.

Post-harvest technologies to extend the freshness of cut flowers on the market

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Summary

Maintaining the freshness of cut flowers depends on post-harvest, including optimal storage conditions and supply chains. Key factors are temperature (0–2 °C for most species), high humidity and control of ethylene, which accelerates senescence. Rapid cooling, e.g. by pre-cooling, slows down the plant metabolism and prevents the development of microorganisms. Chemical treatments with sugars improve hydration and prolong freshness. Mechanical damage and bacteria can lead to wilting, so correct handling is crucial. Digitalization and blockchain enable supply chain tracking, reduce losses and improve the sustainability of the cut flower industry.

Key words: cut flowers, post-harvest, storage, cooling, temperature

Introduction

The cut flower market plays a key role in global trade and business, as flowers often travel long distances before reaching their final consumers (Raynolds, 2012). The appearance of cut flowers, such as stem length, flower color and shape, is of paramount importance. The cut flower industry is a global business with major production locations in North America, South America, Central America, East Africa, Europe, the Middle East, Asia, Australia and New Zealand (Faust and Dole, 2021). The most popular flower varieties on the global market include roses, followed by chrysanthemums and carnations. Globalization, the introduction of new technologies and improved supply chains have enabled the worldwide distribution of flowers, often over distances that require several days or weeks of transportation. While this process enables worldwide access to flowers, it also poses a challenge to maintaining quality, as the time between harvest and delivery greatly affects the flowers shelf life and appearance. Therefore, it is of paramount importance to understand the factors that impact quality maintenance during distribution, as well as the main post-harvest technologies used in the processing and storage of flowers (Malakar et al., 2023). Post-harvest technology is a key sector that includes efficient and constructive systems for considering new packaging, storage and transportation management, post-harvest pest, disease and aging control and market access with supply chain optimization for monitoring systems to ensure delivery of the highest quality crops to the end consumer (Majumder and Sharangi, 2024). According to van Meeteren (2008), the quality of flowers for the end consumer is most influenced by transportation and supply chain conditions. However, one of the biggest challenges in the cut flower sector is the short shelf life of the flowers. As a rule, it is desirable to pick flowers early in the morning when temperatures are low, the water content of the plant is high and the whole day is available for processing cut flowers. However, after cutting, the flowers naturally wilt and rot and therefore need special care to maintain their freshness. According to Cevallos and Reid (2000), the quality and shelf life of cut flowers can be improved by mixing them with a sugar solution after harvesting. Cut

flowers can also be treated with various preservatives containing silver thiosulfate (STS), 1-methylcyclopropene (1-MCP), 8-hydroxyquinoline citrate (8-HQC) and/or sucrose to extend their postharvest shelf life (Serek et al., 1994; Doi and Reid, 1995; Skutnik et al., 2021). In addition to post-harvest factors, the actual shelf life of cut flowers is also influenced by pre-harvest growing conditions (Gudin, 1992; Paull et al., 1992; Gorsel, 1992; van Meeteren et al., 2003; Byung-Chun et al., 2007) and end-user conditions. The expected shelf life of cut flowers is likely to be the result of the influence of air temperature and humidity at different stages of the supply chain on the aging rate, germination of *Botrytis conidia* and water balance of cut flowers, which will be explained later in this paper. From all this, it can be concluded that in order to reduce losses and prolong freshness, it is necessary to apply special handling, storage and processing methods, including technological innovations that extend the shelf life of flowers.

Post-harvest technologies for processing and storing flowers

The quality of cut flowers is highly dependent on storage conditions, especially temperature, water, ethylene, volatiles and light (Goszczyńska and Rudnicki, 1988; Krička et al., 2022; Matin et al., 2024). A key element of post-harvest processing of flowers is the control of temperature and humidity during storage to reduce the rate of plant metabolism and delay senescence (Matin et al., 2021). The processing and storage temperature influences physiological processes (flower development and senescence, plant response to stem injury) and physical processes (water loss, condensation/drying) that lead to quality loss (Buanong and Uthairatanakij, 2020). Cut flowers should be stored in an atmosphere with a relative humidity of more than 95% to minimize water loss (Fanourakis et al., 2012). Maintaining optimal conditions slows down the natural aging processes and reduces moisture loss, which can significantly extend the life of the flowers. Most species are best stored at temperatures between 0 and 2 °C, while cold-sensitive species are more stable at temperatures above 10 °C (Krička et al., 2022). Storing flowers at high temperatures accelerates the respiration process, which increases nutrient consumption and leads to faster wilting. Stress at low temperatures not only affects their ornamental characteristics such as flower shape and color, but also reduces their ornamental quality (Li et al., 2009), and annual production is affected by limiting their growth and development (Ao et al., 2019). At high temperatures, ethylene synthesis in plants is accelerated, which also accelerates senescence (Jones, 2001). For example, a flower stored at 29 °C ages up to 45 times faster than a flower stored at temperatures between 1 and 2 °C (Goszczyńska and Rudnicki, 1988; Krička et al., 2022).

Even if flowers lose a considerable amount of water, they can be completely rehydrated if water uptake into the stem is not hindered by air bubbles, bacterial blockages, plant debris or dirt (Jones, 2001). Water is constantly lost from cut flowers through transpiration, and water loss also accelerates ripening, senescence, browning of leaves and increases plant susceptibility to cold damage (Teixeira da Silva, 2015). The cells at the cut site excrete sugars, amino acids, proteins and other substances that are excellent food for the bacteria that are always present on the surface of the plants (Capozzi, et al, 2009). If the cut stems are kept dry, the bacteria cannot grow, as they need water to grow, and the cell contents excreted at the cut site dry out quickly. If the number of microbes at the base of the flower stalk in the water rises above a certain level, clogging occurs, the movement of water in the stalk is impeded and wilting occurs. A large number of bacteria can be found in the soil, which is why it is important that there is no soil residue on the stems (Kögel-Knabner, 2002). As the plant ages, ethylene synthesis also increases, which eventually leads to rotting and the end of the flower's life. Since ethylene is a gas, ethylene synthesis in a wilting plant can also affect neighboring plants. Many plants, such as carnations, are more sensitive to the effects of ethylene, which causes accelerated senescence (an Altvorst and Bovy, 1995). Large amounts of ethylene are produced mainly in the petals a

few days after the flower has fully opened. The increased ethylene production promotes petal curling and wilting (Sato et al., 2008). In open carnation flowers, ethylene production increases on the fifth day after harvest, with the highest levels occurring on the sixth day after harvest (Jones, 2001).

The presence or absence of light during storage is also very important, but is generally not a cause for concern unless the leaves begin to yellow. The leaves of some varieties, such as chrysanthemums, can yellow if stored in the dark at higher temperatures (Teixeira da Silva et al., 2015). Starch and sugars stored in the stem, leaves and petals provide most of the nutrients needed to open and maintain the cut flower. Carbohydrate content is highest when plants are grown in bright light and with proper crop management (Ferrante and Mariani, 2018). In fact, the carbohydrate content is highest in the late afternoon, after a full day of sunshine. Mechanical damage and bacterial infections pose a significant threat to flower quality during transportation and storage. Damage to petals, stems and leaves can lead to faster spoilage, while bacteria can block water uptake and accelerate wilting (Majumder and Sharangi, 2024). Losses during distribution can be up to 30%. Common causes are improper handling of flowers, poor storage and transportation conditions and unsuitable processing methods. Timely pruning, an adequate supply of nutrients and the prevention of microbial growth are key to maintaining the freshness of flowers. Some flower species, such as carnations and chrysanthemums, are harvested when the buds are still partially closed, which allows for easier packaging and reduces mechanical damage (Faust and Dole, 2021). This also slows down the aging process, allowing the flowers to stay on the market longer. In addition, damaged tissue generally has higher respiration and ethylene synthesis, which further shortens the lifespan of the flowers. Cut flowers are very susceptible to disease, not only because of their fragile petals, but also because of the sap secreted by the stems, on which pathogens feed (Muñoz et al., 2021). When flowers are moved from cold storage to a warmer place, water often condenses on the surface of the flower.

Digital technologies in logistics and quality monitoring

Digitalization plays a key role in optimizing the supply chain for cut flowers. The development of blockchain allows for greater transparency in the supply chain, as consumers can trace the origin of the flowers and ensure that the technology has been grown according to organic and ethical standards. These innovations are helping to improve product quality, reduce waste and increase consumer confidence in the cut flower industry. The use of modern technologies such as temperature monitoring sensors and automatic climate control systems can help to maintain the quality of flowers throughout the supply chain (Verdouw et al., 2013). In addition to technological innovations, sustainability is becoming an increasingly important factor in the cut flower industry. The use of environmentally friendly packaging materials, the reduction of carbon dioxide emissions during transportation and the application of sustainable cultivation methods contribute to the long-term conservation of natural resources and the protection of the environment. A modern approach to digitizing and optimizing the supply chain enables fast delivery of fresh flowers, reduces losses and contributes to the sustainability of the entire industry (van Beuningen, 2017). By using innovative solutions, producers and retailers can increase their efficiency, while consumers can be sure that they are receiving high-quality and ethically produced floral arrangements.

Conclusions

To successfully maintain the quality of cut flowers, careful management of post-harvest procedures is required. This includes the control of temperature, humidity and ethylene as well as the use of blast chilling and chemical treatments. These methods significantly extend the shelf life of the flowers, reduce losses and prevent the growth of bacteria. Proper handling and protection from mechanical damage are also crucial to maintaining freshness. With the

development of digital technologies and blockchain, the cut flower industry has the opportunity to reduce waste, increase transparency and contribute to sustainability, ensuring long-term success and economic profitability.

References

- Ao, N., Ma, J., Xu, T. T., Su, J. S., Yang, X. C., Guan, Z. Y. (2019). Genetic variation and QTL mapping for cold tolerance in a chrysanthemum F₁ population at different growth stages. *Euphytica*. 215(5): 88.
- Buanong, M., and Uthairatanakij, A. (2020). Postharvest Technology of Cut Flowers of Orchids. *Orchid Biology: Recent Trends & Challenges*. 95-117.
- Byung-Chun, I., Motomura, S., Inamoto, K., Doi, M., Mori, G. (2007). Multivariate analysis of relations between preharvest environmental factors, postharvest morphological and physiological factors, and vase life of cut 'Asami Red' roses. *Journal of the Japanese Society for Horticultural Science*. 76(1): 66–72.
- Capozzi, V., Fiocco, D., Amodio, M. L., Gallone, A., Spano, G. (2009). Bacterial stressors in minimally processed food. *International journal of molecular sciences*. 10(7): 3076-3105.
- Cevallos, J. C., and Reid, M. S. (2000). Effects of temperature on the respiration and vase life of *Narcissus* flowers. *Acta Hort*. 517:335–342.
- Doi, M., and Reid, M. S. (1995). Sucrose improves the postharvest life of cut flowers of a hybrid *Limonium*. *HortScience*. 30(5): 1058-1060.
- Fanourakis, D., Carvalho, S. M., Almeida, D. P., van Kooten, O., van Doorn, W. G., Heuvelink, E. (2012). Postharvest water relations in cut rose cultivars with contrasting sensitivity to high relative air humidity during growth. *Postharvest Biology and Technology*. 64(1): 64-73.
- Faust, J. E., and Dole, J. M. (2021). The global cut flower and foliage marketplace. *Cut flowers and foliage*. 27: 1-47.
- Ferrante, A., and Mariani, L. (2018). Agronomic management for enhancing plant tolerance to abiotic stresses: High and low values of temperature, light intensity, and relative humidity. *Horticulturae*. 4(3): 21.
- Gorsel, R. V. (1992). Postharvest quality and water relations of *Bouvardia* cut flowers as affected by preharvest temperature and season. *Physiological Basis of Postharvest Technologies*. 343: 309-312.
- Goszczyńska, D. M., and Rudnicki, R. M. (1988). Storage of cut flowers. *Horticultural reviews*. 10: 35-62.
- Gudin, S. (1992). Effect of preharvest growing temperatures on the development of cut roses. *Postharvest biology and technology*. 2(2): 155-161.
- Jones, R. (2001). *Caring for cut flowers*. Landlinks Press.
- Kögel-Knabner, I. (2002). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil biology and biochemistry*. 34(2): 139-162.
- Krička, T., Matin, A., Grubor, M. (2022). Tehnologija dorade i skladištenja cvijeća. Udžbenik, Sveučilište u Zagrebu Agronomski fakultet
- Li, Y., Yang, J. S., Zhang, G., Chen, D. F. (2009). Effects of low temperature exercise on physiological activity of *Chrysanthemum* under low temperature stress. *J. J. North China Agric. Sci*. 24 (04): 179–182.
- Malakar, M., Paiva, P. D. D. O., Beruto, M., Cunha Neto, A. R. D. (2023). Review of recent advances in post-harvest techniques for tropical cut flowers and future prospects: *Heliconia* as a case-study. *Frontiers in Plant Science*. 14, 1221346.
- Matin, A., Pavkov, I., Grubor, M., Jurišić, V., Kontek, M., Jukić, F., Krička, T. (2021). Influence of harvest time, method of preparation and method of distillation on the qualitative properties of organically grown and wild *Helichrysum italicum* immortelle essential oil. *Separations*, 8(10), 167.

- Matin, A., Brandić, I., Krička, T., Matin, B., Jurišić, V., Špelić, K., Grubor, M., Antonović, A., Jovičić, N. (2024). Sustainable disposal of waste from the flower industry. *Environmental Engineering-Inženjerstvo okoliša*, 11(1-2): 57-61.
- Majumder, J., and Sharangi, A. B. (2024). Advances in Postharvest Technology of Flowers, Medicinal, and Aromatic Herbs. In *Recent Advances in Postharvest Technologies*, 2: 247-276.
- Muñoz, M., Cieniewicz, E., Faust, J. E. (2021). Diseases and disease management. *Cut flowers and foliages*. 258.
- Paull, R. E., Higaki, T., Imamura, J. S. (1992). Season and fertilization affect the post-harvest flower life of anthurium. *Scientia horticultrurae*. 49(1-2): 125-134.
- Raynolds, L. T. (2012). Fair trade flowers: Global certification, environmental sustainability, and labor standards. *Rural sociology*. 77(4): 493-519.
- Satoh, S., Watanabe, M., Chisaka, K., Narumi, T. (2008). Suppressed leaf senescence in *Chrysanthemum* transformed with a mutated ethylene receptor gene mDG-ERS1 (etr1-4). *Journal of Plant Biology* 51: 424-427.
- Serek, M., Sisler, E. C., Reid, M. S. (1994). Novel gaseous ethylene binding inhibitor prevents ethylene effects in potted flowering plants. *Journal of the American Society for Horticultural Science*. 119(6). 1230-1233.
- Skutnik, E., Łukaszewska, A., Rabiza-Świder, J. (2021). Effects of postharvest treatments with nanosilver on senescence of cut lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) flowers. *Agronomy*. 11(2): 215.
- Teixeira da Silva, J. A., Kim, H., Engelmann, F. (2015). *Chrysanthemum* low-temperature storage and cryopreservation: a review. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 120: 423-440.
- van Altvorst, A. C., and Bovy, A. G. (1995). The role of ethylene in the senescence of carnation flowers, a review. *Plant Growth Regulation*. 16: 43-53.
- van Beuningen, J. P. C. M. (2017). How to design a profitable logistical supply chain for distributing fresh flowers in urban areas, while optimizing the societal values Entrepreneurship, Environment, Education and Emotion?.
- van Meeteren, U., van Gelder, A., van Ieperen, W. (2003). Effect of growth conditions on post harvest rehydration ability of cut chrysanthemum flowers. In *VIII International Symposium on Postharvest Physiology of Ornamental Plants* 669: 287-296.
- van Meeteren, U. (2008). Causes of quality loss of cut flowers-a critical analysis of postharvest treatments. In *IX International Symposium on Postharvest Quality of Ornamental Plants*. 847: 27-36.
- Verdouw, C. N., Beulens, A. J. M., Van Der Vorst, J. G. A. J. (2013). Virtualisation of floricultural supply chains: A review from an Internet of Things perspective. *Computers and electronics in agriculture*. 99: 160-175.

ORIGINAL SCIENTIFIC PAPER

DuPont analysis of the profitability of selected wineries in the Republic of Croatia

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Summary

Wine production has a long tradition in the world. The favourable agro-ecological and pedological conditions in Croatia enable the cultivation of different grape varieties. For wineries to operate profitably, entrepreneurs need to know how to manage and respond to changes in market conditions and customer needs in time to direct their resources to the most profitable opportunities. The aim of the paper is to determine the profitability and success of the wineries Krauthaker Ltd. from Slavonia, Vina Matošević Ltd. from Istria and Saints Hills Ltd. from Dalmatia using the DuPont indicator system. The results of the paper show that the Matošević winery is the most profitable, followed by the Saints Hills winery and finally the Krauthaker winery.

Key words: DuPont analysis, winery, financial analysis

Introduction

People have been making wine since time immemorial. The oldest archaeological evidence of viticulture comes from Georgia and dates back to around 6000 BC. In Croatia, the cultivation of grapevines began around 400 BC. The modern development of viticulture and winemaking in Croatia, which began in the late 1990s, has resulted in a relatively large number of small and medium-sized producers that emphasise the diversity and specificity of the different regions (Preiner et al., 2021). The European Union is the largest producer, consumer and exporter of wine in the world. It accounts for around 50% of global wine production, with three quarters of the wine produced in the EU coming from Spain, France and Italy, which also lead the way in wine exports (Eurostat, 2024).

According to the Croatian Bureau of Statistics (2024), wine production in Croatia is declining and will reach 612,000 hl in 2023, a decrease of 24% compared to 2020. Croatian producers are following the global trend of declining wine production. The International Organisation of Vine and Wine (OIV) reported that wine production in the EU will fall by 10% in 2022, the second-lowest figure since the beginning of the century. Extreme climatic conditions such as drought, extreme temperatures, hailstorms, frost and oxidative stress have a profound negative impact on production (Jogaiah, 2023). Climate change has a significant impact on viticulture worldwide, including in Croatia. It affects all activities related to wine production, including grape cultivation, winemaking, wine economy and environmental concerns (Ollat et al., 2016). In 2022, 275 Croatian companies active in wine production and grape growing submitted annual financial reports to FINA. This group of entrepreneurs achieved a positive financial result of 1.59 million euros in 2022 (FINA, 2023). The value of wine exports in 2023 totalled 19.3 million euros, while imports reached 45.65 million euros (DZS, 2024a).

According to the rating agency CompanyWall, the 100 most successful wineries generated sales of 146.3 million euros and a profit of 5.8 million euros in the same period, continuing the growth trend of recent years. The Croatian Agricultural Act recognises the wine sector as one of the country's strategic sectors (Bedeck and Njavro, 2016). Čop et al. (2019) analysed the production

and business performance of wineries in continental and Adriatic Croatia and concluded that wineries in the continental region perform better than those in the Adriatic region. Large wineries (with assets of more than 2 million euros) have better financial ratios compared to small wineries. Borisov and Radev (2020) used the DuPont system to analyse and evaluate the economic profitability factors of 55 wineries in southern Bulgaria. Their research found that return on sales and asset turnover are the most important factors influencing economic success, with marketing efforts playing a crucial role in sales performance. In addition, maintaining product quality is critical to profitability and requires higher production costs, resulting in higher fixed costs that must be adjusted to market prices. Szerb and Szerb (2020) analysed the profitability of Hungarian wineries using the DuPont analysis. Their results show that newer wineries tend to achieve higher profitability than established wineries, which is mainly due to innovative approaches to attract younger consumers.

Material and methods

This study used the DuPont indicator system (Table 1), a method that has been in use since 1919. At that time, it was developed to analyse the corporate performance of the American company Du Pont de Nemours & Co. The primary goal of every company is to generate profits and thus satisfy first the shareholders and then the other stakeholders. The DuPont formula provides management with a top-down view of company performance and leads to the calculation of return on equity (ROE), which is of greatest interest to shareholders (Bigel, 2022). By applying the DuPont indicator system, managers gain real insight into the effective utilisation of the company's assets, allowing them to manage assets more rationally and make better-informed decisions. DuPont integrates information from two basic financial reports: the balance sheet and the income statement. At the top of the pyramid of indicators (the key performance indicator) is return on assets, which reflects the fundamental business objective and defines the requirements to be met at the lower operational levels. In addition to the return on assets, the return on equity (ROE), which indicates the return per unit of invested capital, is often included in the analysis (Sesar et al., 2015).

Table 1. DuPont indicator system

Indicator	Numerator	Denominator
Profit Margin	Net Profit	Revenue
Asset Turnover	Revenue	Total Assets
ROA (Return on Assets)	Profit Margin	Total Asset Turnover Ratio
Leverage	Total Assets	Equity
ROE (Return on Equity)	ROA	Equity Multiplier

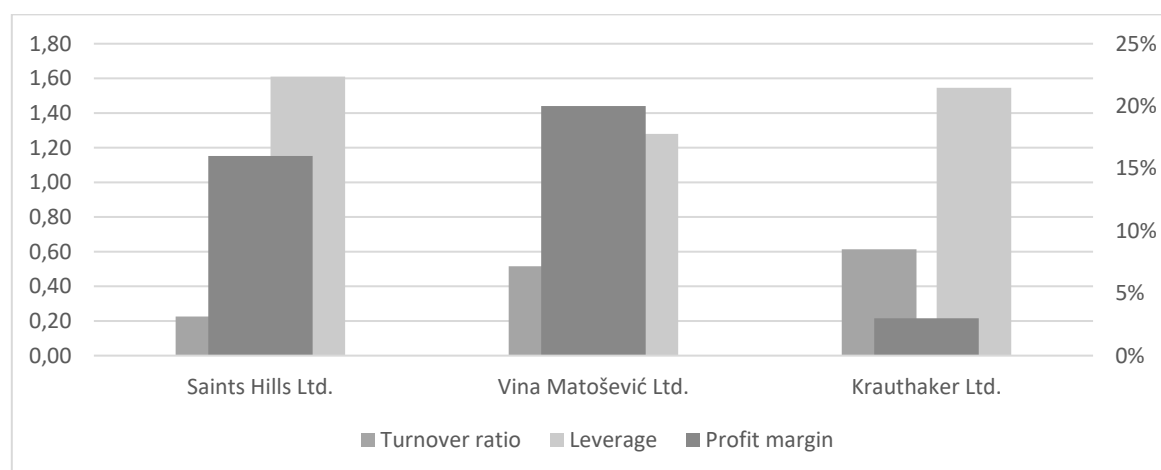
Source: Authors on the basis of Žager et al. (2021)

The equations of the DuPont system were applied to the financial statements of three wineries in Croatia, Krauthaker Ltd. from Slavonia, Vina Matošević Ltd. from Istria and Saints Hills Ltd. from Dalmatia, to assess their business performance in the period from 2019 to 2023. Wineries from different wine-growing regions of Croatia were selected to identify potential regional differences/similarities in business practises.

Results and discussion

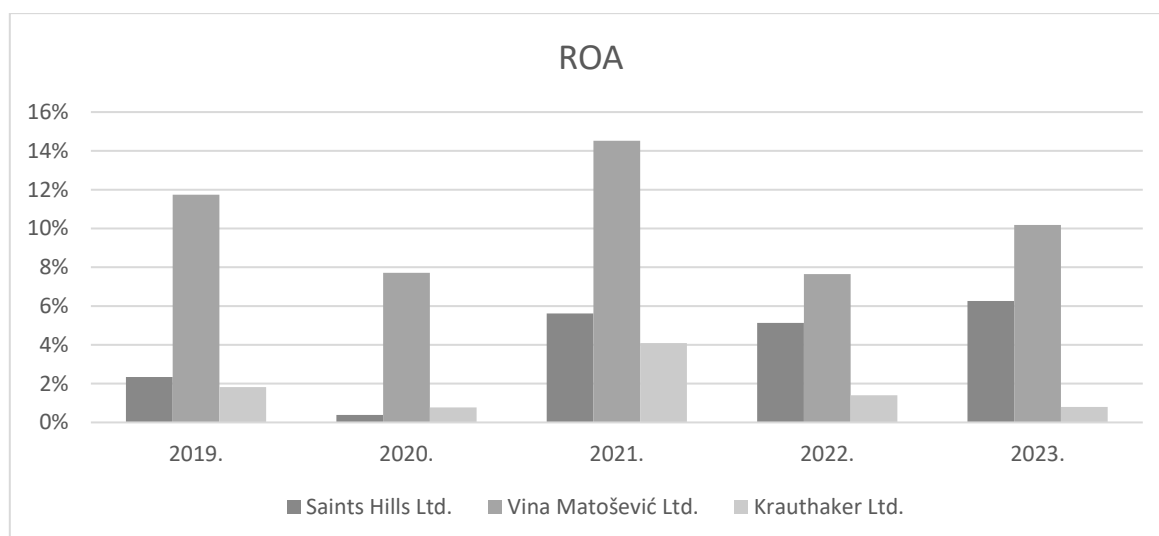
The first component of the DuPont system is the profit margin, which indicates the percentage of profit generated from a business that should be as high as possible. In the case of Saints Hills Ltd., a significantly lower profit margin was observed in 2020 than in the other years analysed, which was probably a direct consequence of the COVID-19 pandemic and the resulting market

disruptions. In the following years, however, the winery achieved exceptionally favourable values for the profit margin, which averaged 20%. At Vina Matošević Ltd. on the other hand, the opposite trend was observed. The highest profit margin was achieved in 2021, followed by a significant decrease, which was 11.4% in 2022. This decline was likely due to a significant increase in expenses in that year. A similar trend was observed at Krauthaker Ltd. which recorded its lowest profit margin in 2023 (only 1.09%). Although the winery generated higher revenue than the previous year, expenses also increased significantly. The turnover ratio measures how efficiently a company uses its assets to generate revenue. Ideally, this ratio should be greater than 1. At Saints Hills Ltd, the values remained below the desired threshold throughout the five-year period, although a positive trend towards improvement was observed. Vina Matošević Ltd. also did not optimally utilise its assets during the observed period, but the values of the ratio were higher than those of Saints Hills Ltd. Krauthaker Ltd. recorded the highest values for asset turnover, but this winery also did not reach the optimal value. The leverage reflects the extent to which a company relies on financial leverage. Saints Hills Ltd. showed a decreasing reliance on debt over the observed period, with the highest use of financial leverage recorded in 2019. A similar trend was observed at Vina Matošević Ltd, which relied most heavily on debt capital in 2020. Of all the wineries analysed, Vina Matošević Ltd. was the least indebted. Krauthaker Ltd., on the other hand, showed the least fluctuation in this indicator (Graph 1).



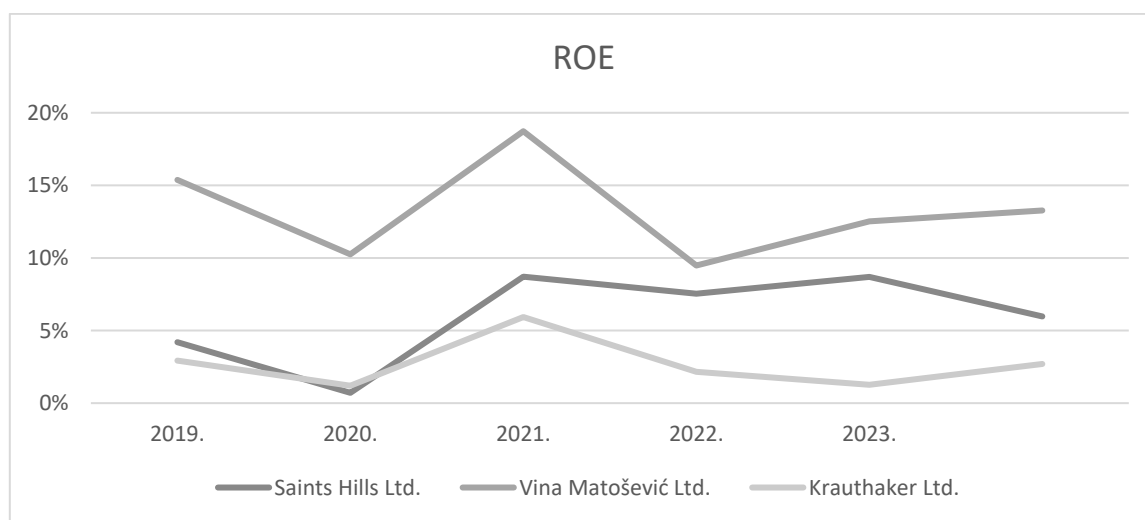
Graph 1. Average profit margin, turnover ratio and leverage for the period 2019. - 2023.

Graph 2 shows the return on assets (ROA) of the selected wineries. On average, Vina Matošević Ltd. recorded the highest ROA (10.36%), while Krauthaker Ltd. recorded the lowest (1.78%). All wineries recorded their lowest ROA in 2020, followed by a significant increase in 2021. While Vina Matošević and Saints Hills continued to show an upward trend in ROA in the last two years observed, Krauthaker Ltd. showed a downward trend. If we consider a theoretically satisfactory ROA level of 5%, we can see that only Vina Matošević Ltd. has consistently exceeded this threshold in all observed years, while Saints Hills Ltd. exceeded it from 2021 onwards.



Graph 2. Return on assets (ROA) of selected wineries

The last component of the DuPont ratio system of key figures is the return on equity (ROE), which should ideally be as high as possible. Here too, Vina Matošević Ltd. has the highest ROE with an average of 13.27%, while Krauthaker Ltd. has the lowest at 2.70% (Graph 3). If we consider the theoretically desirable threshold value for this indicator to be 10-15%, Vina Matošević Ltd. once again stands out.



Graph 3. Return on equity (ROE) of selected wineries

Saint Hills Ltd. is the youngest winery in this study investing significantly in marketing activities and located in the Dalmatia region. It has seen increasing profitability in recent years. Founded in the 1990s, Vina Matošević Ltd. invests heavily in marketing and modernisation, giving its wines modern names and designs (e.g. the Funky line) to attract younger consumers with higher purchasing power. In addition, the winery is located in Istria, a major tourist destination, which contributes to better wine sales. Krauthaker Ltd. is the oldest selected winery with over 30 years of business tradition. In recent years, it has seen declining trends in return on assets and return on equity. According to Szerb and Szerb (2020), despite their importance in the market, traditional wineries often lose business because new, younger and more attractive competitors appear. It is therefore recommended that this winery continues to invest in marketing activities to improve its market positioning.

Conclusions

Many Croatian entrepreneurs have ventured into wine production, but the highly competitive market, unpredictable climatic changes and occasional market disruptions pose major challenges for their businesses. To remain profitable, entrepreneurs need to manage effectively and respond quickly to market changes and customer needs, directing their resources towards the most profitable opportunities. This study analyses three wineries, Saint Hills Ltd, Vina Matošević Ltd and Krauthaker Ltd, over the period from 2019 to 2023 using the DuPont indicator system. Vina Matošević Ltd. achieved the highest average return on assets (ROA - 10.36%) and return on equity (ROE - 13.27%). This winery also had the lowest level of debt and the highest profit margin. The lowest average return on assets (ROA - 1.78%) and return on equity (ROE - 2.70%) were recorded by Krauthaker Ltd., which still achieved positive results but were below the theoretically desirable values. Saint Hills Ltd. ranked in the middle with an average ROA of 3.95% and ROE of 5.97%.

References

- Bedek Ž., Njavro M. (2016). Risks and Competitiveness in Agriculture with Emphasis on Wine Sector in Croatia. APSTRACT: Applied Studies in Agribusiness and Commerce, AGRIMBA. Vol. 10 (1): 1-8. DOI: <https://doi.org/10.22004/ag.econ.244439>
- Bigel K.S. (2022). Introduction to Financial Analysis, Pressbooks. Available from: <https://pressbooks.pub/introductiontofinancialanalysis/>
- Borisov P., Radev T. (2020). Drivers of the Economic Performance of Bulgarian Wineries. Assessment Based on Dupont Analysis. Journal of Bio-based Marketing. Vol. 2: 60-65. DOI: <https://doi.org/10.5281/zenodo.3736301>
- CompanyWall business. Available from: <https://www.companywall.hr/>
- Čop T., Juračak J., Njavro, M. (2019). Production and business results of wine producers in continental and Adriatic Croatia. Proceedings in Food System Dynamics, 93-101. DOI: <https://doi.org/10.18461/pfsd.2019.1910>
- Croatian bureau of Statistic (DZS) (2024). Agricultural Production, 2023. Statistical Reports. Available from: <https://podaci.dzs.hr/media/pb2dl2w1/si-1727-poljoprivredna-proizvodnja-u-2023.pdf>
- Croatian bureau of Statistic (DZS) (2024a). Wine balance sheet. Statistical Reports. Available from: [https://web.dzs.hr/PxWeb/pxweb/hr/Poljoprivreda,%20lov,%20%C5%A1umarstvo%20i%20ribarstvo vo/Poljoprivreda,%20lov,%20%C5%A1umarstvo%20i%20ribarstvo__Bilanca%20vina/BV1.px/](https://web.dzs.hr/PxWeb/pxweb/hr/Poljoprivreda,%20lov,%20%C5%A1umarstvo%20i%20ribarstvo/Poljoprivreda,%20lov,%20%C5%A1umarstvo%20i%20ribarstvo__Bilanca%20vina/BV1.px/)
- Eurostat (2024). Wine trade. Available from: <https://agridata.ec.europa.eu/extensions/DashboardWine/WineTrade.html>
- FINA (2023). Business results of entrepreneurs in wine production and grape growing in the year 2022. Available from: <https://www.fina.hr/novosti/rezultati-poslovanja-poduzetnika-u-proizvodnji-vina-i-uzgoju-grozda-u-2022.-godini>
- International Organisation of Vine and Wine (OIV). Available from: <https://www.oiv.int/>
- Jogaiah S. (2023). Vineyard management strategies in scenario of climate change - A review. Grape Insight. 1 (1): 11-22. DOI: <https://doi.org/10.59904/gi.v1.i1.2023.7>
- Ollat N., Touzard J.M., van Leeuwen C. (2016). Climate Change Impacts and Adaptations: New Challenges for the Wine Industry. Journal of Wine Economics. 11 (1): 139-149.
- Preiner D., Jagatić Korenika A.M., Marković Z., Jeromel A. (2021). Modern trends in viticulture and wine production in Croatia. Glasilo biljne zaštite. 21 (3): 323-332.
- Sesar V., Buntak K., Borlinić, M. (2015). Application of the DuPont system in the management process. Tehnički glasnik. 9 (1): 99-103. Available from: <https://hrcak.srce.hr/138003>
- Szerb B., Szerb A.B. (2020). The Effects of Wine Regions on the Profitability of the Hungarian Winers. Regional and Business Studies. 12 (2): 65-76. DOI: <https://doi.org/10.33568/rbs.2520>
- Žager K., Mamić Sačer I., Sever Mališ S., Pavić I., Brozović M., Žager, L. (2021). Analysis of financial statements: essential concepts and applications, Zagreb.

The influence of demographic factors on the use of Rural Development measures on family farms in Croatia

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Summary

This paper analyses the correlation between the demographic characteristics of family farm holders in Croatia and the use of measures from the Rural Development Program 2014–2020, focusing on Measure 10 (Agriculture, Environment, and Climate Change), Measure 11 (Organic Farming), and Measure 13 (Payments in Areas with Natural Constraints). Data were collected from the Paying Agency for Agriculture in 2022, and statistical analysis was performed using Pearson's chi-squared test. The results show that the majority of users of all three measures belong to the 41–65 age group and hold secondary education degrees. Younger and highly educated farmers are significantly underrepresented among beneficiaries. The findings highlight the need for policy interventions aimed at increasing the participation of younger and more educated farmers in rural development programs, as well as adapting agri-environmental measures to the specific needs of different farm types, which could enhance the overall effectiveness and sustainability of rural policy implementation.

Key words: family farms, demographic factors, rural development measures, environmental measures

Introduction

Family farms represent a fundamental element of agricultural systems that are more resilient to the effects of climate change (Chao, 2024). In Croatia, the emergence of family farms dates back to 1848, when the abolition of feudalism encouraged the individualization of the peasantry and the transition from household cooperatives to family holdings (Zupančić, 2005). During the 20th century, the rural economy developed through two models: traditional peasant farms focused on self-sufficiency, and market-oriented farming enterprises. Despite modernization, including mechanization since 1967 (Defilippis, 2005), the number of registered family farms significantly declined, from 165,167 in 2016 to 128,290 in 2022 (APPRRR, 2016, 2022). Simultaneously, demographic trends indicate that the majority of the workforce today consists of individuals over 65 years old (27.56%), while those under 25 account for only 5.92% (DZS, 2020).

In addition to demographic challenges, family farms face financial difficulties such as dependence on short-term loans, lack of business skills, and unresolved property issues (Ministry of Agriculture, 2015). Nevertheless, the legislative framework (Narodne Novine, 2018, 2023) recognizes their strategic importance and regulates their operations.

Family farms play a central role in implementing nature conservation measures within the European Union's Common Agricultural Policy (CAP), owing to their adaptability to local agro-ecological conditions. These farms often rely on traditional practices that integrate sustainable production with the preservation of natural resources, thereby supporting biodiversity and minimizing environmental impacts (Wuepper et al., 2019). In Croatia, such measures were introduced during the 2014–2022 programming period following EU accession, primarily through greening under Pillar I and a set of targeted interventions within the Rural

Development Programme (Narodne Novine, 2022). Greening measures included crop diversification, maintenance of permanent grasslands, and the preservation of ecologically significant areas, with annual payments granted for compliance (Ministry of Agriculture, 2015). Complementary support under Pillar II, specifically through Measure 10 (Agri-environment and climate), Measure 11 (Organic farming), and Measure 13 (Areas with Natural Constraints), contributed to broader environmental and climate objectives. While greening was mandatory for direct payment recipients, participation in agri-environment-climate measures (AECMs) was voluntary and subject to five-year commitments, including annual CAP applications, training, and documentation of implemented practices (Narodne Novine, 2022). However, uptake of Measure 10 was relatively limited. In 2021, programs focused on high-nature-value grasslands and the protection of species such as the corncrake and grassland butterflies covered approximately 26,000 hectares - only 36% of the initial target (Ministry of Agriculture, 2023a). Low participation may be attributed to farmers' perceptions of certain operations as ineffective or burdensome. Research indicates that adoption of AECMs is positively associated with higher education levels, contractual flexibility, peer networks, and access to advisory services (Mikuš et al., 2020). Measure 11 proved particularly effective in expanding organic farming, offering increased financial support - 20% higher during the conversion phase - to offset yield reductions and the inability to market products as organic during the transition. This incentive significantly boosted organic land, which grew from 2,660 hectares in 2004 to 129,000 hectares by 2022, representing 9% of the total utilised agricultural area (Blaće et al., 2020; Ministry of Agriculture, 2023). Measure 13 provides compensatory support to farms operating in areas with natural limitations, which account for roughly 41% of Croatia's agricultural land - often overlapping with Natura 2000 and other protected areas. In 2022, 49,197 family farms (60% of those analysed) received this support. Eligible beneficiaries include both individuals and legal entities registered in the Farm Register, with payments ranging from 82 to 226 EUR per hectare. The objective of this measure is to sustain agricultural activity, protect biodiversity, support rural employment, and mitigate depopulation trends in disadvantaged areas (Narodne Novine, 2022).

Given the strategic importance of family farms in the implementation of EU rural development policies, this paper aims to explore how demographic characteristics of farm holders - specifically age and education level - affect the uptake of selected rural development measures in Croatia. The study focuses on three key measures: Measure 10 (Agri-environment-climate), Measure 11 (Organic Farming), and Measure 13 (Payments in Areas with Natural Constraints). The main research questions are: (1) Is there a statistically significant relationship between the age of family farm holders and their use of these measures? and (2) Is the education level of farm holders associated with the adoption of these measures?

Material and methods

The aim of the research was to examine the correlation between the demographic characteristics of family farm holders in Croatia and the use of measures from the Rural Development Program of the Republic of Croatia 2014-2020, with a special focus on Measure 10 (Agriculture, Environment, and Climate Change), Measure 11 (Organic Farming), and Measure 13 (Payments in Areas with Natural Constraints). Data for this analysis were collected from the Agency for Payments in Agriculture, Fisheries and Rural Development for the year 2022, and data processing was conducted using the statistical software R Studio. The data include various variables, including MIBPG (unique agricultural identification number), gender, date of birth, and education level of family farm holders, as well as information on the measures applied by users. For the purposes of analysis, the variables date of birth and education level were categorized as follows: age into three age groups: 1 - up to 40 years, 2 - from 41 to 65 years, and 3 - over 65 years, to reflect common demographic segments used in agricultural policy

discussions and to capture the differences between younger, working-age, and retirement-age farmers. The education level of users was categorized based on the highest level of education attained, with categories being: 1 - Incomplete primary school, 2 - Primary school, 3 - Secondary school, 4 - Higher education, 5 - University or Higher Education based on the official Croatian education classification system, allowing for consistent interpretation across statistical and policy frameworks (Narodne novine, 2001). To analyse the correlation between different demographic factors and the application of agri-environmental measures, statistical tests based on Pearson's chi-squared test were used. This method was chosen because it is appropriate for testing relationships between categorical variables, such as age and education categories and the use of specific rural development measures. Its non-parametric nature makes it suitable for the type and scale of the data analysed in this study, where variables are grouped into discrete, non-ordinal categories. The Pearson's chi-squared test was selected due to its suitability for testing the independence between categorical variables, in this case, demographic characteristics (age and education) and the use of specific rural development measures. For each variable, contingency tables were created, followed by the application of Pearson's chi-squared test to test the statistical significance of the association between variables.

Results and discussion

In 2022, out of a total of 128,290 family farms (N=128,290), 81,912 utilized at least one form of agricultural support, and this group is the subject of further analysis. A detailed analysis revealed that women as farm holders were represented in 21,900 cases (26.7%), while men constituted the majority with 60,012 holders. The age structure of family farm holders shows significant differences among categories. The largest share of holders belongs to category 2 (41 to 65 years old), accounting for 48.1%, while category 1 (up to 40 years old) represents 15.6%, and category 3 (over 65 years old) accounts for 36.3%. The youngest holder was 19 years old, while the oldest respondent was 101 years old, with the average age of farm holders being 58.3 years. According to available data on the educational structure for 56,030 family farms, secondary education indicates the dominance, constituting 39% of the total. This is followed by the group of holders with completed primary education (18.6%), while a significantly smaller proportion of holders have incomplete primary education (3.4%). Higher education was completed by 1.9% of holders, while 5.5% of holders have a university degree or higher education.

The subject of analysis in this study included Measure 10 - Agriculture, Environment, and Climate Change, Measure 11 - Organic Farming, and Measure 13 - Payments in Areas with Significant Natural and Other Constraints. Consequently, the following results are based on examining the correlation between the demographic characteristics of family farm holders and the use of these measures.

Analysis of Measure 11 - Organic Farming

Payments under Measure 11 (Organic Farming) between 2014 and 2022 were relatively successful, with the share of agricultural land under organic practices increasing from 0.2% in 2003 to around 9% in 2022 (Blaće et al., 2020). In that year, 4,904 out of 81,912 family farms receiving CAP support used Measure 11, with 28.18% of users being women and 71.82% men. The age of users ranged from 20 to 95, with an average age of 57.73 years. Chi-square tests showed a significant correlation between age and the use of organic farming, with most users (53.75%) in the 41-65 age group, followed by those up to 40 years old (26.49%) and those over 65 (19.76%). There was also a significant correlation between education level and the use of Measure 11. The majority of users (44.03%) had completed secondary school, while only 0.49% had incomplete primary education and 3.59% had higher education, though 30.53% did not declare their education level. These results are consistent with existing research, such as the

study by Sikandar Azam and Banumathi (2015), which found that higher education levels positively influence the adoption of organic farming practices.

Analysis of Measure 10 - Agriculture, Environment, and Climate Change

A similar analysis was conducted for Measure 10, which was used by 14,226 family farms in 2022. Of these, 24.82% were owned by women and 75.18% by men. Users ranged in age from 19 to 99, with an average age of 55.11 years. The Chi-square test confirmed a significant correlation between age and use of the measure ($X^2 = 2103.1$, $p < 2.2e-16$), with the majority of users (50.84%) aged 41–65, followed by those over 65 (28.65%) and those up to 40 (20.51%). This age distribution reflects long-term family and business motivations for engaging in environmental practices, such as preserving the family farm, as noted by Farmar-Bowers and Lane (2009), and supported by findings from Meert et al. (2005) and Siebert et al. (2006). Education level also showed a statistically significant correlation with use of Measure 10 ($X^2 = 12155$, $p < 2.2e-16$). Most users (43.82%) had completed secondary school, while only 1.43% had incomplete primary education and 2.81% held higher education degrees. The frequency of specific operations within Measure 10 varied widely. Some practices, such as mechanical weed control in perennial crops (5,829 users) and the use of pheromone, visual, and food traps (2,743 users), were widely adopted. Others, including integrated production and soil tillage on slopes for annual crops, saw no uptake at all. The butterfly protection pilot measure had only five users, suggesting limited awareness or relevance. These differences point to diverse agronomic, ecological, and socio-economic factors influencing the adoption of specific practices within the measure.

Analysis of Measure 13 - Payments in Areas with Significant Natural and Other Constraints

Measure 13, which supports farms in areas with natural and specific constraints, is vital for sustaining agriculture in rural regions. In 2022, it was used by 49,197 family farms, with 73.88% of users being men and 26.11% women. Users ranged from 19 to 101 years old, with an average age of 58.92. A Chi-square test confirmed a significant correlation between age and use of the measure ($X^2 = 8031.5$, $p < 2.2e-16$), with the majority of users aged 41–65 (46.77%), followed by those over 65 (38.31%), while the under-40 group accounted for only 14.92%. Education also significantly influenced use of Measure 13 ($X^2 = 38877$, $p < 2.2e-16$), with most users having secondary education (40.45%) and the fewest having higher education (2.16%). These findings reflect similar patterns observed by Papić et al. (2022), who emphasized the importance of compensatory and agri-environmental measures for the economic survival of farms in constrained areas, where producers often depend on stable production systems to secure support.

Conclusions

The analysis of the use of Measures 10, 11, and 13 reveals clear patterns related to the age and educational structure of the beneficiaries. The largest share of users of all three measures consists of farmers aged 41 to 65, while the younger population, particularly farm holders under the age of 40, is significantly underrepresented. This data highlights the challenges of generational renewal in agriculture, with older farmers more frequently utilizing support measures, which can be attributed to their need for income stability and long-term farm development planning. Regarding the educational structure, the majority of beneficiaries have completed secondary education, while the proportion of highly educated farmers is relatively low. This aligns with previous research showing that education positively influences the adoption of new agricultural practices, but also indicates the need for additional incentives to encourage greater participation of highly educated farmers in rural development programs. In

conclusion, the results of this study emphasize the need for targeted policies that will encourage younger generations to take over agricultural holdings, increase the level of education within the sector, and ensure better adaptation of measures to specific regional needs. Future policy interventions should focus on enhancing support for younger farmers, such as through financial incentives, mentorship programs, and facilitating access to land. Moreover, improving educational opportunities and promoting professional development for farmers could foster the adoption of innovative and sustainable farming practices. Key factors for the long-term sustainability of Croatian agriculture include improving access to information and education, which could result in greater efficiency and better utilization of available support. Specifically, future studies should explore targeted educational programs designed to increase the participation of younger and highly educated farmers in rural development measures. Such programs could be tailored to address the unique challenges faced by these groups, including access to funding, knowledge transfer, and networking opportunities. Additionally, more research is needed to understand the barriers to the adoption of agri-environmental measures and explore how they can be better tailored to specific farm types, regions, and farmer motivations.

References

- Azam M., Banumathi M. (2015). The Role of Demographic Factors in Adopting Organic Farming: A Logistic Model Approach. *International Journal of Advanced Research*. 3: 713-720.
- Blaće A., Čuka A., Šiljković, Ž. (2020). How Dynamic Is Organic? Spatial Analysis of Adopting New Trends in Croatian Agriculture. *Land Use Policy*. 99: 105036.
- Chao K. (2024). Family Farming in Climate Change: Strategies for Resilient and Sustainable Food Systems. *Heliyon*. Vol. 10 (7): e28599.
- Croatian Bureau of Statistics. (2020). Number of Agricultural Holdings, General Overview, Republic of Croatia and HR NUTS 2 (HR_NUTS 2021). Available from: <https://web.dzs.hr>
- Defilippis J. (2004). Development of Family Farms in Croatia and Cooperatives. *Sociologija Sela*. 167: 43-59. Available from: <https://hrcak.srce.hr/file/50906>
- Farmer-Bowers Q., Lane R. (2009). Understanding Farmers' Strategic Decision-Making Processes and the Implications for Biodiversity Conservation Policy. *Journal of Environmental Management*. 90(2): 1135–1144. Available from: <https://pubmed.ncbi.nlm.nih.gov/18565641/>
- Ministry of Agriculture (2015). Rural Development Program. Ministry of Agriculture, Zagreb. Available from: <https://ruralnirazvoj.hr>
- Ministry of Agriculture (2023). Annual Report on the State of Agriculture in 2022. Ministry of Agriculture, Zagreb. Available from: <https://poljoprivreda.gov.hr>
- Mikuš O., Kovačiček T., Hadelan L., Jež Rogelj M. (2020). Agri-Environmental Indicators and Their Application in the EU Common Agricultural Policy (CAP). *Glasnik Zaštite Bilja* 6: 13-20.
- Meert H., Van Huylenbroeck G., Vernimmen T., Bourgeois M., & Van Hecke E. (2005). Farm Household Survival Strategies and Diversification on Marginal Farms. *Journal of Rural Studies*. 21: 81-97.
- Narodne novine (2018). Family Farm Act. OG 29/2018. Available from: <https://narodne-novine.nn.hr>
- Narodne novine (2023). Regulation on the Register of Family Farms. OG 80/2023. Available from: <https://narodne-novine.nn.hr>
- Narodne novine (2022). Regulation on the Implementation of Direct Payments in Agriculture and IACS Rural Development Measures for 2022. OG 27/2022. Available from: <https://narodne-novine.nn.hr>

- Narodne novine (2001). Metodologija nacionalne standardne klasifikacije obrazovanja. Available from: <https://narodne-novine.nn.hr>
- Papić Milojević R., Bogdanov N., Todorović S. (2022). How Could the CAP Measures Impact Family Farms in Areas with Natural Constraints in Serbia. *Journal of Agricultural, Food and Environmental Sciences*. 76: 62-75.
- Siebert R., Toogood M., Knierim A. (2006). Factors Affecting European Farmers' Participation in Biodiversity Policies. *Sociologia Ruralis*. 46(4): 318-340.
- Wuepper D., Wimmer S., Sauer J. (2020). Is Small Family Farming More Environmentally Sustainable? Evidence from a Spatial Regression Discontinuity Design in Germany. *Land Use Policy*. 90: 104360
- Župančić M. (2005). Family Farms and Rural Development in Croatia. *Sociologija i Prostor*. 43(167): 171-194. Available from: <https://hrcak.srce.hr/file/52321>

The impact of changes in food costs on the structure of consumer expenditure in the period 2020-2024: Case study Croatia

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Summary

The article aims to analyse the effects of retail food prices on the expenditure structure from the consumer's perspective. The analysis is carried out using the example of the Republic of Croatia and consumers in Croatia, with input data from the Croatian Statistical Office. The methodology of econometric modeling was applied, whereby the developed model passed a series of statistical and econometric tests that excluded multicollinearity, autocorrelation, and other negative factors that could affect the accuracy and adequacy of the developed model.

Key words: consumer prices, consumers, econometric modelling, expectation elasticity, Croatia

Introduction

Croatia joined the EU Schengen area in a landmark development and adopted the euro on January 1st, 2023. This has boosted Croatia's tourism industry, which has a typical contribution to GDP of 25% and accounts for a similar share of employment. We expect tourism to be robust in 2024, with tourism receipts having already surpassed pre-pandemic levels (Economist Intelligence, 2025). In 2024, Croatia's GDP is projected to grow by 3.6%. Growth is expected to be mainly driven by private consumption, boosted by substantial real wage and employment growth and sustained investment (European Commission, 2025).

Consumer preferences change according to the level of personal income, lifestyle, and characteristics of the country in which they live. We can analyse them from the point of view of the structure of the distribution of consumer spending. On the other hand, such analysed data can help us understand the habits of a group of consumers and predict their reactions to consumption in the event of an increase in the prices of consumer goods. So far, some studies have been conducted, mainly analyzing how consumers change their preferences for protected product labels (Jelnikar, 2010), how consumer preferences can be satisfied (Peteros, 2017), and consumers' attitudes toward local food (Hristov et al., 2023). In addition to analysing consumer preferences for personal income consumption (economic aspect), the analysis presented in this paper also presents Croatian consumers' attitudes towards food and food-related consumption (social aspect). We expect these results to be helpful for a broader understanding and for preventive measures to mitigate the negative consequences in the agri-food sector. With the results of this research, decision-makers will be able to understand better consumers' expected reactions to changes in consumer goods' prices and for the possible preparation of proposals for managing and forecasting general consumption. The good contribution of the study can be linked to the fact that the analysis is based on publicly available data from the National Institute of Statistics, which we know is of limited use for scientific research purposes due to its scope, the method of analysis, and the complexity of data collection. However, due to their size, they can provide a perfect representative sample for the analysis, which cannot be achieved with our

data collection methods. According to the research findings and the econometric model developed, we have successfully overcome the above limitations and achieved resounding results.

Material and methods

The analysis included data from 2020 to 2024, forming the basis for developing a predictive econometric model. The data were obtained from the Croatian Statistical Office (CBS, 2025) and were processed to represent the dependent and independent variables used in the econometric prediction model (Table 1).

Table 1. Description of the variables used in the predictive econometric model

Variable	Definition / description of the variable	Number of the observed unit	Unit
Specification of dependent variable			
$Y = a/b$			
Y	a = Harmonized index of consumer prices – total / b = Food and non-alcoholic beverages (the impact of retail food prices on the overall structure of expenditure from the consumer's point of view)	62	Monthly rates of change, % (previous month = 100)
Specification of independent variable			
X ₁	Alcoholic beverages and tobacco	62	Monthly rates of change, % (previous month = 100)
X ₂	Housing, water, electricity, gas, and other fuels	62	Monthly rates of change, % (previous month = 100)
X ₃	Furnishings, household equipment, and routine household maintenance	62	Monthly rates of change, % (previous month = 100)
X ₄	Health	62	Monthly rates of change, % (previous month = 100)
X ₅	Transport	62	Monthly rates of change, % (previous month = 100)
X ₆	Communication	62	Monthly rates of change, % (previous month = 100)
X ₇	Recreation and culture	62	Monthly rates of change, % (previous month = 100)
X ₈	Education	62	Monthly rates of change, % (previous month = 100)
X ₉	Restaurants and hotels	62	Monthly rates of change, % (previous month = 100)
X ₁₀	Miscellaneous goods and services	62	Monthly rates of change, % (previous month = 100)

For the definition of the dependent variable (y), the Harmonized Index of Consumer Prices (HICP) was used, which is the European measure of inflation and measures changes in retail prices of products and services from the point of view of the expenditure structure of (domestic

and foreign) consumers for the purchase of consumer goods on the Slovenian territory (principle of domestic consumption). Harmonized consumer price indices are calculated by all member states of the European Union (EU), which makes it possible to compare consumer price trends between EU member states.

Results and discussion

We tested four different model forms based on a combination of linear and logarithmic forms of the dependent and independent variables (LIN, LOG, LIN-LOG, LOG-LIN). Statistical and econometric criteria showed the best selection and suitability of the developed model as a linear equation form. The form of the model developed is as follows:

$$Y = 0,331 + 0,749 * x_1 - 0,143 * x_2 + 0,486 * x_3 + 0,028 * x_4 + 0,439 * x_5 + 0,178 * x_6 - 0,614 * x_7 + 0,073 * x_8 + 0,221 * x_9 - 0,400 * x_{10} + u_t$$

The results of the specified model, which was developed using the SPSS statistics program, are shown in Table 2.

Table 2. A summary of the properties of the specified model

Model summary data					
Type	R value	R ² value	Adj. R Square	St. error of the estimate	Durbin-watson test (DW)
LIN	0.584	0.341	0.206	1.65	1.80

The specified model was tested in various tests with which we analyzed the following:

Autocorrelation in the residuals violates the independence assumption and leads to biased standard errors and invalid hypothesis tests. We used the Durbin-Watson test to test the model for autocorrelation. The Durbin-Watson (DW) test is a statistical test used in regression analysis to determine the presence of autocorrelation (especially first-order serial correlation) in the residuals of a regression model. Autocorrelation is present when the residuals (errors) are not independent but are correlated with each other. $DW \approx 2$ means no autocorrelation; $d < 2$ indicates positive autocorrelation, and $d > 2$ means negative autocorrelation. The optimum value of the DW test could be between 1.8 and 2.2. The value of the DW test of the developed model is shown in Table 2 and is 1.80. There is no autocorrelation in the specified model.

Variable selection and multicollinearity problem (multicollinearity is the non-isolate relationship between independent variables and dependent variable) - The variance inflation factor (VIF) measures multicollinearity in regression analysis. It indicates the extent to which the variance of the regression coefficient is inflated by multicollinearity between the independent variables.

Interpretation of the VIF value (Prišenk et al., 2025):

- VIF = 1: No multicollinearity (the variable is not correlated with other independent variables)
- $1 < VIF \leq 5$: Moderate multicollinearity, generally acceptable
- VIF > 5: High multicollinearity, may need to be investigated
- VIF > 10: Very high multicollinearity, problematic, and the variable may need to be removed or treated (e.g., by regularization or dimensionality reduction).

The values of the VIF test are presented in the Table 3.

Table 3. Values of VIF test.

Independent variable	VIF values – Collinearity statistics
X ₁	1.212
X ₂	2.055
X ₃	1.767
X ₄	1.335
X ₅	1.966
X ₆	1.162
X ₇	2.684
X ₈	1.285
X ₉	2.539
X ₁₀	1.342

For the discussion, the elasticities of the individual independent variables were calculated according to the equation $b_i, i=1,2,...,10 \cdot (x'/y')$, where b_i represents the coefficients of the variables that can be seen from the model specification in subsection above, and multiplied by the ratio between the mean value of the corresponding independent variable and the mean value of the dependent variable. The results of the calculated elasticities are shown in Table 4. The variables were inelastic in all cases of the calculations (below 1 or above -1). By calculating the elasticity, we assessed the impact of price changes for different consumer goods groups on food purchases.

Table 4. Elasticity results with a description of the substantial explanatory power.

Independent variable	Elasticity value	Description of the explanatory power
Alcoholic beverages and tobacco – x ₁	0.46	increased influence
Housing, water, electricity, gas and other fuels – x ₂	-0.07	no influence
Furnishings, household equipment and routine household maintenance – x ₃	0.24	increased influence
Health – x ₄	0.01	no influence
Transport – x ₅	0.20	increased influence
Communication – x ₆	0.01	no influence
Recreation and culture – x ₇	-0.34	reduced influence
Education – x ₈	0.02	no influence
Restaurants and hotels – x ₉	0.24	increased influence
Miscellaneous goods and services – x ₁₀	-0.24	reduced influence

Positive values express the increased influence of retail food prices on the overall expenditure structure from the consumer's perspective in the event of price changes (increase) in the groups "x₁, x₃, x₅ and x₉". Food costs are expected to account for a larger share of total consumer expenditure (Table 4). Consumers will react to price increases in these groups by using some of the reduced funds to increase consumption in the food and non-alcoholic beverages group. Negative values express the inverse impact of retail food prices on the overall expenditure structure from the consumers' perspective in the event of price changes (increase) in the groups "x₇ and x₁₀". The share of food costs in the structure of total consumer expenditure is expected to decrease (Table 4). This means that consumers will not react to price increases in groups "x₇" and "x₁₀" with increased consumption at the expense of food consumption but will shift

their reduced consumption to other consumer goods groups. However, for price changes (increases) in groups x_4 , x_6 , x_8 , and x_2 we do not expect any significant or more pronounced effects of food price changes on consumer preferences. Thus, there are cost groups related to education, health, communication, and essential goods (water, electricity, etc.) whose price changes do not impact consumers' willingness to spend more or less on food purchases.

The study results are surprising in specific segments, such as the "communication" segment, which, in terms of consumer preferences, is categorized as an "important" life need, such as health and education. In this case, the consumer is not prepared to reduce his expenditure in other consumption segments in the event of a change in expenditure on food and non-alcoholic beverages. An interesting and expected result is that the consumer reacts to restaurant price increases by using part of the reduced funds to purchase food and non-alcoholic beverages. It would be helpful to examine this result further for a connection with the tourist capacity, which accounts for a very significant proportion of the country's total income.

Conclusions

The results of the research carried out will be useful, and we expect that they will attract the public's attention and help different sectors make their decisions based on the expected reactions of consumers. The problem is particularly relevant from the point of view of current price increases and the high inflation rate in Croatia, which reduces the population's purchasing power.

References

- Croatian Bureau of Statistics (CBS) (2025). Consumer price indices. Available from: <https://podaci.dzs.hr/en/>.
- Economists Intelligence 2025. Croatia. Available from: <https://country.eiu.com/croatia>.
- European Commission 2025. Economic forecast for Croatia. Available from: https://economy-finance.ec.europa.eu/economic-surveillance-eu-economies/croatia/economic-forecast-croatia_en
- Hristov H., Erjavec K., Pravst I., Juvančič L., Kuhar A. (2023). Identifying Differences in Consumer Attitudes towards Local Foods in Organic and National Voluntary Quality Certification Schemes. *Foods*. 12(6): 1132. Available from: <https://doi.org/10.3390/foods12061132>
- Jelnikar K. (2010). The impact of organic labels on consumer preferences. Graduation thesis. University of Ljubljana, University of Social Sciences, Ljubljana, 100.
- Peteros A. (2017). Searching for the best or satisfying preferences - Analysis of decision-making methods on the example of Slovenian consumers. Master's thesis. University of Ljubljana, Faculty of Social Sciences, Ljubljana, 102.
- Prišenk J., Zidar N., Turk J. (2025). Development of a Theoretical Model for the Price Formation of Agri-Food Products in the Food Supply Chain: A Slovenian Case Study. *Foods*. 14(3): 415. Available from: <https://doi.org/10.3390/foods14030415>.

ORIGINAL SCIENTIFIC PAPER

Economic justification of the application of conservation tillage in corn production

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Summary

The paper analyzed the economic efficiency and cost price of various conservation tillage technologies in corn production with conventional and reduced fertilization in comparison to conventional fertilization and soil cultivation at two locations of the Pannonian agricultural region. The relatively highest yields are with normal fertilization and reduced with the addition of soil conditioners at one location. The lowest cost prices were recorded with normal fertilization, where there is no effect of soil cultivation. Highest economic coefficients were recorded with variants with loosening, both with normal and reduced fertilization.

Keywords: cost effectiveness, soil conservation tillage, corn, cost price

Introduction

Conservation tillage is a term that, according to Jug et al. (2017), can be defined in two ways: as a tillage system in which plant residues are retained on or near the soil surface with the aim of suppressing and/or mitigating erosion and achieving more favourable soil moisture, and as a tillage system where harvest residues cover more than 30% of the soil surface in order to reduce erosion by 50%. The basis of conservation agriculture is management based on three fundamental postulates, which contextually unite climate-soil-plant, while taking into account agro-ecological and socio-economic differences (Jug et al., 2018). Jug (2005) in his research of the reduced tillage impact on the yield of winter wheat, corn and soybeans in the agro-ecological conditions of northern Baranja, states that the investigated indicators, especially the yield of winter wheat grain, were almost equally influenced by weather conditions and soil cultivation methods. On a three-year average, the highest wheat grain yields were recorded in the multiple discing variant, while the highest corn and soybean yields were achieved in standard tillage. The conservation tillage system achieves almost the same corn yields as conventional tillage, but with 37.5% less energy consumption (Košutić et al., 2005). Žugec et al. (2002) explain that some tillage treatments on chernozem, compared to conventional tillage, provide more favourable effects in the form of cost reductions ranging from 3.5 to 37.3% with increased profits from 3.6 to 25.8%.

Material and methods

The paper analysed data from the experimental sites Čačinci and Križevci where research was conducted in 2024 as part of the HRZZ project "Evaluation of conservation tillage as an advanced method of growing crops and preventing soil degradation - ACTIVEsoil". In this paper, the income calculation was made according to the yield levels and the average selling price immediately after the harvest. Average direct payments for agricultural production of €298.63 are also included in the income. The contribution to the coverage of variable costs (Gross margin) is calculated as the difference between the value of production and variable

costs. The cost price was calculated according to a simple divisional calculation, and the calculations were made according to the direct costing method with the calculation of only variable (direct) production costs. For the calculations, apart from the prices of input and output in agriculture in 2024 published in the database of the Market Price Information System (TISUP), the reports of the Central Bureau of Statistics (DZS) and the announcements of suppliers and buyers on the Croatian market were used. Production costs were obtained by multiplying material and labour costs with current prices per unit of consumption. Material costs (seeds, protective agents and mineral fertilizers) were obtained according to actual data from experiments, while labour costs per work operation were estimated from standards (technological norms) according to previous research.

The cost per hour of operation of tractors and implements (Table 3) is calculated according to the following scheme:

- A: price of a new machine on the market
- B: price after the depreciation period
- C: price difference ($A - B$)
- D: depreciation period
- E: annual depreciation amount (C / D)
- F: average working hours per year
- G: depreciation per hour of operation (E / F)
- H: annual interest on invested funds (7%)
- I: interest per hour of operation (H / F)
- J: price of blue diesel
- K: fuel consumption per hour of operation
- L: fuel cost per hour of operation ($J * K$)
- M: maintenance cost (30% of fuel costs)
- N: cost per hour of operation ($G + I + L + M$)

The labour costs of people and machines were estimated according to the technological requirements of the crop and the technical characteristics of the mechanization. The standards are achieved under average production conditions and with average effort. The labour costs of the mechanization per unit area are determined by the speed of work and the area covered, which define the theoretical effect, and the practical effect is determined by the degree of utilization of working time, which in crop farming ranges from 35 to 60% (Grgić et al., 1999). Calculations were made for yields recorded in experiments, according to different soil cultivation and fertilization systems. Three different basic soil cultivation systems are presented (soil cultivation by ploughing and deep loosening and shallow loosening), two fertilization methods (according to the recommendation and half as much) combined with the application of soil conditioners (Geo2). Accordingly, the cost prices of corn were obtained at the level of the sum of the total costs of mechanization and variable costs (seeds, fertilizers, protective agents). Fixed farm costs (rent, insurance, etc.) are not included. Costs for seeds, fertilizers, and protective equipment are included, excluding VAT.

Results and Discussion

The data refer to the prices of seeds at the time of sowing, mineral fertilizers and protective agents at the end of the first quarter of the current year, and selling prices immediately after harvest in the current year (Table 1).

Table 1. Overview of input prices, subsidies and sales prices in corn production

Description of input and output	Year	Seed price €/ha	State support €/ha	Selling price €/kg
Maize	2023	146.00	280.00	0.16
(seed 250 kg/ha)	2024	160.59	280.00	0.18
Fertilizer price	€/kg	2023	2024	
Urea		0.50	0.53	
KAN		0.42	0.43	
NPK 0:20:30		0.51	0.53	
NPK 15:15:15		0.45	0.52	
Pesticides price	€/unit	2023	2024	
Lancelot	33 gr	19.95	19.97	
Palas	200 gr	19.91	20.24	
Elatus	0.7 lit	57.07	59.99	
Poleci plus	0.6 lit	7.35	8.28	
Faster	0.5 lit	4.14	4.60	
Custodia	1 lit	31.85	33.21	
Other				
Price of blue diesel	€/litre	0.96	0.85	
Price of labour	€/hour	3.40	5.34	

Source: TISUP and DZS

Average purchase prices of mechanization for agricultural production were used for the calculation of the hourly cost of mechanization and the calculation of the cost of mechanization for corn, the purchase of which is recommended and technologically well-utilized on arable land of farms up to 50 ha (Table 2).

Table 2. Overview of used own agricultural machinery and purchase prices in 2020

	Purchase price, €	Years of use	Depreciation, €
Tractor (114 kw)	39,847.24	10	3,437.52
Tractor plough	2,389.01	12	154.84
Tractor seeder	2,787.18	12	210.14
Fertiliser spreader	1,513.04	12	103.97
Atomiser	2,070.48	12	133.83
Subsoiler	2,787.18	12	188.02

Source: Average values of used machinery – according to pull

According to the scheme presented in the materials and methods, the costs per hour of tractor and implement operation for 2024 were calculated and presented by work operations (Table 3).

Table 3. Costs per hour of tractor and implement operation by work operation

Operation	Amount €/hour
Ploughing	35.53
Closing the furrow	35.53
Loosening	48.16
Pre-sowing preparation	45.91
Sowing	34.79
Cultivation	34.79

Fertilization	36.60
Liming	36.60
Protection	34.62
Transportation	39.29
Other	34.62

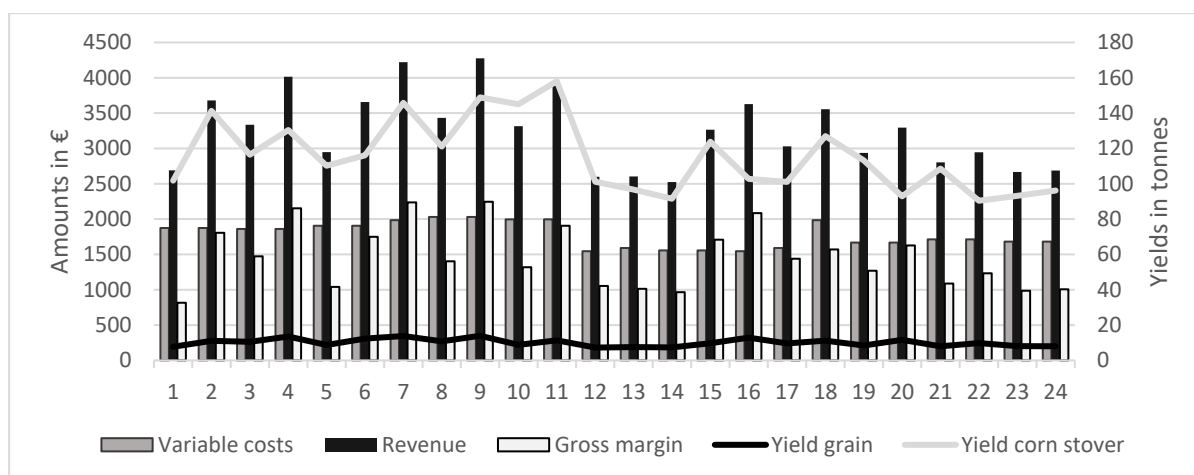
The costs of mechanization (Table 4) were calculated based on actual labour hours at two locations (Čačinci and Križevci) for 3 levels of soil cultivation: A1 is conventional soil cultivation by ploughing, A2 includes deep soil loosening, and A3 is cultivation with shallow soil loosening.

Table 4. Costs of mechanization at experimental locations (€/ha)

Description of operation	Costs					
	Čačinci			Križevci		
	A1	A2	A3	A1	A2	A3
Ploughing	166.88	166.88	166.88	166.88	166.88	166.88
Closing the furrow	136.85	0.00	0.00	136.85	0.00	0.00
Loosening	0.00	182.92	149.47	0.00	182.92	149.47
Pre-sowing preparation	61.28	61.28	61.28	75.50	75.50	75.50
Sowing	71.90	71.90	71.90	79.27	79.27	79.27
Cultivation	79.44	79.44	79.44	79.44	79.44	79.44
Fertilization	82.75	82.75	82.75	82.75	82.75	82.75
Liming	0.00	0.00	0.00	0.00	0.00	0.00
Protection	112.70	112.70	112.70	112.70	112.70	112.70
Transportation	127.91	127.91	127.91	170.54	170.54	170.54
Other	75.13	75.13	75.13	75.13	75.13	75.13
TOTAL	914.84	960.92	927.46	979.07	1,025.14	991.68

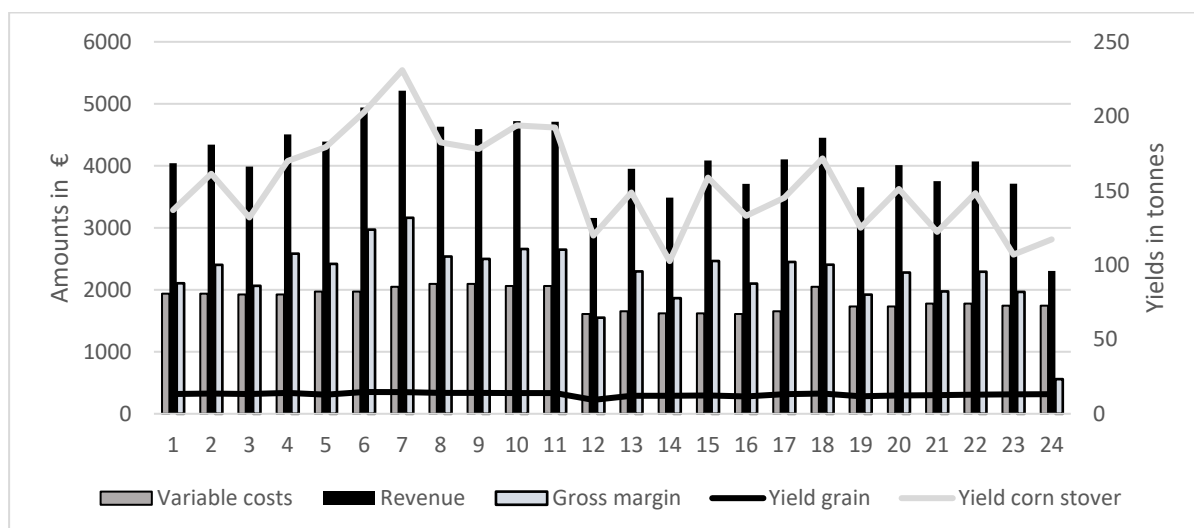
For each system, 8 fields were tested. Within each tillage system, normal and reduced (half-less) fertilization, as well as the application of soil conditioners, were additionally analysed. In the experimental fields of Čačinci and Križevci, greater differences in grain yield were recorded, which directly determines the value of production, but is not related to the selected agricultural technique (Graphs 1 and 2). The differences in yield are by tillage systems, but also within them, depending on the intensity of fertilization. The average yields at the Čačinci location are 10.08 t/ha (deviation from 7.27 to 13.93 t/ha), while the average yield at the Križevci location is 13.01 t/ha with fluctuations from 9.34 to 14.63 tons. Production costs fluctuate depending on the selected technology. There is no connection between the level of yield and technology, because relatively higher and lower yields occur in each technology. Income and costs are not mutually related, because yields and income do not necessarily increase with the intensity of costs. In all fields, regardless of the applied agricultural techniques, positive contributions to coverage were achieved, meaning production was economical.

At the Čačinci location, the highest yield was achieved with classic agrotechnics with the use of soil conditioners, and the highest cost-effectiveness with reduced fertilization. The lowest cost price was recorded with the use of conditioners at both fertilization levels.



Graph 1. Yields, income, costs and contribution to coverage at the Čačinci location

At the Križevci location, the highest yields are achieved with conventional fertilization, without and with soil conditioners, regardless of the use of mechanization. Income and costs are not interconnected, because yields and income do not necessarily increase with the intensity of costs. In the cost structure, the greatest impact is exerted by the cost of fertilization and mechanization, and differences in technology affect the total cost. The application of mineral fertilizers has a smaller effect on the contribution to coverage, and the application of soil conditioners in the reduced tillage technology did not have a detrimental effect on the cost-effectiveness of production, since significantly higher yields and income were recorded. In all fields, regardless of the applied agricultural technology, positive contributions to coverage were achieved, i.e. production is economical. The greatest cost-effectiveness is achieved with conventional fertilization and reduced tillage, and the lowest cost price is achieved with the same agricultural technology but with the use of soil conditioners.



Graph 2. Income, costs and contribution to coverage at the Križevci location



Graph 3. Relationships of economy and cost of production

According to the economic criterion (the lowest cost price and the highest production economic efficiency), the best systems are reduced tillage with shallow soil loosening, in all forms of fertilization (normal, reduced and reduced with soil conditioners).

Conclusions

There are large differences in the average yield values between the experimental sites. The average yields at the Čačinci site are 10.08 t/ha, and at Križevci 13.01 t/ha. Since the same procedures were carried out at the two sites, and the yields at these sites are different regardless of additional inputs (amount of fertilizer, soil improver) or the choice of technology (shallow or deeper soil cultivation with and without loosening), it is recommended to conduct additional research, since the level of grain yield and investment in production directly determine economic efficiency.

Based on the results achieved in the ACTIVEsoil project research, it can be concluded that the cost-effectiveness of the application of conservation tillage, which is classified as a technology for mitigating climate change in crop production, largely depends on productivity, that is, on the amount of yield achieved. Cost-effectiveness is achieved at all yields and applied technologies. The lowest cost price and the highest production cost are achieved with reduced tillage with shallow soil loosening, in combination with all forms of fertilization.

Acknowledgement

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References

- Grgić Z., Šnajder I., Košutić S. (1999). Korištenje poljoprivredne mehanizacije na obiteljskom gospodarstvu. Aktualni zadaci mehanizacije poljoprivrede, Opatija 2.-5.02.1999., Zagreb: Agronomski fakultet. 29-34.
- Jug I. (2005). Utjecaj agroekoloških uvjeta i krupnoće sjemena na rani porast OS hibrida kukuruza. Magistarski rad. Sveučilište J. J. Strossmayera. Poljoprivredni fakultet u Osijeku. Osijek.

- Jug D., Jug I., Vukadinović V., Stipešević B., Đurđević B. (2018). The role of conservation agriculture in mitigation and adaptation to climate change. *Poljoprivreda* 24 (1): 35-44. Available from: <https://doi.org/10.18047/poljo.24.1.5>
- Jug D., Jug I., Vukadinović V., Đurđević B., Stipešević B., Brozović B. (2017). Konzervativna obrada tla kao mjera za ublažavanje klimatskih promjena. Sveučilišni udžbenik (na hrvatskom). Hrvatska organizacija za istraživanje obrade tla, Osijek.
- Košutić S., Filipović D., Gospodarić Z. (2001). Maize and winter wheat production with different soil tillage systems on silty loam. *Agricultural and Food Science in Finland* 10(2): 103-112.
- Košutić S., Filipović D., Gospodarić Z., Husnjak S., Kovačev I., Čopeć K. (2005). Effects of different soil tillage systems on yield of maize, winter wheat and soybean on albic luvisol in North-West Slavonia. *JCEA*. 6(3): 241-248.
- Ministarstvo poljoprivrede. Tržišno informacijski sustav u poljoprivredi (TISUP). Available from: <http://www.tisup.mps.hr/>
- Žugec I., Jurić I., Jug D., Kanisek J. (2002). Obrada tla u održivom uzgoju ratarskih kultura Istočne Hrvatske. Ekoinženjerstvo, Plitvice.

Telematics systems and big data in agriculture

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Summary

The paper presents an analysis and comparison of various telematics systems in modern agriculture. Telematics systems enable the collection and analysis of data on crops, weather conditions and machine operation, thereby optimizing resource management. The paper analyzes several leading systems such as *John Deere FarmSight*, *Claas Telematics*, and similar ones. The systems have specific characteristics from real-time machine monitoring to cost optimization and yield increase. The aim of the paper was to compare their key features in order to assess their contribution to the efficiency and sustainability of agrotechnical operations. The paper concludes with the importance of telematics in the modernization of agricultural practice and concludes that the application of these technologies significantly improves work in agricultural production.

Key words: telematics, telematic systems, precision agriculture, data, information

Introduction

Telematics is an interdisciplinary field that integrates telecommunications, informatics and data transmission technology to provide a wide range of services and applications. The word "telematics" comes from the combination of the words "telecommunications" and "informatics". Telematics systems enable remote monitoring, control and exchange of information, using integrated systems that include sensors, data acquisition devices, communication networks and software applications (Bonsall and Bell, 1987). Goel (2007) states that the combination of telecommunications and informatics is known as "telematics", and is of key importance for future vehicles. Zagorda and Kielbasa (2023) state that using the system it is possible to identify operating parameters and energy input. According to Saidani et al. (2024), telematics is increasingly promising and is being adopted to increase the productivity and efficiency of systems in various industrial sectors, while according to Pohl et al. (2019) telematics and ICT have an important potential for reducing emissions in the environment and increasing the energy and resource efficiency of work operations. Pietikäinen (2014) observes that there a lot useful information available in agricultural machinery. The telematics system provides information to be extracted from the machine for use by other. According to Erickson and Widmar (2015), one of the biggest changes between the 2011, 2013, and 2015 survey of agricultural service providers is the use of telematics for field-to-home office communication. In 2011, only 7% of service providers offered telematics data services, but this percentage increased to 15% by 2013, and to 20% in 2015. Bennett (2016) states that if vehicles and telematics systems are properly configured, electronic alerts can be sent for action, such as notifying the dealer/repairer of a diagnostic trouble code or alerting law enforcement that the machine has been moved outside the expected operating area. Zimmer et al. (2015) state that telematics in agriculture enables the wireless transmission of all collected data from farms to the end user. Kanižai (2013) states that there are a large number of different telematics systems that consist of different combinations of three basic components: hardware components

(devices installed on the vehicle to collect data), data transfer components (collected data is transferred from the vehicle to the data collection service) and control software (collected data is converted into useful information necessary for the successful operation of the company). According to (Link 1.), the hardware devices most commonly used in telematics systems are OBU (On Board Units), communication module and GNSS navigation receiver. The authors (Pałczyński, and Pawelski, 2010 and Zimmer et al., 2015) state that the OBU is the most important part of the telematics system of an agricultural aggregate and consists of logic circuits that are used to determine the position of the aggregate, communicate data with the central information system and monitor the operation of the agricultural aggregate. It is an electronic unit that contains software for reading and storing data from the vehicle and reading data from the GNSS receiver, and also controls data transmission.

Main Components of a telematics system

For the successful implementation of a telematics system, the OBU is indispensable as the central part of the system that serves to connect all other devices into a single functional unit. In addition to the OBU, a communication module is required that serves as an intermediary between the telemetry equipment installed in the agricultural aggregate and the communication network. Data is transmitted and received to the central information system via the GSM network through a data protocol that enables data exchange within the GSM network (Zimmer et al., 2015). The communication module serves as an intermediary between the telemetry equipment installed in the agricultural aggregate and the communication network. Data is transmitted and received to the central information system via the GSM network through a data protocol that enables data exchange within the GSM network. A GNSS (eng. *Global Navigation Satellite System*) navigation receiver is used to read the position of the agricultural machine. The navigation receiver used in telematics systems is a separate electronic module that is installed in the cabin of the agricultural machine and is connected to the OBU. The control software is one of the components of the telematics systems, and is used to convert the collected data into a series of information that is used to operationally manage the transport process or the work of the vehicle and driver in a more efficient way. The software provides reports on the work, and can also be used as equipment for graphic and textual display, or it can be a monitoring system. It can be managed using a single computer via a computer network or via the Internet.

Comparison of telematics systems

The *John Deere FarmSight* telematics system enables operators to achieve complete automation and intelligent management. *John Deere FarmSight* is based on three key elements: wireless communication, remote control, and hardware. Wireless communication enables instant collection of information on machine logistics, receiving alerts, and monitoring preventive maintenance of machines using *JDLINK* (Figure 1). Using wireless communication, operators have insight into which machines are working and which are idle, i.e.: monitoring the condition of machines, extending their lifespan through preventive maintenance, simplifying maintenance schedules and documentation, increasing uptime through warnings about possible interruptions, and documenting the productivity of machines and operators or workers. One of the intelligent solutions is *JDLINK Select*, which enables monitoring of operating hours, location, and maintenance of machines in different operating modes (24/7/365). Using *JDLINK Select*, geozones can be created around the machine to be notified if the machine goes outside its virtual boundary, which indicates a high level of security when using the aforementioned telematics system. The *JDLINK Ultimate* intelligent solution is used for detailed insight into engine diagnostics and monitoring of fuel consumption, speed selection, component pressure and temperature, as well as detailed download of diagnostic information. The *JDLINK* application

works by combining a controller that includes mobile communication with GPS capabilities. Machine data is collected via the controller and transmitted wirelessly to the data server, where it is available via the *JDLink* website. *Service ADVISOR Remote* enables the dealer to alert the operator and/or farmer of machine problems and initiate service diagnostic code refresh solutions. *Service ADVISOR Remote* (Figure 2) can wirelessly send software updates to the aggregate. The price for payroll employee is 29 \$/month and for employer of record 599 \$/month.



Fig 1. Data Management JDLink

Source: Link 1.



Fig 2. Service ADVISOR Remote

Source: Link 2.

Claas telematics is a digital data transmission system that continuously collects and documents operating data, track data and yield data from connected machines such as combines, forage harvesters and tractors. All data is transmitted via the mobile network to the Telematics portal where it is processed and stored. The Telematics system supports the optimization of agricultural processes and resource management by providing real-time data insights and historical data analysis. One of the intelligent solutions is called *Remote Service*, which enables remote diagnostics, problem identification and reduction of downtime. Another intelligent solution is the Cemos automatic system for automatic optimization of combine operation. Using the data collected via the Telematics system, Cemos automatic (Figure 3) can automatically adjust the combine settings to ensure maximum efficiency and minimum losses. This system continuously analyzes the operating conditions and adjusts parameters such as rotor speed, air flow and forward speed. *FieldNav* is a navigation system integrated with Telematics that helps operators navigate in the field. The system uses GPS data to provide optimal routes for machine movement across the field, reducing overlap and optimizing coverage. This is particularly useful for large production areas where precise movement is essential for efficient operation. *AFS Connect* by Case IH is an advanced telematics system designed to optimize the management of agricultural operations and vehicle fleets. This system allows farmers to remotely monitor and manage their equipment through an intuitive interface available on any mobile devices. *AFS Connect* system components include *AFS Connect* portals that enable remote monitoring and management of farm operations, providing operators with access to key fleet information and attachment performance (Figure 4).

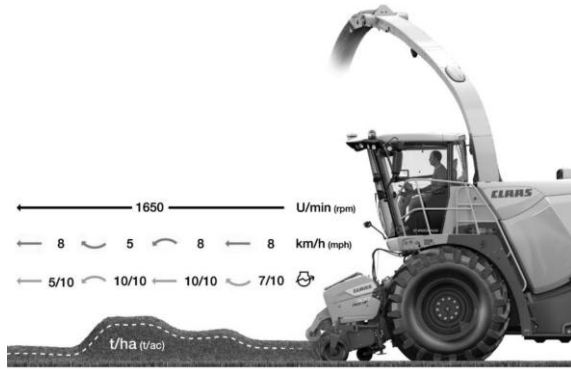


Fig 3. CEMOS AUTOMATIC
Source: Link 3.

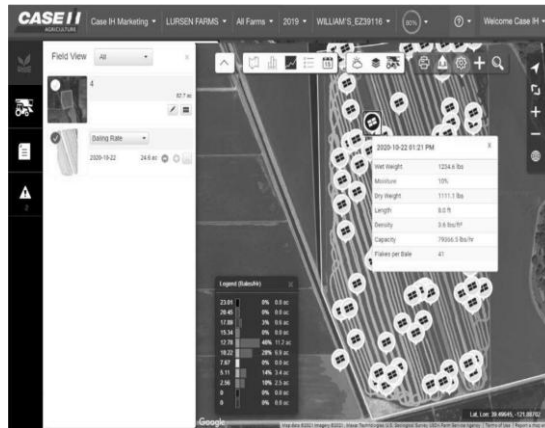


Fig 4. AFS Connect Farm App
Source: Link 4.

For the precise application of protective agents and the management of agrotechnical operations, the manufacturers like *Miller* telematics system (Figure 5) and *New Holland* (Figure 6) created an intelligent solution called the Intelligence Series. The system combines technology and engineering design to provide farmers with efficient application, monitoring and machine control solutions. The *My PLM* (Precision Land Management) platform enables farmers to access and record machine data from a remote location, and enables dealers to access diagnostic codes and perform remote diagnostics. One of the key components of the system is the *Raven XRT Sprayer Height Control*, which uses radar sensors to maintain the optimum application height. The *IntelliSpray II* system provides advanced nozzle control, reducing chemical consumption and ensuring precise and consistent droplets during application, which is essential for effective crop protection. The latest *Miller Nitro 7000* series sprayers has *Bilberry* identification technology. It uses an embedded camera network, artificial intelligence and deep learning software to identify weeds in-crop or in Green-on-Green applications.



Fig 5. IntelliSpray II™ system
Source: Link 5.



Fig 6. New Holland IntelliSpray
Source: Link 6.

The *New Holland PLM* (eng Precision Land Management) system is a telematics system designed to optimize agricultural operations through connectivity and data management. The system uses the *MyPL Connect* portal, which enables remote monitoring and management of machines and data. *PLM Connect* offers three types of connectivity: Customer-to-Vehicle, Dealer-to-Vehicle and Advisor-to-Vehicle. The mentioned types of connection enable the management of agricultural operations and the constant monitoring of machines, as well as the use of advanced diagnostics and software updates on the production surface.

Conclusion

Telematics systems have become an indispensable tool in modern precision agriculture, enabling farmers to precisely monitor, manage and optimize their machinery and agrotechnical operations. The use of telecommunications and IT in the management and control of aggregates in Republic of Croatia is increasingly available through navigation systems, which improves processes and enables cost reduction. These systems combine wireless communication, GPS technology and remote control to provide a comprehensive overview of the operation of agricultural machinery. These systems offer innovative solutions that are directly designed for modern agricultural production, more precisely optimized operation and integration into the use of advanced technologies. These systems enable the collection and analysis of data from various aspects of agricultural production, which significantly increases efficiency, work precision and consequently productivity. Also new systems can also be installed on older agricultural machinery, which still exists in the Republic of Croatia.

Literature

- Bennett, J. (2016): Value of Map Sharing Between Multiple Vehicles In The Same Field While Using Automated Section Control. MS Thesis. Kansas State University.
- Erickson B, and Widmar DA (2015): 2015 Precision Agricultural Services Dealership Survey Results. August 2015. Available from: <https://www.agecon.purdue.edu/cab/ArticlesDatabase/articles/2013PrecisionAgSurvey.pdf>
- Goel, A. (2007): Telematics. In: Goel, A. (ed.), Fleet Telematics – Real-time management and Planning of Commercial Vehicle Operations. Springer, New York.
- Pałczyński, T., Pawelski, Z. (2010): Onboard unit (OBU) in an ITS system on the basis of coopers project. Archives of Transport System Telematics, 3(3), 25-31.
- Pohl J, Hilty LM, Finkbeiner M. How LCA contributes to the environmental assessment of higher order effects of ICT application: A review of different approaches. Journal of cleaner production. 2019 May 10;219:698-712.
- Pietikäinen, S. (2014): Remote isobus telematics in agricultural environment.
- Saidani, M., Bolowich, A., Bednářová, S., Gutiérrez, T. N., Benetto, E. (2024): Life cycle assessment of a telematics box with ICT impact allocation and quantification–Application to precision agriculture technology and robotics. Procedia CIRP, 122, 455-460.
- Zagórda, M., Kielbasa, P. (2023): The use of telematics systems to optimize the operation of agricultural machinery. Przegląd Elektrotechniczny, 99(2).
- Link 1. JDLink™Connectivity Available from: <https://www.deere.com/en/technology-products/precision-ag-technology/data-management/jdlink/> (14.04.2025.)
- Link 2. Inventory Gallery Available from: www.crossimplement.com (14.04.2025.)
- Link 3. Claas and Myanmar Available from: <https://www.myanmar.claas.com> (14.04.2025.)
- Link 4. Farm Equipment Available from: <https://www.americafem.com/> (14.04.2025.)
- Link 5. Miller Sprayers: Miller Machines Available from: <https://www.millersprayers.com> (14.04.2025.)
- Link 6. New Holland IntelliSpray™ Available from: <https://www.newhollandrochester.com/precision-farming/new-holland-intellispray/> (14.04.2025.)

SCIENTIFIC REVIEW

Autonomous robots in smart warehouses

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Summary

This paper discusses the role of autonomous mobile vehicle systems within smart warehouses. It outlines the rationale behind the implementation of autonomous mobile robot systems, detailing their development and operational methodologies. The paper also delves into warehouse management systems, elucidating the motivations for their adoption in warehouse environments. The primary objective of this study is to showcase contemporary autonomous robots and their applications in advanced warehouse settings. The aim of this article is using modern systems within smart warehouses leads to enhanced efficiency in warehousing processes and considerable time savings. This study examines systems in smart warehouses, focusing on LiDAR and efficiency gains. Results show a 30% reduction in operational costs.

Key words: autonomous mobile robots, smart warehouses, AGV system, LiDAR

Introduction

Warehouses and inventories are an integral part of the supply chain, which is managed through various methods, either manually or automatically (Khan et al. 2022). According to Tompkins et al. (2010), the warehouse affects the process of material flow from suppliers to end consumers for finished goods, and to production for raw materials. Therefore, special attention is paid to the warehouse because storage requirements change from day to day. Berg (2007) states that warehousing operations include various activities such as transportation, storage, inbound delivery, outbound delivery, physical inventory count, quality inspection, and packaging, and each of the above activities is considered automated in order to achieve high efficiency in terms of redundant operations and reducing time in performing necessary activities in warehousing operations. Vukelić (2020) notes that the next phase of warehouse automation is enabled by technological advances. In addition to technological advances, the use of robots in warehouses is greatly influenced by business opportunities that put newer technology at the forefront, and such technology represents an adequate solution to new business requirements. According to Majchrzak (2020), autonomous mobile robots (AMR) represent a relatively new technology that is gradually finding its place in the warehouse industry and smart warehouses. According to Vukelić (2020), warehousing operations are an indispensable part of the supply chain, and are represented in a large number of its links. By increasing the level of services and developing the business itself that warehouses provide to clients, new, more modern warehouse operations have been introduced. Most warehouse operations are suitable for performing certain types of mechanization, and by connecting mechanization using a control system, an automated system is obtained. The main reasons for using automated systems in warehouses are to increase efficiency, quality and safety, reduce labor requirements and reduce production costs. Garc (2017) states that optimizing warehouse systems and processes enables fast flow of goods and reduces costs incurred due to the construction of warehouse capacities and warehousing processes. Shorter storage time results in smaller quantities of goods in the warehouse, and therefore lower warehouse costs. One of the major developments is the evolution of automated vehicles and AMR. Le Ahn and Koster (2006) state that the development of AMR was driven

by the need for greater flexibility, not only in navigation capabilities but also in other services they can provide. AMRs can provide numerous services beyond transportation and material handling operations, such as patrolling and collaboration with operators. Zhen and Haolin (2021) note that the rapid development of technology has intensively appeared in the fields of logistics, more precisely, smart warehouses have become a product of smart technologies, triggering a wave of transformation of the warehousing industry. Smart warehouse systems equipped with newer storage technologies are increasingly attracting the attention of industrial and technological giants, and such a way of storage represents a solution for the future of warehousing development. Custodio and Machado (2020) state that technology is advancing at an alarming rate, while Iqbal et al. (2016) state that the use of automation instead of manual labor allows employees to be relieved. De Ryck et al. (2020). note that automated systems can work flawlessly if they are programmed in the right way and if proper maintenance is carried out. Moravec (2020) lists robotics as a field that covers a large number of technological sciences. According to Pakter (2021), the world is increasingly using information technologies that have a great impact on the growth, development and progress of society. Obligations are most often solved through modern technologies, and such technologies advance and develop every day. For serious companies and branches, it is necessary to follow the development of newer technologies in order to remain competitive in business. Daley (2022) states that autonomous robots are robots that perform their work without depending on human operators. They use their sensors to recognize or learn about the environment and make the best decisions to perform the tasks for which they are intended. Min (2023) states that a smart warehouse is actually an intelligent warehouse of the future that creates flexible and agile warehouse environments by automating warehouse activities and running diagnostics for any need, thus enabling the repair and improvement of warehouse equipment and information technology. Wang et al. (2015) state that smart warehouses are managed using database-based software applications such as the Inventory Management System (IMS), the Advance Planning and Scheduling System (APS), the Transportation Management System (TMS), the Warehouse Management System (WMS) and the ERP (Enterprise Resource Planning) system. Culler and Long (2016) state that automated vehicle guidance systems (AGVS) are used in smart warehouses, whose movements are guided by sensors and therefore have greater flexibility during rotation and shipping.

Material and Methods

In accordance with the PRISMA methodology, a systematic literature review was performed using the Web of Science database to investigate the role of autonomous mobile robots (AMRs) in smart warehouse systems, particularly within agricultural contexts. The initial search strategy was based on the inclusion of the following keywords: "smartwarehouse", "agriculture", and "AMR". This query yielded a total of 410 scientific publications. To refine the dataset and enhance relevance, an additional inclusion criterion was applied, focusing on studies that demonstrated a close conceptual or practical connection to agriculture. As a result, the number of eligible studies was narrowed down to 100 publications. Through further screening and content analysis of these sources, it became evident that the implementation of autonomous mobile robots in smart warehouses represents a frequent and emerging need, especially in addressing the increasing demand for automation and precision within agricultural logistics and supply chains. This filtered body of literature formed the empirical basis for evaluating current technological trends, practical applications, and the integration challenges associated with AMRs in smart agricultural warehousing. A systematic review of AMR technologies in smart warehouses was conducted, analyzing case studies from agriculture and industry. Data on efficiency gains were synthesized from Fragapane et al. (2021) and Min (2023). A significant advancement is the progression of autonomous vehicles and automated mobile robots. Artificial

intelligence has the potential to serve as the primary management system for an intelligent warehouse. According to Kamali (2019), the emergence of advanced warehousing technologies has prompted numerous companies to alter their business strategies, adopting these innovations to minimize expenses and labor demands.

Autonomous robots

The evolution of mobile robots is categorized into three distinct generations (I, II, and III). These robots predominantly operate within industrial settings, particularly in automotive manufacturing and agricultural machinery production. Equipped with advanced sensors, they are designed to adapt to the varying conditions of their operational environments. Commonly, these robots are utilized in specific areas such as paint shops (Figure 2) or assembly stations for particular components. The most recent generation of robots is distinguished by their integration of sophisticated computing systems and finely-tuned software, featuring a variety of sensors and artificial intelligence capabilities. This enables them to perceive their surroundings, assess their interactions, and learn from past errors. Third-generation Autonomous Mobile Robots (AMRs) incorporate LiDAR technology and artificial intelligence to facilitate real-time decision-making (Figure 3), in contrast to earlier generations that were restricted to predetermined pathways as shown in Figure 1.



Fig 1. First generation robots

(Source: Mtg-transform, 2016)



Fig 2. Second generation robots

(Source: Oreilly, 2025)



Fig 3. Third generation robots

(Source: Design World Online, 2023)

Autonomous mobile robots are actually a new derivative of automated guided vehicles (AGVs) and are significantly more efficient and smarter in terms of their working characteristics because they use a higher level of artificial intelligence. The great advantage of the aforementioned is that they work in space by mapping the working environment, which makes them faster than previous AGVs because they use laser sensors and use advanced features such as tracking a specific person. A web interface like Servant is used for all AMR settings and settings, which is created according to the needs of each robot and working environment. The main element of AMR is a complex navigation system that actively participates in creating a route from the starting to the destination point. AMRs are autonomous to the extent that they comply with rules, regulations and restrictions set by company. The level of application of artificial intelligence together with sensors is necessary so that AMR can independently perform work tasks/storage and detect system errors and react to them in a timely and correct manner. Figure 4 shows a diagram of the working principle of AMR navigation with the use of external and internal sensors when encountering an obstacle. AMRs use warehouse maps for orientation and created obstacle maps along with the basic navigation sensor LiDAR (Light Detection and Ranging), a laser that can detect objects at a distance of up to several tens of meters, usually with a wide field of view (Figure 6).

Smart warehouses

Smart warehouses are i-warehouses of the future that have a high level of automation of warehouse activities with real-time diagnostics, which enables repair and improvement of warehouse equipment and information technology. Smart warehouses use the integration of the Internet of Things (IoT), artificial intelligence, machine and deep learning, and AMRs. IoT sensors in smart warehouses enable real-time inventory tracking via RFID, reducing stockouts by 20 % (Wang et al., 2015). AI algorithms optimize storage layouts dynamically. (Figure 5.)

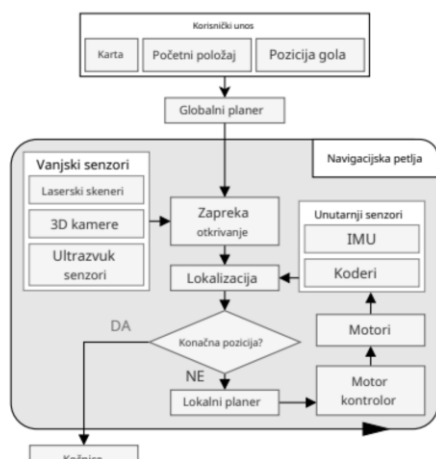


Fig 4. AMR navigation behavior in the presence of obstacles
(Source: Gibas, 2021.)

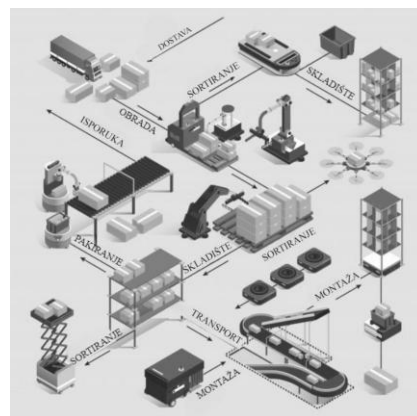


Fig 5. i-warehouse operation scheme
(Source: Bizbloqs, 2021)

Results and Discussion

According to Gondchawar et al. (2016), by using new technologies in warehousing, operating costs could be reduced by 30% in the next few years, and inventory losses could be reduced by up to 75%. Also, inventory holding costs usually range from 20 - 40% of the value of inventory. This finding is consistent with research by Alicka et al. (2017), which states that by using new technologies in warehouses, costs can be reduced by up to 30%, and inventory losses by up to 75%. According to Hollnagel and Woods (2005), automation will not achieve its full potential if users do not adopt it appropriately, while Negrello et al. (2020) also clarify the same issue of acceptance by mentioning that the acceptance of automation depends on culture and nation.

Conclusion

The implementation of warehouse automation significantly streamlines logistics operations within the agricultural sector. The integration of automated warehouse systems and AMRs into storage facilities enhances smart agricultural practices. These systems are among the most innovative automated solutions currently available in the agriculture. As robotic technology and automation continue to evolve, the emergence of smart warehouses that optimize business operations is becoming increasingly prevalent in this sector. The demand for intelligent warehouses is evident across various industries, including agriculture. Specifically for smart warehouses, the adoption of AMRs offers significant contributions by enabling dynamic and flexible material handling, optimizing storage space utilization through intelligent navigation, and improving overall operational efficiency by reducing manual labor and minimizing errors. Furthermore, AMRs in smart warehouses can facilitate real-time inventory management and order fulfillment, leading to more responsive and agile supply chains within the agricultural domain.

References

- Alicke, K., Rexhause, D., Seyfert, A. (2017): Supply chain 4.0 in consumer goods.
- Berg, J. (2007): Integral Warehouse Management: The Next Generation in Transparency, Collaboration and Warehouse Management Systems.
- Bizbloqs (2021): What is a warehouse management system? Retrieved from www.bizbloqs.com/what-is-a-warehouse-management-system/, last visit April 25. 2025
- Culler, D., Long, J., (2016): A Prototype Smart Materials Warehouse Application Implemented Using Custom Mobile Robots and Open Source Vision Technology Developed Using EmguCV. *Procedia Manufacturing*.
- Custodio, L., Machado, R., (2020): Flexible automated warehouse: a literature review and an innovative framework. *International Journal of Advanced Manufacturing Technology*.
- Daley, S. (2022): Robotics Technology, Available from: <https://builtin.com/robotics>
- De Ryck, M., Versteyhe, M., Debrouwere, F. (2020): Automated guided vehicle systems, state-of-the-art control algorithm and techniques. *Journal of manufacturing systems*.
- Design World Online (2023). SLAM Technology Improves AMR Autonomy. Retrieved from <https://www.designworldonline.com/slam-technology-improves-amr-autonomy>, last visit April 25. 2025
- Fragapane, G.; Hvolby, H.H.; Sgarbossa, F.; Strandhagen, J.O (2020): Autonomous Mobile Robots in Hospital Logistics. In *Proceedings of the Advances in Production Management Systems*.
- Garc, R. (2017): Skladišni sustav kao logistički sustav poduzeća, Završni rad, Veleučilište u Požegi. User Guide for MiR100 Autonomous Mobile Robots. Available online: https://gibas.nl/wp-content/uploads/2021/01/mir100-user-guide_31_en.pdf. last visit April 25. 2025
- Gondchawar, N., & Kawitkar, R. S. (2016): IoT based smart agriculture. *International Journal of advanced research in Computer and Communication Engineering*, 5(6), 838-842.
- Hollnagel, E., & Woods, D. D. (2005): Joint cognitive systems: Foundations of cognitive systems engineering. CRC press
- Iqbal, J., Islam, R.U., Abbas, S.Z., Khan, A.A., Ajwad, S.A. (2016): Automating industrial tasks through mechatronic systems – review of robotics in industrial perspective
- Kamali, Ali. (2019): Smart Warehouse vs. Traditional Warehouse - Review. *Automation and Autonomous System* 11
- Khan, MG; Huda, NU; Zaman, UK. (2022): Pametni sustav upravljanja skladištem: Arhitektura, implementacija u stvarnom vremenu i dizajn prototipa.
- Majchrzak, M. (2020): AMR market expands rapidly: The market for autonomous mobile robots (AMRs) is growing fast, and there is a lot of demand globally for them in traditional automation, in non-automotive sectors. *Control. Eng.*
- Le-Anh, T., De Koster, M. (2006): A review of design and control of automated guided vehicle systems. *European journal of operational research*, 171(1), 1-23.
- Min, H. (2023): Smart Warehousing as a Wave of the Future. *Logistics*.
- Min, H. (2022). *The Essentials of Supply Chain Management: Theory and Applications*; Amazon Direct Publishing: Seattle, WA, USA, 2022.
- Moravec, H. (2020): Robotics, Available from: <https://www.britannica.com/technology/robotics>
- Mtg-transform (2016): The 3 Most Common Challenges with Manufacturing Robots. Retrieved from www.cmc-consultants.com/blog/the-3-most-common-challenges-with-manufacturing-robots, last visit April 25. 2025
- Negrello, Francesca, Stuart, Hannah & Catalano, Manuel. (2020): *Hands in the Real World*.
- Oreilly (2025): Second-generation robots. Retrieved from <https://www.oreilly.com/library/view/keras-2x-projects/9781789536645/9ddca1b0-997d-483d-951e-0f88b83c7ee0.xhtml>, last visit April 25. 2025
- Pakter, M. (2021).: Razvoj i primjena robotike, Završni rad, Sveučilište Jurja Dobrile u Puli.
- Vukelić, D. (2020): Primjena robota u skladišnom poslovanju, Diplomski rad, Sveučilište Sjever.

- Wang, Q.; Alyahya, S.; Bennett, N.; Dhakal, H. (2015): An RFID-enabled automated warehousing system. *Int. J. Mater. Mech. Manuf.*
- Tompkins, J., White, J., Bozer, Yavuz., Tanchoco, J.. (2010): *Facilities Planning*.
- Zhen, L., Li, H. (2022): A literature review of smart warehouse operations management. *Frontiers of Engineering Management*, 9(1), 31-55.

Session 6

Youth and Education in Life Sciences

PRELIMINARY COMMUNICATION

Preparation of LSP Teaching Materials in Life Sciences

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Summary

Language for Specific Purposes (LSP) materials are becoming crucial in modern education due to an increase in academic and professional demands. Life sciences students require scientific communication proficiency, alongside their specialized subject area. In order to design LSP teaching materials, linguistic features, discipline-specific content and pedagogical approaches are required. This paper reviews the recent developments in the production of LSP materials for life sciences. A few challenges are presented: scientific knowledge is dynamic and changes constantly, the terminology is specialized, and the context-driven materials are required. Recommendations for the best practice in the future design of LSP material are included, as are the strategies like task-based learning, interactive content and multimedia integration.

Key words: Language for Specific Purposes (LSP), life sciences, task-based learning, authentic scientific texts, multimedia integration

Introduction

Language for Specific Purposes (LSP) implies language teaching for specific purposes in both academic and professional or vocational settings. The LSP materials are designed to help the students who are learning to understand scientific texts. The students are expected to be able to read scientific literature, write scientific papers, and present research findings. The role of LSP in life sciences education has risen in the last years. Scientific research has become more complex and „the ability to communicate in English has become a necessity to collaborate worldwide“ (Baştürkmen and Bocanegra-Valle 2018, p. 18). To create the LSP materials, it is crucial to have knowledge of linguistic theory and a life sciences discipline. This paper will discuss a theoretical framework, challenges, and strategies in the development of LSP materials for life sciences students. Research on language learning with a subject-specific content is also presented. The effective ways of teaching academic English and scientific knowledge are also highlighted. The paper also examines how technological and pedagogical advances affect the effectiveness of LSP resources.

Theoretical Framework for LSP in Life Sciences

In order to teach LSP in life sciences several pedagogical approaches are taken. Hyland (2020) recognizes a genre-based teaching (GBT) as a main way of development of the LSP teaching/learning material. In this approach a student is told how to approach and produce certain types of academic writing, such as research articles, laboratory reports, and conference presentations. Each of these genres has its own structure, terminology, and argumentation techniques, which are of great importance for an effective communication in life sciences (Triastuti, 2022). As stated by Baştürkmen and Bocanegra-Valle (2018) the life sciences LSP materials should combine the language skills with a domain-specific knowledge. This integration is important for the students to be able to write and speak about a scientific field in a detailed way and also in compliance with the rules of a particular discipline. Since life sciences are usually very technical and specialized, the LSP materials for this field do not only have to teach vocabulary, but they also have to explain to the students how language is used to express

scientific concepts and structures. For example, when instructing the students how to compose a research paper or a laboratory report, the teachers will have to address the lexical choices (passive voice, nominalization), as well as the disciplinary conventions (data presentation, argumentation structures).

The latest research has also highlighted the role of a task-based language teaching (TBLT) in the life sciences LSP. According to Van der Branden (2016), a task-based teaching makes the learners confront with the real life-situations they are likely to encounter in their future professions. The tasks like writing research proposals, analyzing experimental data, or designing experiments give the students a chance to use the language structures in lifelike situations. The TBLT also involves critical thinking because the students have to produce the language, solve problems, and make decisions within the context of their scientific field (Jung, 2016).

Challenges in Preparing the LSP Teaching Materials for Life Sciences

The development of LSP materials for life sciences is also characterized by certain typical problems, the major one being the technical language of science. Life sciences texts are characterized by technical vocabulary, complex syntax, and genre-specific features that may pose problems to the students, particularly those who are learning English as a second language (Najmus, 2024). For example, the language used in biology, chemistry, and physics is quite different and sometimes uses different terms to denote the same thing within the same university (Flowerdew, 2018).

Another important issue is a dynamic nature of life sciences research. Every day, new discoveries are made, which influences the terminological and conceptual structure of the discipline, exerting an impact on the language used in teaching and learning processes. It is crucial to update the LSP materials to reflect the current developments to ensure that the material is up-to-date. In addition, the educators have to refresh their respective vocabulary list; what is more, they also have to consider how new research methodologies, or the technological advancements, influence the linguistic features of scientific communication (Fitria, 2020). This evolution means that the LSP materials must be versatile and active and may need to be revised from time to time to reflect a changing world of life sciences (Baştürkmen and Bocanegra-Valle, 2018).

In addition, the life sciences students can have different linguistic and cultural backgrounds, which can make the development of LSP materials complicated. The students may also have the different levels of academic English proficiency and may be on different levels of command concerning their comprehension of scientific matters (Cummins, 2014). Consequently, the materials should be developed with some form of differentiation in order to provide a sort of content progression to help all students, irrespective of their initial learning capability, to be able to comprehend and benefit from a learning experience. This also implies that the materials should provide both the autonomous and guided learning opportunities so that the students can not only learn how to study on their own but also how to work in a group.

The Methods to Prepare Effective LSP Teaching Materials in Life Sciences

To this end, a few strategies can be recommended for the development of LSP materials for life sciences. Among the most common strategies is the use of realistic scientific texts. This makes it possible to introduce the students to the real language of academic writing through such sources as research articles, laboratory reports, and case studies. Therefore, the students are able to encounter the actual linguistic forms and the particular characteristics of academic communication in the life sciences (Baştürkmen, 2014). For example, they can be informed about the structure of a scientific paper, how language is used in scientific writing and how data are presented. Besides, the authentic texts allow the a transfer of learning from a lecture room

to a real-world scientific communication. Another important strategy is the inclusion of multimedia components in the LSP materials (Živić 2023); for instance, interactive simulations, videos, and online resources. Hence, interactive and engaging multimedia components can be used to explain scientific concepts and help the learners, who learn better through visuals, to understand abstract concepts. Reinhard (2019) argued that „using digital tools like interactive 3D models, virtual labs, and other similar tools help students understand the concept of biological processes, chemical reactions, and scientific processes more easily.“ Furthermore, these tools can be simultaneously used for different learners with different learning styles, thus making the learning environment more equitable for all students. Task-based language teaching (TBLT) is another very successful way of developing the LSP materials. In his paper on learning and teaching languages, Van der Branden (2016) pointed out that a task-based teaching requires the students to perform realistic scientific tasks in real language. The tasks such as writing research reports, conducting experiments, analyzing data, and presenting the findings are similar to what the students will be doing in their academic life and working careers. The TBLT is a good way of helping the students to learn academic language and discipline-specific skills, because a learned content is directly related to what the students will be doing in their future careers (Jung 2016). Lastly, cooperative learning can also be applied to enhance the development of LSP materials. In pairs, the students can criticize each other's work, work on group projects, and present a group work to the class, all of which gives the students a way of practicing scientific writing and critical thinking in a group environment. As Flowerdew (2016) points out, collaborative tasks help the students develop their language command, as well as their teamwork and interpersonal skills, which are important both throughout their schooling and in their future professions.

Guidelines for the Development of LSP Teaching/Learning Materials in the Life Sciences

The following are some of the guidelines that should be followed in the development of LSP teaching materials for the life sciences. Swales (2019) claims that „the materials should be based on real scientific data which is relevant to the future professional lives of the students. Besides the traditional tasks, such as reading and writing, other tasks, which simulate the real scientific activity, like data analysis and laboratory work should also be applied.“ Furthermore, the materials should be versatile to meet the needs of different students, with different language abilities and knowledge of the content to be learned. Some of the techniques that can be used imply the usage of glossaries, summaries, and guided questions to make complex content more understandable. In addition, the materials should include various multimedia resources and interactive elements that can improve the understanding of difficult concepts and engage the students actively (Fitria, 2020). For instance, the use of videos to explain how to do a laboratory experiment or three-dimensional models to explain a biological process would be very helpful. This paper also suggests that collaborative tasks should be incorporated in the curriculum to help students develop their communication skills and work in groups. Finally, feedback and assessment should be continuous in the development of LSP materials. As Douglas (2012) suggests, formative assessments including peer reviews, quizzes, and group discussions should be used to provide immediate feedback to the students to help them improve their language skills and understanding of the content. The final assessments should mimic real life—tasks, for instance, writing a research proposal or presenting scientific findings to determine language competence, as well as the discipline expertise.

Conclusion

The development of LSP teaching materials for the life sciences is not straightforward and is still evolving, which makes it necessary to consider linguistic and disciplinary aspects. In order to design effective LSP materials, language learning has to be linked with content-area learning, which involves using real scientific texts, technological aids, and task-based learning strategies. The issues such as technical terms, new scientific knowledge, and students' diversity are continually evolving, and hence the teaching materials need to be updated and altered regularly. The LSP materials can be developed to the level of language and scientific literacy that will enable the students to gain the necessary skills for further academic and professional development in the field of life sciences using the best practices and the latest pedagogical technologies.

References

- Baştürkmen, H. (2014). LSP teacher education. *Ibérica*. (28): 17-34 <https://www.revistaiberica.org/index.php/iberica/article/view/237>
- Baştürkmen, H., Bocanegra-Valle, A. (2018). Materials Design Processes, Beliefs and Practices of Experienced ESP Teachers in University Settings in Spain. In: Kırkgöz, Y., Dikilitaş, K. (eds) *Key Issues in English for Specific Purposes in Higher Education*. English Language Education. Springer, Cham. 11: 13-27 https://doi.org/10.1007/978-3-319-70214-8_2
- Cummins, J. (2014). Beyond language: Academic Communication and student success. *Linguistics and Education*. 26: 145-154. <https://doi.org/10.1016/j.linged.2014.01.006>
- Douglas, D. (2012). ESP and assessment. *The handbook of English for specific purposes*, Paltridge B., Starfield S. (ed.): 367-383. <https://doi.org/10.1002/9781118339855.ch19>
- Fitria, T. N. (2020). Teaching English for Specific Purposes (ESP) to the Students in English Language Teaching (ELT). *Journal of English Teaching*. 5(1): 55-66 <https://doi.org/10.36456/jet.v5.n01.2020.2276>
- Flowerdew, J. (2016). English for Specific Academic Purposes (ESAP) Writing. *Writing & Pedagogy*. 8(1): 1-4. DOI: 10.1558/wap.v8i1.30077
- Hyland K. (2024) Ken Hyland's essential bookshelf: Academic writing. *Language Teaching*. 57(3): 399-407. doi:10.1017/S0261444822000118
- Jung Y., Long, M. (2016): Second Language Acquisition and Task-Based Language Teaching. **Applied Linguistics**. 37(3): 438–441 <https://doi.org/10.1093/applin/amv074>
- Najmus S., Rakhshinda J. (2024). Challenges and Predicaments in Teaching “English for Science” in an ESP Classroom: A Research Study to Pinpoint Problems and Solutions. *International Journal of English Language Teaching*. 12(1): 80-89. DOI: 10.37745/ijelt.13/vol12n18089
- Reinhard, J. (2019) Social Media in Second and Foreign Language Teaching and Learning: Blogs, Wikis, and Social Networking. **Language Teaching**, 52(1): 1–39. <https://doi.org/10.1017/S0261444818000356>.
- Swales, J. M., Feak, C. B. (2023) Task evolution in English for Academic Purposes writing materials: The case of “Information Transfer” to “Critical Commentary”. *Journal of Second Language Writing*. 61: 101017. <https://doi.org/10.1016/j.jslw.2023.101017>
- Triastuti, Anita, et al. (2022) Genre-Based Teaching Cycle and Instructional Design for Teaching Texts and Mandated Curriculum Contents. *Indonesian Journal of Applied Linguistics*. 2(1): 1-15. doi:10.17509/ijal.v12i1.46563
- Van der Branden K. (2016) Task-Based Language Teaching. In *The Routledge Handbook of English Language Teaching*, Hall G. (ed.), chapter 17, London, UK. <https://doi.org/10.4324/9781315676203>
- Živić, Tihomir (2023) Multimedia Toolset Deployment in the English-Course Presentation of Digital Agriculture. Second International Conference on Advances in Science and Technology: Proceedings COAST 2023, Djordje Jovanović et al. (ed.), Fakultet za menadžment Herceg Novi: 1361-71.

EXPERT PAPER

Student Engagement Beyond the Classroom through the Gardening Group at the University of Zagreb

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Summary

The University of Zagreb Faculty of Agriculture has recognized the importance of formalizing and acknowledging student participation in extracurricular activities, which are officially recorded in the diploma supplement. Among the various forms of engagement, research-specialist groups play an important role. The aim of this paper is to provide a brief review of the Gardening Group as an example of good practice. This extracurricular activity complements academic learning with practical experience in horticulture, sustainability, and community engagement, enabling students to develop both key and specialized competencies, such as working with children.

Key words: extracurricular activities, higher education, Faculty of Agriculture, key competencies

Introduction

In the context of higher education, extracurricular activities (ECA) are structured, voluntary activities that take place outside the formal academic curriculum but are supported or organised by the institution. Although they are usually not credited, they play a crucial role in students' overall development as they provide them with the opportunity to gain practical experience, build social networks and acquire transferable skills (Thompson et al., 2013; Clark et al., 2015; Tran, 2017). While academic education remains the foundation of university learning, extracurricular engagement significantly enriches the student experience by fostering personal initiative, collaboration, and the development of interdisciplinary perspectives (Brown-Liburd and Porco, 2011; Kotlar and Čulum, 2014).

In today's increasingly competitive job market, students need to demonstrate value beyond their academic achievements in order to improve their employability (Chapman et al., 2023; Ribeiro et al., 2023). Employers, both internationally and in Croatia, are increasingly recognizing the importance of skills and experience gained outside the curriculum. In many cases, these additional qualities become crucial when selecting applicants, especially if the academic qualifications are similar. This is also relevant when considering that many graduates pursue careers outside of their academic field, making general competencies such as teamwork, communication, leadership and critical thinking particularly important (Thompson et al., 2013; Clark et al., 2015; Tran, 2017). In addition, they provide hands-on learning experiences that are not always accessible in traditional courses and help bridge the gap between theoretical knowledge and real-world applications (Chapman et al., 2023; Javed and Srivastava, 2024).

Kotlar and Čulum (2014) argue that current education systems often fail to adequately prepare students for modern societal and professional challenges, citing deficiencies in the areas of teamwork, project-based learning, risk assessment and lifelong learning strategies. In response, many higher education institutions are developing frameworks to recognize and formally validate extracurricular engagement (Thompson et al., 2013; Tran, 2017). This shift reflects broader educational policy trends, including those promoted by the European Union that

emphasize stronger links between education, research and the labor market (EACEA, 2024). According to Ribeiro et al. (2023) and Javed and Srivastava (2024), higher education institutions should actively promote learning experiences that extend beyond curricular objectives, as extracurricular activities contribute to a more holistic development of students by supporting both mental health and a sense of belonging within the academic community.

Extracurricular activities at the University of Zagreb Faculty of Agriculture

The University of Zagreb, including its Faculty of Agriculture (FAZ), recognizes the importance of ECA as an integral part of higher education (UNIZG, 2024; AGR, 2025). In response to the growing involvement of students in activities outside the formal curriculum, the FAZ has formally introduced regulations for the recognition of ECAs (AGR, 2015). This institutionalization was a natural consequence of increasing student engagement through the years and the need to provide a structured framework for the recognition of such contributions. Following a comprehensive analysis of student involvement in extracurricular activities conducted in the 2013/2014 academic year, the FAZ began to officially record recognized ECAs in the diploma supplement from the 2014/2015 academic year. The types of activities eligible for recognition include various academic awards (such as the Rector's Award, Dean's Award and Academic Excellence Awards), involvement in teaching and student support roles (e.g. demonstrators and student tutors), participation in registered student associations, contributions to the organization of academic and professional events, faculty promotional activities and involvement in research-specialist groups. There are currently 23 officially recognized research-specialist groups at the FAZ, of which 19 were active in the 2023/2024 academic year, involving 17 departments and a total of 142 students (Table 1).

Table 1. Research-specialist groups at FAZ as part of extracurricular activities

Group	Department	Number of students enrolling in 2023/2024
3D modeling	Information Science and Mathematics	4
Agrochemical group	Chemistry	5
Agroenergetic group	Sustainable Technologies and Renewable Energy Sources	6
Ampelographic group	Viticulture and Enology	5
Art in phytomycology	Plant Pathology	4
Botanical group	Agricultural Botany	10
Entomological group	Agricultural Zoology	7
Field crops group	Field Crops, Forage and Grassland	3
Gardening group	Vegetable Crops	11
Greening of common interiors	Ornamental Plants, Landscape Architecture and Garden Art	8
Group focusing on data in agriculture	Animal Science	11
Hunting group	Fisheries, Apiculture, Wildlife management and special Zoology	17
Intriguing World of Weeds	Weed Science	6
Mechanization group	Mechanization and Autonomous Systems in Agriculture	10
Pomology group	Pomology	8
Research group for grape and wine analysis	Viticulture and Enology	10
Research group for meat processing	Animal Science and Technology	6
Small student distillery	Viticulture and Enology	5
Wine group	Viticulture and Enology	6

This reflects the strong interest of students and the growing popularity of this form of extracurricular engagement (AGR, 2025). The activities undertaken in these groups are outside the formal academic workload of enrolled courses and provide valuable opportunities for students to develop both transferable and subject-specific key competencies. These experiences allow students to deepen and apply the knowledge gained in formal education in a practical, collaborative and innovative context. The Faculty of Agriculture provides institutional support through dedicated funding for research-specialist groups, thereby enabling the smooth implementation of planned activities without financial limitations. All students participating in these activities can apply for formal recognition of their acquired competencies by completing the Student Extracurricular Activities Application Form. The application must be submitted with the approval of the activity supervisor and forwarded to the Extracurricular Activities Committee along with the required supporting documentation.

The Gardening group

An example of good practice among the activities of the research-specialist groups is the Gardening Group (*Vrtlarska grupa*). This initiative provides students with hands-on experience in horticulture, sustainability and community engagement. Formally established in the 2016/2017 academic year, the group's roots go back further. It grew out of a student-led initiative that involved volunteer gardening at children's homes and kindergartens, as well as participation in faculty promotional events. This organic development shows how extracurricular activities can arise from the genuine interests of students and the needs of the local community.

The group is active on a weekly basis, with approximately 20 hours per semester in the winter and 40 hours in the summer. To be eligible for official credit on the diploma supplement, students must participate in at least 80 % of the activities. Supervision by academic staff from the Department of Vegetable Crops ensures structured, consistent and guided skills development. Additionally, the group coordinators continuously engage in professional development related to pedagogy and student engagement, participating in workshops organized by the *Forum for Freedom in Education* and webinars provided by the *University Computing Centre*. At the end of each academic year, a detailed report on the group's activities and outcomes is submitted to the Faculty's Extracurricular Activities Committee, which evaluates the quality and effectiveness of the activities, including the acquisition of key competences by the students. Group activities go beyond classroom learning and promote the integration of academic knowledge with practical experience and community engagement (Figure 1, a–d). Practical tasks include planning and maintaining vegetable gardens with aromatic and medicinal herbs in kindergartens and elementary schools in Zagreb County and beyond (a), harvesting and drying medicinal plants for the production of essential oils, herbal tea blends and scented sachets, composting, growing lesser-known vegetables and microgreens; preparing juices from wild and cultivated medicinal plants and producing natural cosmetic products (b). Other activities include candying edible flowers, growing grafted vegetable seedlings (c) and organizing educational programs for children in kindergartens and science festivals (d), as well as participating in faculty promotional events. The group provides a platform to apply the knowledge acquired in class to real-life contexts while strengthening teamwork, communication and problem-solving skills. Collaboration with kindergartens, elementary schools and retirement homes encourages civic engagement and social responsibility among students. The activities also give students an insight into vegetable production that goes beyond basic growing techniques and they are encouraged to consider the entire value chain from cultivation to the creation of value-added products.



Figure 1. Some of the activities of the Gardening group: maintaining vegetable gardens with aromatic and medicinal herbs in kindergarten (a); making of natural cosmetics (b); grafting vegetable seedling (c); educational workshop for children at the Science festival (d)

Student participation varies from year to year. Table 2 provides an overview of enrollment numbers and participants who have met the threshold for diploma supplement recognition. In years when more than 15 students enroll, enrollment is capped on a first-come, first-served basis, with 10 participants considered optimal for effective implementation. However, initial enrollment is often higher, with a slight decrease expected by the summer semester.

Table 2. Student participation in the Gardening Group by academic year

Academic year	Number of enrolled students	Number of students acquired diploma supplement
2017/2018	14	14
2018/2019	6	6
2019/2020	13	12
2020/2021	8	6
2021/2022	6	2
2022/2023	13	9
2023/2024	11	5

Despite its success, the group faces some challenges. Attendance tends to be higher in the winter semester, while engagement declines in the summer, likely due to increased academic commitments such as more practical classes and internships. There are also scheduling conflicts between classes and Gardening Group activities, a problem that Thompson et al. (2013) point out in general. However, these challenges are effectively overcome through transparent communication, structured planning and strong faculty support.

The Gardening Group also encourages interdisciplinary collaboration as students from different degree programs regularly participate. Notably, many interested students are not enrolled in the Horticulture program, demonstrating the cross-disciplinary appeal and integrative nature of well-designed extracurricular activities.

The structure of extracurricular activities such as the Gardening Group is intentionally designed to deviate from the standard academic curriculum, focusing on the development of key competencies that complement and enhance formal education. Some of these skills, formally listed in the diploma supplement include building new social relationships, coping with unfamiliar situations, applying theoretical knowledge in practical contexts, translating ideas into practice, encouraging creativity and innovation, and contributing effectively to group dynamics. In addition to these general skills, the diploma supplement also recognises specific competencies related to the focus of the group, such as techniques for making natural cosmetics

(e.g. soaps, balms, tinctures), techniques for planting in aromatic gardens, grafting vegetables (e.g. watermelon and melon), sowing and transplanting vegetables and medicinal plants, making seed bombs with seeds of aromatic and medicinal plants, and conducting educational workshops for children.

Conclusions

Extracurricular activities should be considered an essential part of higher education because they provide a variety of benefits beyond academic achievement. The Gardening Group is a compelling example of how a well-designed extracurricular activity can foster the development of various skills that enrich academic learning and contribute to lifelong learning, career preparation, and community engagement while enhancing students' academic identity and personal growth.

References

- AGR (2025). Sveučilište u Zagrebu Agronomski fakultet. <https://www.agr.unizg.hr/hr/group/121/Izvannastavne+aktivnosti> - accessed on Feb 25 2025.
- Brown-Liburd, H. L., Porco, B. M. (2011). It's what's outside that counts: Do extracurricular experiences affect the cognitive moral development of undergraduate accounting students?. *Issues in Accounting Education*, 26(2): 439-454.
- Chapman, G., Emambocus, W., Obembe, D. (2023). Higher education student motivations for extracurricular activities: evidence from UK universities. *Journal of Education and Work*, 36(2): 138-152.
- Clark, G., Marsden, R., Whyatt, J. D., Thompson, L., Walker, M. (2015). 'It's everything else you do...': Alumni views on extracurricular activities and employability. *Active learning in higher education*, 16(2): 133-147.
- EACEA (2024). European Commission Eurydice. Validation of non-formal and informal learning in higher education in Europe. Eurydice report. Luxembourg: Publications Office of the European Union.
- Javed, I., Srivastava, A. K. (2024). An Analysis of Role of Extracurricular Activities (ECA) in Higher Education. *Revista Review Index Journal of Multidisciplinary*, 4(1): 66-73.
- Kotlar, V., Čulum, B. (2014). Vrednovanje izvannastavnih aktivnosti: pogled iz studentske perspektive. *Suvremene teme: međunarodni časopis za društvene i humanističke znanosti*, 7(1): 30-55.
- Ribeiro, N., Malafaia, C., Neves, T., Menezes, I. (2023). The impact of extracurricular activities on university students' academic success and employability. *European Journal of Higher Education*, 14(3), 389–409. <https://doi.org/10.1080/21568235.2023.2202874>
- Thompson, L. J., Clark, G., Walker, M., Whyatt, J. D. (2013). 'It's just like an extra string to your bow': Exploring higher education students' perceptions and experiences of extracurricular activity and employability. *Active Learning in Higher Education*, 14(2): 135-147.
- Tran, L. H. N. (2017). Developing employability skills via extra-curricular activities in Vietnamese universities: Student engagement and inhibitors of their engagement. *Journal of Education and Work*, 30(8): 854-867.
- UNIZG (2024). University of Zagreb. Pravilnik o dodjeli i priznavanju bodova prema ECTS-u izvannastavnim aktivnostima Sveučilišta u Zagrebu. Zagreb, Croatia.

REVIEW ARTICLE

Molecular insights into gene expression in dairy cattle: Techniques and trends

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Summary

Gene expression is a complex biological process influenced by both genetic and environmental factors, involving a series of biochemical reactions. Production traits are significantly affected by gene expression, and understanding these mechanisms allows for production optimization, reduced disease occurrence, improved reproduction, and overall enhancement of production quality. Whole-genome sequencing enables the identification of individual gene functions within the genome and their role in the production performance of an individual. By regulating gene expression, cells can precisely control the production of functional proteins, ensuring optimal physiological conditions. The main methods used to study gene expression in livestock include microarray hybridization, RNA sequencing (RNA-Seq), and real-time PCR (qPCR). Microarrays allow many genes to be analyzed at once using special cDNA chips. RNA-Seq measures the activity of all genes in a sample and gives a detailed picture of gene expression. Because of its accuracy and wide range of uses, RNA-Seq is now considered the most powerful tool for gene expression analysis.

Key words: gene expression, RNA-Seq, cattle, milk production

Introduction

In intensive dairy cattle systems, the primary objective is to achieve adequate milk fat and protein quality along with a proportionally high daily milk yield. Milk production is a technologically complex process involving the interaction between cattle, the production environment, and humans, with its outcomes being influenced by numerous genetic and environmental factors that determine the expression of an individual's genetic potential. Gene expression is the process by which genetic information is translated into functional molecules, such as RNA and proteins, enabling cells to perform specific functions, and it is influenced by both genetic and non-genetic factors (Cassar-Malek et al., 2008). This involves the transcription of DNA into RNA and the subsequent translation of RNA into proteins. Gene expression regulation is crucial for cell differentiation and organism development, and plays a significant role in regulating physiological and production traits, including the nutritional composition of milk and lactation parameters (Gao et al., 2013). Although gene expression data is not routinely incorporated into genomic evaluations, it is highly valuable in scientific research for identifying and classifying genomic sequences associated with production and reproductive traits, thereby providing more accurate information on specific genes and traits to enhance breeding programmes (Fang et al., 2025).

The connection between gene expression and milk traits of cows

Towards the end of the last century and the beginning of this one, molecular biology emerged as a key research area in livestock breeding (Cassar-Malek et al., 2008). Moving beyond mere identification of DNA sequences has enabled a more precise definition of gene functions at the whole-genome level. Organizing sequences into functional groups is based on their expression, which lays the foundation for further experiments aimed at the detailed characterization of the function of the final gene product (Moody, 2001). Gene expression is determined by both genetic and environmental factors. The efficiency of gene expression research is achieved by analyzing phenotypic variability within and between breeds, as well as by comparing animals with significant deviations in traits such as growth dynamics, body condition, and production quality parameters. Genetic research has significantly contributed to the understanding of numerous physiological processes and organ functions (Cassar-Malek et al., 2008). A large number of gene expression studies have been conducted in the field of dairy cattle production, leading to the key insights. According to Gao et al. (2013), the mammary gland is the only organ that, after the maturation process, undergoes cycles of proliferation and involution, characterized by phases of increased cell growth followed by eventual cell regression. Epithelial cells of the mammary gland are characterized by their ability to produce and secrete milk (Canovas et al., 2014). Understanding the molecular processes in these cells can significantly improve management and breeding technologies for dairy cattle.

Breeding programs have significantly influenced the metabolic balance of dairy cows, with the aim of achieving high milk production driving nutritional demands far beyond the animals' natural intake capacity (McCabe et al., 2012). This process has resulted in a disturbance of the physiological status known as negative energy balance (NEB), a physiological condition with distinctly adverse effect on animal health and fertility, further highlighting its economic importance in milk production (McCabe et al., 2012). Like most mammals, dairy cows enter a period of negative energy balance shortly after calving, as their nutrient requirements during lactation exceed their intake from food (Lor et al., 2006). After calving, the transition from colostrum to milk production involves major changes in gene expression in the mammary gland. These changes play an important role in starting and supporting lactation. During this period, called secretory activation, genes responsible for lactose production become more active. Notably, the increased expression of LALBA and B4GALT1 genes is positively correlated with higher milk and lactose production (Shangraw and McFadden, 2024). Increased expression of several genes is commonly observed during the periparturient period, although this pattern may not always be consistent. The transcriptomic profile of mammary secretory cells provides direct insights into key genes that may act as limiting factors for high milk production (Shangraw and McFadden, 2024).

Thus, in the studies by Bionaz and Lor (2011), increased expression of the LALBA, CSN3, FASN, and LPIN1 genes was observed in the mammary gland 15 days after calving, compared to the prepartum period. It is known that increased milking frequency during the postpartum period positively affects milk yield, particularly during the secretory activation phase (Wall and McFadden, 2007). By measuring gene expression during the period of intensive milking, key genes responsible for lactose biosynthesis and increased milk production could be identified (Shangraw and McFadden, 2024). The discovery of transcriptomic profiles of mammary gland epithelial cells (MEC) during lactation is crucial for identifying candidate genes associated with milk production traits (Yang et al., 2015). In addition to MECs, mammary gland tissue also includes myoepithelial and mesenchymal cells. As a result, RNA isolated from mammary epithelial cells (MECs) may not accurately reflect the complete gene expression profile of these cells. Given that milk fat globules (MFGs) are derived exclusively from MECs, RNA obtained from MFGs offers a more reliable representation of gene expression in the mammary epithelium (Maningat et al., 2009). Research on gene expression during lactation using MFGs represents

a simpler alternative to MEC analysis. MFGs can be easily collected at any time during lactation, whereas mammary gland tissue biopsy is invasive, costly, and disrupts the natural course of lactation (Yang et al., 2015). A significant number of differentially expressed genes have been identified using RNA-seq sequencing. In cows with higher milk, fat, and protein yields at peak production, a large proportion of these genes exhibited lower expression. Specifically, 91.01% of the differentially expressed genes in a specific interval showed lower expression during peak production (Yang et al., 2015). The findings of Cui et al. (2014), studying the differential gene expression in the mammary gland tissue of cows with extremely high and low fat and protein content, align with the findings of Yang et al. (2015), further supporting the hypothesis of a connection between gene expression dynamics and milk containing higher fat and protein levels. Using RNA-seq sequencing technology, Yang et al. (2015) investigated differential gene expression in MFGs of lactating cows with significantly different production outcomes.

Experimental methods for gene expression analysis

The regulation of gene expression enables cells to adapt to different conditions and ensures that the synthesized functional product, usually a protein, is produced at the right time and in appropriate amounts. Numerous methodologies are available for gene expression analysis, with microarray hybridization, RNA sequencing (RNA-Seq), and real-time quantitative PCR (qPCR) being the most commonly used as highly reliable (Sun et al, 2012).

The microarray hybridization method allows the simultaneous analysis of gene expression for a large number of genes and is divided into three main types: 1) oligonucleotide chips, which are created by synthesizing oligonucleotides directly onto a glass slide; 2) oligonucleotide chips, which are made by placing previously synthesized oligonucleotides onto glass slides or nylon membranes; and 3) cDNA chips, which are created by inserting PCR-amplified inserts from cDNA library copies onto glass slides or nylon membranes (Moody, 2001). Hybridization-based approaches usually involve the incubation of fluorescently labeled cDNA with microarrays which can be either custom-made or commercially available high-density oligonucleotide microarrays (Clark et al., 2002.).

Moody (2001) conducted an experiment using two mRNA samples to demonstrate the fundamental principles of the microarray hybridization technique. In this procedure, messenger RNA (mRNA) from both samples is reverse transcribed into complementary DNA (cDNA) and labeled with fluorescent markers. The newly formed cDNA is hybridized onto a microarray containing sequences from thousands of genes. The hybridization signal, once detected is proportional to the amount of mRNA transcript in the original sample. By comparing the hybridization signals of individual genes across different samples, differences in gene expression can be identified (Moody, 2001). The analysis of microarray data typically involves three main steps: 1) the identification and quantification of hybridization intensity, 2) data visualization for pattern recognition, and 3) the application of clustering techniques to classify gene expression profiles. However, hybridization-based methods face several limitations, such as the requirement for prior knowledge of the target genome sequences, high background noise which arises from cross-hybridization, and a dynamic range which is restricted to some extent for detecting gene expression across various transcript levels (Ciaramella and Staiano, 2019). Finally, comparing expression levels between different experiments may require complex normalization processes for results and data (Wang et al., 2009).

Real-time quantitative PCR (qPCR) is a molecular technique that enables the simultaneous amplification and quantification of target DNA in real time (Pryor and Wittwer, 2006). This method quantifies the initial amount of target DNA in a sample by monitoring fluorescence during the PCR reaction, enabling for both absolute and relative quantification (Singh and Roy-

Chowdhuri, 2016). Owing to its high sensitivity and specificity, qPCR is extensively employed in genetic analyses of specific genes, genotyping, gene expression profiling, and the identification of polymorphisms and mutations. The primary limitation of qPCR lies in its dependence on prior knowledge of target sequences, which prevents its use in the discovery of novel genes (Nola et al., 2006). One of the main limitations of qPCR is its high sensitivity to sample contamination and primer-dimer formation, which can result in inaccurate outcomes. Consequently, its dynamic range is narrower than that of sequencing-based methods, making it less effective for detecting extremely low or high expression levels across a wide array of genes (Smith and Osborn, 2009). *RNA-Seq* is a powerful high-throughput sequencing approach that allows comprehensive and quantitative profiling of the entire transcriptome (Wang et al., 2009). It relies on next-generation sequencing technologies to convert RNA molecules into complementary DNA (cDNA) fragments, which are then sequenced and mapped to a reference genome. One of the key advantages of *RNA-Seq* is its high sensitivity and low background noise, as reads can be uniquely assigned to specific genomic regions. Unlike hybridization-based methods, *RNA-Seq* offers a broad dynamic range of detection, with expression levels directly reflecting the number of sequence reads per transcript. This enables both the detection of highly expressed genes and the identification of transcripts with low abundance. As the technology advances, future developments are expected to improve the resolution of complex transcriptomes, particularly in distinguishing alternative splicing events and rare RNA isoforms (Wang et al., 2009).

Conclusions

Gene expression is a complex mechanism influenced by both genetic and environmental factors. Understanding the expression of individual genes can lead to improvements in production efficiency by optimizing genetic pathways that enhance desirable traits. In addition to production outcomes, understanding and controlling gene expression can improve the health condition of animals, their fitness, and reduce the frequency of certain diseases. Over the past two decades, numerous experimental methods for gene expression analysis have been developed, significantly influencing breeding programs. These methods enable more precise quantification and comparison of gene expression with high sensitivity. They vary in their analytical approaches, sample types and the quantity of genes studied. Among all methods, *RNA-Seq* stands out due to its comprehensive approach and precise data processing. As technological advancements and bioinformatics tools continue to evolve, transcriptome analysis will become even more refined, potentially leading to the integration of new gene expression insights into breeding programs for livestock species.

References

- Bionaz, M., Looor J.J. (2008). Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics*, 31(9): 366.
- Bionaz, M., Looor, J.J. (2011.) Gene networks driving bovine mammary protein synthesis during the lactation cycle. *Bioinformatic and Biology Insights*, 5: 83-98.
- Canovas, A., Rincon, G., Bevilacqua, C., Islas-Trejo, A., Brenaut, P., Hovey, R.C., Boutinad, M., Morgenthaler, C., VanKlombenberg, M.K., Martin, P., Medrano, J.F. (2014). Comparasion of five different RNA sources to exmaine the lactating bovine mammary gland transcriptome using *RNA-Sequencing*. *Scientific Reports*, 4, 5297.
- Cassar-Malek, I., Picard, B., Bernard, C., Hocquette, J.-F. (2008). Application of gene expression studies in livestock production systems: a European perspective. *Australian Journal of Experimental Agriculture*, 48: 701-710.
- Ciaramella, A., Staiano, A. (2019). The Role of Clustering and Visualization Techniques in Gene Microarray Dana. *Algorithms*, 12(6): 123.

- Clark, T.A., Sugnet, C.W., Ares, M Jr. (2002). Genomewide analysis of mRNA processing in yeast using splicing-specific microarrays. *Science*, 3, 296(5569): 907-10.
- Cui, X., Hou, Y., Yang, S., Xie, Y., Zhang, S., Zhang, Y., Zhang, Q., Lu, X., Liu, G.E., Sun, D. (2014). Transcriptional profiling of mammary gland in Holstein cows with extremely different milk protein and fat percentage using RNA sequencing. *BMC Genomics*, 15: 226.
- Fang, L., Teng, J., Lin, Q. *et al.* (2025). The Farm Animal Genotype–Tissue Expression (FarmGTEx) Project. *Nature Genetics*, 57: 786–796.
- Gao, Y., Lin, X., Shi, K., Yan, Z., Wang, Z. (2013). Bovine mammary gene expression profiling during the onset of lactation. *PLoS One* 8.
- Loor, J.J., Dann, H.M., Guretzky, N.A., Everts, R.E., Oliveira, R., Green, C.A., Litherland, N.B., Rodriguez-Zas, S.L., Lewin, H.A., Drackley, J.K. (2006). Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. *Physiol Genomics*, 3, 27(1): 29-41.
- Maningat, P.D., Sen, P., Rijnkels, M., Sunehag, A.L., Hadsell, D.L., Bray, M., Haymang, M.W. (2009). Gene expression in the human mammary epithelium during lactation: the milk fat globule transcriptome. *Physiological Genomics*, 3, 3(1): 12-22.
- McCabe, M., Waters, S., Morris, D., Kenny, D., Lynn, D., Creevey, C. (2012). RNA-seq analysis of differential gene expression in liver from lactating dairy cows divergent in negative energy balance. *BMC Genomics*, 13: 193.
- Moody, D.E. (2001). Genomics techniques: An overview of methods for the study of gene expression. *Journal of Animal Science*, 79: 128–135.
- Nolan, T., Hands, R.E., Bustin S.A. (2006). Quantification of mRNA using real-time RT-PCR. *Nature Protocols*, 1(3): 1559-82.
- Pryor, R.J., Wittwer, C.T. (2006). Real-time polymerase chain reaction and melting curve analysis. *Methods in Molecular Biology*, 336: 19-32.
- Shangraw, E.M., McFadden, T.B. (2024). Regulation of gene expression in milk secretory pathways during secretory activation in Holstein cows. *Journal of Dairy Science*, 107(12): 11761-11773.
- Singh, C., Roy-Chowdhuri, S. (2016). Quantitative Real-Time PCR: Recent Advances. *Methods in Molecular Biology*, 1392: 161-76.
- Smith, C.J., Osborn, A.M. (2009). Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiology Ecology*, 67(1): 6-20.
- Sun, Z., Kuczek, T., Zhu, Y. (2012). Statistical Calibration of qRT-PCR, Microarray and RNA-Seq Gene Expression Data with Measurement Error Models. *The Annals of Applied Statistics*, 8(2).
- Wall, E.H., McFadden, T.B. (2007). The milk yield response to frequent milking in early lactation of dairy cows is locally regulated. *Journal of Dairy Science*, 90(2): 716-20.
- Wall, E.H., McFadden, T.B. (2012). Triennial Lactation Symposium: A local affair: How the mammary gland adapts to changes in milking frequency. *Journal of Animal Science*, 90(5): 1695-707.
- Wang, Z., Gerstein, M., Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Review Genetics*, 10(1): 57-63.
- Yang, J., Jiang, J., Liu, X., Wang, H., Guo, G., Zhang, Q., Jang, L. (2015). Differential expression of genes in milk of dairy cattle during lactation. *Animal Genetics*, 47(2): 174-80.

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