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EDITORIAL BOARD

CONTENT

IN HONOUR OF THE 25th ANNIVERSARY
OF THE PUBLISHING ACTIVITY OF THE FACULTY OF AGRICULTURE,
GOCE DELCEV UNIVERSITY – STIP

The Publishing activity of the Faculty of Agriculture has a profound tradition and own specific history, more than two decades old. Volume 23, Number 1 of the Journal of Agriculture and Plant Science, is dedicated to the 25-years publishing tradition of the Faculty of Agriculture, Goce Delcev University - Stip.

The beginnings of the Publishing activity of the Faculty of Agriculture date back to 2000 with the release of the Collection of Abstracts 1990-2000 of, in that time, Public Scientific Institution Institute for Southern Agricultural Crops - Strumica. From 2001 to 2006, the institute continued its publication activities through publication of the Annual Yearbooks of the Institute for Southern Agricultural Crops - Strumica.

The Institute for Southern Agricultural Crops - Strumica was merged with Goce Delcev University as part of the Faculty of Agriculture following the law on the establishment of the State University Goce Delcev - Stip, adopted by the Parliament of the Republic of Macedonia on March 27, 2007. Since then, the goals of the Faculty of Agriculture have been based on the long-standing experience and rich tradition of our Macedonian agricultural production. Therefore, it is understandable that we have continued to nurture the rich tradition through specific educational, research and publishing activities.

The first issue of the Annual Yearbook of the Faculty of Agriculture, Goce Delcev University - Stip was published in 2007, the year of University's establishment. The continuous publication activity is indisputable proof of the publication of scientific and research activities, primarily of the research staff of the faculty, but also of the wider agricultural community in the country and the region. The Annual Yearbooks of the Faculty of Agriculture was published continuously from 2007 to 2016 in Macedonian language.

On April 6, 2017, the Teaching and Scientific Board of the Faculty of Agriculture launched an initiative to transform the Annual Yearbook of Faculty of Agriculture into an international journal published in English with Macedonian abstracts. Consequently, the first number of Volume 15 was published in June 2017 as a continuation and successor of the Annual Yearbook of Faculty of Agriculture.

Today, we can proudly state that JAPS is an international journal that integrates the latest scientific research results in papers of researchers from our faculty, the country, the region and beyond. JAPS is an indexed journal in the EBSCO and DOAJ which contributes to its visibility and internationalization. These achievements reflect the continuous growth and development of the publishing activity of Faculty of Agriculture.

The JAPS Editorial Office expresses its profound gratitude to Prof. d-r Sasa Mitrev, whose initiative as Director of the Institute of Southern Agricultural Crops - Strumica marked the beginning of the Publishing Activity, and whose distinguished leadership as the first Rector of Goce Delcev University significantly strengthened the Publishing Department and laid the foundations for future academic advancement.

The JAPS Editorial Office express its deepest gratitude the members of Editorial Board for their commitment and support towards internationalization of the journal. The gratitude is expanded to all authors for their interest in publishing in our journal. Last but now least, the thankfulness is conveyed to all reviewers and language and technical editors who have contributed to the high quality of the journal publications since its very beginnings.

Each passing decade brings the challenge of the next!

May the next decade bring new quality, new bloom and new challenges in the Publishing activity of the Faculty of Agriculture, Goce Delcev University – Stip.

Editorial Board,
June, 2025

Editor in chief,
Prof. Liljana Koleva Gudeva, PhD

INTRODUCTION

In recent years, climate change has emerged as a key driver influencing pest population dynamics and crop health. Warmer winters, extended growing seasons, and irregular rainfall patterns have altered the timing, abundance, and distribution of several economically important pests. During the current production season, notable changes in pest incidence have been observed across both vegetable and cereal crops, with several species showing significant deviations in abundance compared to previous years. These shifts are likely influenced by a combination of climatic conditions, crop phenology, and the presence or absence of natural enemies.

In vegetable production, the presence of thrips (Thrips tabaci Lindeman and Frankliniella occidentalis Pergande) has shown a marked increase, estimated at approximately 10 – 15% higher than in prior seasons. Thrips are recognized as important pests due to their direct feeding damage and their role as vectors of various plant viruses. This upward trend is of concern, as it may indicate more favorable environmental conditions for their reproduction and survival. Similarly, leaf aphid populations (Homoptera: Aphididae) have risen substantially, with recorded numbers about 30% higher than last year. Aphids, like thrips, pose a dual threat through direct sap-feeding and transmission of viral pathogens, meaning that their population increase could have significant implications for crop health and yield.

The occurrence of the tomato leafminer (Tuta absoluta Meyrick) in spring tomato production remained at levels similar to the previous year. However, a dramatic situation was recorded in summer production, where intense infestations led to severe damage, with some crops experiencing 100% yield loss. Also, two economically important moth pests, Autographa gamma L. (silver Y moth) and Helicoverpa armigera Hübner (cotton bollworm), were observed. This year, A. gamma was notably more abundant than H. armigera. This shift in prevalence may be attributed to ongoing climate change, which alters pest dynamics, as well as the probable presence of specific natural predators that target H. armigera, thereby reducing its population density. Observations also indicate that moth pest infestations were more frequent and severe in tomato crops than in peppers, suggesting a possible crop preference or differences in pest management practices between these crops.

Soil nematode populations remained stable compared to previous years, continuing to affect nearly 80% of the vegetable production area. Given their persistence and the extent of the affected area, nematodes remain a chronic challenge in vegetable production, requiring ongoing monitoring and integrated management approaches.

In cereal crop production, cereal bugs, particularly Eurygaster species, were recorded at unusually high population densities this year. During the first inspection period (1–15 May), population levels were more than 50% higher than those recorded during the same period in the previous year. These pests can cause substantial yield and quality losses by feeding on developing grains. Interestingly, during the second inspection period (1–15 June), their presence had virtually disappeared. This rapid decline was likely due to accelerated wheat ripening, which shortened the feeding period available to the pests.

In contrast, wheat thrips (Haplothrips tritici Kurdyumov) was entirely absent this season, despite being observed in the previous year. This absence is most likely linked to the unusually high temperatures recorded during the main period of their activity, combined with the rapid maturation of wheat, which limited the window for infestation.

Overall, the data from this year indicate a complex interaction between pest populations, climatic conditions, and crop development stages. While some pests have increased in prevalence, posing new management challenges, others have declined, likely due to unfavorable environmental conditions. Such fluctuations highlight the importance of continuous monitoring and adaptive integrated pest management strategies tailored to local agro-ecological conditions.

June, 2025

On behalf of JAPS Editorial Board,
Ass. prof. Biljana Atanasova
Member of JAPS Editorial Team

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MICROBIOLOGICAL GROUNDWATER QUALITY IN SHALLOW WELLS BEFORE AND AFTER DISINFECTION WITH PERACETIC ACID

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Abstract

The microbiological examination of water is used worldwide to monitor and control the quality and safety of various types of water. Peracetic acid (PAA) has garnered increasing attention as an alternative oxidant and disinfectant in water treatment due to the rising demand to reduce chlorine usage and control disinfection byproducts. The main aim of the research was to assess the well water microbiological quality before and after disinfection with PAA. The water samples were taken from 5 wells in the rural areas of Probishtip and Kocani regions of North Macedonia. Sampling was conducted twice (before and after disinfection) per season during the four seasons of the year. Water samples from 5 shallow wells were analysed for microbiological parameters using reference methods. The results were compared with the quality of control water and the permissible values according to the national legislation. Water quality parameters indicated that all well water samples failed to meet safe drinking water limits. A significant improvement in the microbiological quality of the water was observed during the seasons when a PAA working solution with concentrations of 0.05% and 0.1% was used. The regression statistical model revealed that disinfection with PAA and the seasonal variation in its concentration had a statistically significant influence on the microbiological quality of well water ($p<0.001$). Identification and management of groundwater quality are of utmost importance for maintaining freshwater resources, which are essential for sustainable rural development.

Key words: *water, biological contaminants, microbial safety, risk management.*

INTRODUCTION

Clean water is the most precious resource on planet Earth. Water is the most important compound without which there is no life. Water sources can be surface and underground. Surface waters include streams, rivers, natural and artificial lakes, as well as seas and oceans.

From the aspect of hygienic water quality, underground sources are of the greatest importance for supplying high-quality and safe water. Groundwater is used through wells that can be dug or drilled. Groundwater is formed by the percolation of surface or atmospheric water through permeable layers of soil. When it encounters an impermeable layer, the water is retained, and an underground reservoir is created. There, the water is still moving slowly. In addition, depending on the depth to which the water has reached, groundwater can be shallow (<10m) or deep (>10m), i.e. high or low. Groundwater has the best quality compared to other types of water.

Water quality is a key factor in the use of groundwater for households and agricultural production. Moreover, groundwater quality is largely influenced by the natural processes and anthropogenic activities in the surrounding area. The contamination typically results from polluted surface water seeping through the soil and into underground water reserves (Llopis-González et al., 2014). Rainwater runoff further exacerbates

the problem by carrying microorganisms from the air, roads, household waste, animal waste, and improperly discarded solid materials into both surface and underground water sources. Safe drinking water is considered to be water that does not contain microorganisms, parasites and their forms in a number (concentration) that poses a danger to human health, does not contain physical and chemical substances and radioactive properties that are harmful to human health, and corresponds in terms of organoleptic properties of drinking water.

Groundwater quality, particularly from shallow wells, poses significant challenges for microbial safety in various applications, including agricultural and domestic use. In many rural regions, natural springs and water sources face significant microbial contamination. This issue becomes more pronounced when the water source is located near villages or in areas where livestock farming is prevalent. Communities living nearby often rely on these springs for drinking water, unknowingly exposing themselves to serious health risks. Groundwater can be contaminated with feces if septic tanks are built uncontrolled, without taking into account the groundwater level. The greatest danger for groundwater contamination is municipal wastewater that is discharged uncontrolled, directly or indirectly, into the recipients (rivers, lakes, septic tanks). From the recipients, through the penetration of the water, harmful substances and microorganisms contaminate the groundwater and well waters, thereby changing the quality of the water.

Chlorine and chlorine-based compounds are the most widely used disinfectants in water treatment due to their antimicrobial properties (Song et al., 2019). However, chlorination concerns over the formation of toxic, carcinogenic, mutagenic and teratogenic disinfection by-products (Doederer et al., 2014). As an alternative, peracetic acid (PAA) is recognised for its efficacy as a broad-spectrum disinfectant, making it suitable for treating microbial contaminants in groundwater. PAA exhibits strong oxidizing properties, allowing it to efficiently target a wide range of pathogens in various environmental contexts. Studies have demonstrated that PAA can significantly reduce bacterial counts even in the presence of organic matter, which typically complicates disinfection processes. For instance, Smither et al. (2018) indicate PAA's broad-spectrum activity and effectiveness against various pathogens, supporting its application in the microbiological disinfection of water sources, including shallow wells. Additionally, Zhang et al. (2021) found that PAA had a faster disinfection effect than other disinfectants, underscoring its rapid action and effectiveness.

Wells used by households and the food industry should be protected from pollution, and the microbiological quality of the water should be regularly monitored. Water quality standards are needed to determine whether groundwater of a certain quality is suitable for its intended use. The main objective of the research was to monitor the microbiological quality of well water yielded from shallow wells in two districts of North Macedonia before and after disinfection with different working concentrations of PAA.

MATERIAL AND METHODS

A field survey was undertaken to monitor the water quality from shallow wells to assess seasonal changes over a period of the year. The impact of disinfection methods involving PAA has been studied.

The shallow wells included in the survey are neither lined nor covered and are located close to the surface, near waste dumps or pit latrines, making the water susceptible to high levels of contamination.

The water samples were taken from 5 wells in the rural areas of Probishtip and Kocani regions of North Macedonia. For the assessment of groundwater hygiene quality before and after disinfection with PAA, the following microbiological parameters were analyzed: total number of coliforms, total bacteria count in 1ml at 37°C, total bacteria count in 1 ml at 22°C, faecally derived enterococci, Pseudomonas aeruginosa, Escherichia coli as number /100ml). The well water samples were tested at the Public Health Center - Kocani.

The research and sampling of well water were carried out during one calendar year by seasons of the year, as follows:

• Season 1 or autumn 2023 (months of September, October and November);
• Season 2 or winter 2023/2024 (months of December, January and February);
• Season 3 or spring 2024 (months of March, April and May);
• Season 4 or summer 2024 (months of June, July and August);

To assess the hygienic quality of well water, samples were collected both before and after disinfection, with testing conducted twice during each season of the year. In the first season, a disinfectant was applied at a concentration of 0.01%, equivalent to 100 ml of PAA per 1,000 liters of water. Seven days after treatment, water samples were taken to evaluate the hygienic condition. In the second season, the disinfectant concentration was increased to 0.025% (250 ml PAA per 1,000 liters). The third season was used a concentration of 0.05% (500 ml PAA per 1,000 liters), and during the fourth season, the highest concentration of 0.1% or 1,000 ml PAA per 1,000 liters was used. This gradual increase in disinfectant concentration aimed to identify the optimal concentration of PAA required to achieve the best disinfection efficiency and improvement in the microbiological quality of well water.

The standard methods used for the examination of microbiological parameters are following the Regulation on Water Safety (Official Gazette of the Republic of Macedonia No. 183 /2018). Sterilized 500 ml laboratory glass bottles were used to take water samples for microbiological analysis.

The following microbial analyses for water samples were performed:

- Most probable number of coliform bacteria in 100 ml of water sample (ISO 9308:2006);
- Coliform bacteria of faecal origin in 100 ml of water sample (ISO 9308:2:1990)
- Total number of microorganisms - number of colonies at a temperature of 37°C (ISO 6222:1990);
- Total number of microorganisms - number of colonies at a temperature of 22°C (ISO 6222:1999);
- Enterococci in 100 ml of water sample (ISO7899-2:2000);
- *Pseudomonas aeruginosa* in 100 ml of water sample (ISO 12780:2002)

The statistical General Linear Model (GLM) for repeated measurement was used to determine the influence of water disinfection with PAA, season of year and well location on water microbiological quality.

RESULTS AND DISCUSSION

The Figures 1-6 showed the microbiological quality of well water (W1 – W5) including control water samples (C) and Maximum Permitted Concentration (MPC), regarding the period of sampling (1p before disinfection and 2p after disinfection) and seasons of the year (1_23 - autumn 2023; 2_23 - winter 2023/2024; 3_24 - spring 2024 and 4_24 - summer 2024).

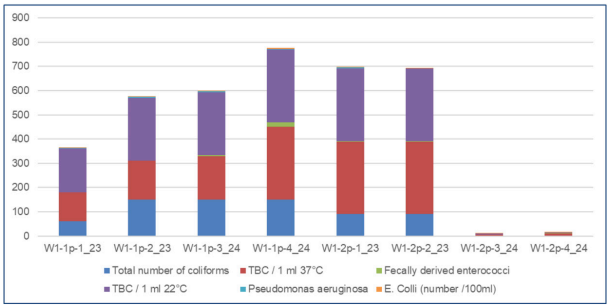

Figure 1. Microbiological quality of water in well 1 before and after disinfection.

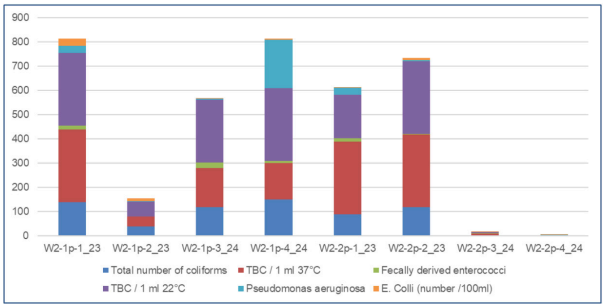

Figure 2. Microbiological quality of water in well 2 before and after disinfection.

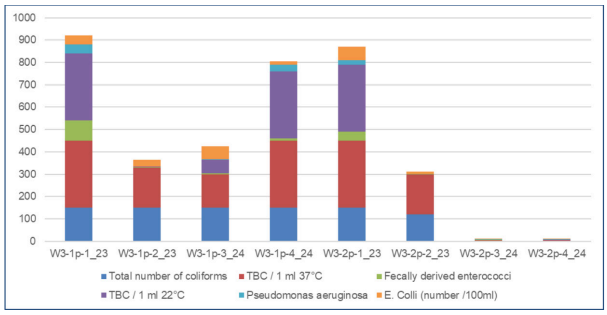

Figure 3. Microbiological quality of water in well 3 before and after disinfection.

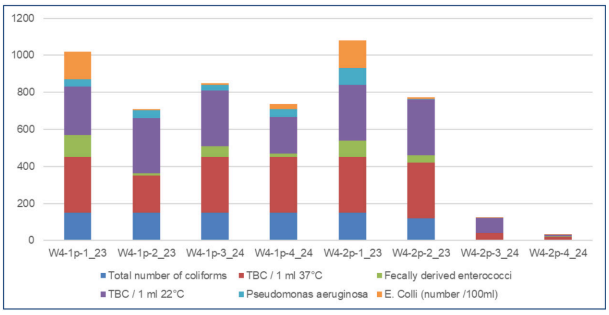

Figure 4. Microbiological quality of water in well 4 before and after disinfection.

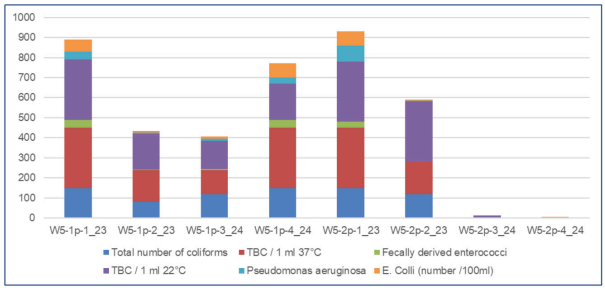

Figure 5. Microbiological quality of water in well 5 before and after disinfection.

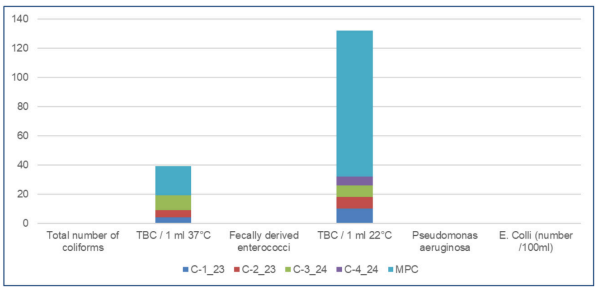

Figure 6. Microbiological quality of control water samples and Maximum Permitted Concentration (MPC).

It can be noted that the microbiological quality of the tested well water samples is not satisfactory from a microbiological point of view, and the obtained values are significantly higher compared to the microbiological quality values of the control water sample and the maximum permitted concentrations (MPC). The results showed that some wells have microbial contamination that can be fatal if the water is consumed untreated. In the water samples from all wells, the highest concentration was determined for the total number of coliform microorganisms and the total number of bacteria, in all four seasons during the year before the well water was disinfected with peracetic acid. The microbiological quality of the control sample in all seasons during the year was within the MPC, which satisfied the needs of drinking water.

Disinfection using PAA and the seasonal variation in its concentration had a statistically significant effect on the microbiological quality of well water ($p<0.001$). In contrast, the location of the shallow wells did not have a significant impact on the microbiological quality of the water.

The microbiological quality of groundwater in shallow wells is a critical public health concern, especially in rural areas where residents often rely on these sources for drinking water.

After the disinfection of the well water with an oxidative disinfectant, a significant improvement in the microbiological quality of the water was observed in the seasons when a PAA working solution with a concentration of 0.05% and 0.1% was used. This concentration of PAA used in the third and fourth seasons gave satisfactory results for the tested parameters, so that the sample complies with the rulebook on the safety and quality of drinking water (Official Gazette of the Republic of Macedonia No. 183/18). However, disinfection with 0.01% and 0.025% PAA did not achieve the required microbiological standards.

Table 1 shows the results of the regression statistical model for the impact of the inter-factor variable of the performed disinfection of well water and the fixed factor variables on the microbiological quality of well water.

The presence of various pathogens in untreated groundwater supplies can result in serious health risks. Bacteria, such as *Escherichia coli* and *Salmonella spp.*, frequently emerge as focal points in assessments of shallow well water quality due to their implications for public health (De Giglio et al., 2017; Olorunleke et al., 2022). *Escherichia coli* and *Enterococci* are indicators of human faecal contamination and are possibly associated with human enteric pathogens.

Table 1. Regression model for the influence of disinfection, season and well location on water microbiological quality.

Dependent variable: Microbiological parameters for water quality			
Source of variations	**df**	**Mean square**	**F-value**
Disinfection	1	125691.743	32.832***
Disinfection*seson of year	3	91572.526	23.920***
Disinfection*well	4	584.165	0.153NS
Error	112	3828.326	

*** significant at level p<0.001; NS non-significant;

As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present. Therefore, coliform (thermotolerant) bacteria are a commonly used bacterial indicator of the sanitary quality of food and water. Kovačić et al. (2017) noted a documented association between drinking water quality and gastrointestinal disease outbreaks, emphasizing the need for precautions against using untreated groundwater

Effective disinfection methods can mitigate these risks significantly. Moreover, the choice of disinfection method plays a significant role in the post-treatment microbial profile of groundwater. Different methods, including chlorination and PAA treatment, have distinct impacts on microbial populations. Similar to our results, the assessment of microbiological quality in groundwater before and after disinfection with PAA has demonstrated significant improvements in microbial contamination levels (Luukkonen & Pehkonen, 2016). The research of Hwang et al. (2012) has identified PAA as a promising disinfectant, noted for its virucidal and bactericidal properties and its efficacy in degrading potential contaminants without harmful residual effects often associated with chlorine-based water treatment. This effectiveness can be attributed to PAA's ability to penetrate biofilms and inactivate bacteria and viruses upon contact (Shen et al., 2016). Cadnum et al. (2016) highlight that ensuring proper concentration measurements of PAA is necessary for effective disinfection without compromising microbial safety. Queiroz et al. (2013) noted that inadequate concentrations of PAA could lead to reduced effectiveness, similar to other disinfectants like sodium hypochlorite. In addition to its disinfectant capabilities, PAA decomposes into non-toxic byproducts, primarily acetic acid and oxygen, enhancing its appeal as a sustainable disinfectant choice (Candeliere et al., 2016).

Variations in treatment efficacy against specific pathogens depend on factors such as water source characteristics and environmental conditions affecting the target pathogen's viability. Source versus household contamination dynamics can also influence disinfection effectiveness. Therefore, both immediate intervention and long-term management strategies must be implemented to sustain water quality improvements (Ercümen et al., 2017).

CONCLUDING REMARKS

Shallow wells are one of the most important types of water supplies for rural areas, mainly due to their low cost and easy way of construction. The groundwater is vulnerable to microbiological contamination due to risk factors such as human activities, lack of well protection structures and the hydrogeological characteristics in the area. The application of PAA in the disinfection of microbial contamination in groundwater, especially from shallow wells, presents numerous benefits. Its broad-spectrum antimicrobial efficacy, rapid action, minimal ecological footprint, and effective degradation into non-toxic components align well with the urgent need to enhance groundwater microbiological quality in various settings. Bridging to adopting and implementing water safety plans, an integrated strategy addressing infrastructure interventions, hydrotechnical protection of water sources, regular monitoring of water quality, and public education and awareness-raising initiatives is needed.

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МИКРОБИОЛОШКИ КВАЛИТЕТ НА БУНАРСКАТА ВОДА ПРЕД И ПО ИЗВРШЕНАТА ДЕЗИНФЕКЦИЈА СО ПЕРОЦЕТНА КИСЕЛИНА

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Резиме

Микробиолошкото испитување на водата се користи за следење и контрола на квалитетот и безбедноста на различните видови вода. Пероцетната киселина привлекува сè поголемо внимание како алтернативно средство за дезинфекција на водата заради трендот на намалена употребата на хлорот кој при дезинфекција на водата формира штетни резидуи. Главната цел на истражувањето беше да се процени микробиолошкиот квалитет на бунарска вода пред и по извршена дезинфекција со пероцетна киселина. Примероците вода беа земени од 5 бунари во руралните области на регионите Пробиштип и Кочани во Северна Македонија. Земањето примероци беше спроведено двапати во секоја од четирите сезони во годината кога беа направени истражувањата, односно во секоја сезона пред и по извршената дезинфекција на водата. Микробиолошките испитувања на примероците бунарска вода беа направени со референтни методи. Резултатите беа споредени со квалитетот на контролните примероци вода и максимално дозволените вредности според националното законодавство. Параметрите за микробиолошкиот квалитет на бунарската вода покажаа дека примероците вода не ги исполнуваат критериумите за безбедна вода за пиење. Значајно подобрување на микробиолошкиот квалитет на бунарската вода беше постигнат во сезоните на годината кога беше извршена дезинфекција на водата со работен раствор на пероцетна киселина во концентрација од 0,05% и 0,1%. Регресиониот статистички модел покажа дека дезинфекцијата и интеракцијата меѓу дезинфекцијата и сезоната во годината имаат статистички значајно влијание врз микробиолошкиот квалитет на бунарската вода ($p<0,001$). Следење и управување со квалитетот на подземните води се од голема значајност за одржување на слатководните ресурси, кои се неопходни за одржлив рурален развој.

Клучни зборови: *вода, биолошка контаминација, микробиолошка безбедност, управување со ризици.*

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A COMPARATIVE STUDY OF CARBON FARMING AND CONVENTIONAL SYSTEMS IN CORN AND SUNFLOWER CULTIVATION: CASE STUDY IN NORTH MACEDONIA

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Abstract

This study conducts a comparative evaluation of carbon farming versus conventional agricultural systems in corn (*Zea mays L.*) and sunflower (*Helianthus annuus L.*) cultivation, examining their impacts on soil carbon and nitrogen dynamics alongside other soil properties during the 2024–2025 growing season. Soil samples were collected at three critical stages—vegetation onset, midseason, and harvest—to quantify total organic carbon (TOC) and total nitrogen (TN) under each management regime.

Results reveal that carbon farming consistently and significantly enhanced TOC and TN compared to conventional agriculture. In corn plots, carbon farming induced a progressive accumulation of both TOC and TN, driven by increased organic matter inputs and stimulated microbial activity—trends consistent with established organic amendment outcomes. Sunflower plots exhibited a delayed but notable increase in soil C and N, likely reflecting the crop's high nutrient uptake and distinct biomass turnover patterns.

Conversely, conventional management displayed stable or declining TOC and TN trends, underscoring the adverse impacts of reliance on synthetic inputs on soil fertility . These findings highlight carbon farming's effectiveness in enhancing soil health by improving nutrient retention and increasing organic matter, aligning with climate-adaptive and regenerative agriculture principles.

In summary, carbon farming presents a promising strategy for boosting soil carbon and nitrogen stocks in cereal and oilseed production systems, offering co-benefits for soil fertility and climate mitigation. Its implementation may advance sustainable crop production and long-term soil resilience.

Key words: *carbon farming, conventional agriculture, soil organic carbon, total nitrogen, corn, sunflower, agroecological practices, soil fertility, climate-adaptive agriculture, sustainable soil management.*

INTRODUCTION

Carbon serves as a vital indicator of soil health in agroecological systems, reflecting the intricate interplay between soil organic matter (SOM), microbial activity, and overall soil functionality (Bienes et al., 2021; Lal et al., 2021; Bhattacharyya et al., 2022). Monitoring carbon levels provides insights into soil fertility, structure, and resilience, all of which are essential for sustainable agricultural practices (Davis et al., 2017). SOM, primarily composed of decomposed plant and animal material, is a key component of soil carbon (Merckx et al., 2001; Lorenz et al., 2018; Javed et al., 2022). Its presence enhances soil aggregation, leading to improved soil structure. Well-aggregated soils have better porosity, facilitating root penetration and water infiltration, which are crucial for plant growth and resilience to droughts (Qi et al., 2022). Additionally, improved soil structure reduces erosion and nutrient leaching, promoting long-term soil fertility (Bronick & Lal, 2005; Ramesh et al.,

2019). Carbon-rich soils have improved water-holding capacity, which is vital for maintaining crop health during dry periods (Usharani et al., 2019). Enhanced water retention reduces the need for frequent irrigation and helps maintain soil moisture levels, contributing to more resilient agricultural systems (Adhikari et al., 2022; Song et al., 2023). Soil carbon contributes to the formation of stable aggregates, which protect the soil from erosion. Cover crops and reduced tillage practices increase soil organic matter, further enhancing soil structure and reducing the risk of soil erosion (Triberti et al., 2016; Nayak et al., 2019). This protection preserves the productivity and long-term use of agricultural land.

Agriculture accounts for nearly a quarter of global greenhouse gas emissions, primarily through methane (CH_4) and nitrous oxide (N_2O) emissions, as well as soil carbon losses (Acharya et al., 2022). Conversely, sustainable agricultural practices such as no-till farming, cover cropping, and agroforestry can sequester significant amounts of carbon in soils and biomass (Meena et al., 2020; Nicoloso & Rice, 2021). To effectively mitigate climate change, it is imperative to establish reliable benchmarks for carbon sequestration in agricultural systems. Several findings indicate that carbon farming practices lead to higher SOC levels compared to conventional methods. For instance, a meta-analysis of cover crop responses in corn systems revealed an average SOC increase of 7.3 % (Joshi et al., 2023). Similarly, studies in Brazil showed that no-tillage systems combined with cover crops resulted in higher carbon stocks and increased maize yields (Besen et al., 2024). The increased SOC in carbon farming systems can enhance soil fertility, water retention, and enhance resiliencies to extreme weather events. However, the effectiveness of these practices can vary based on factors such as soil type, climate, and management techniques (Paustian et al., 2019; Coonan et al., 2020; Dupla et al., 2024). Further research is needed to optimize these practices for different agricultural contexts. Adopting carbon farming practices in corn and sunflower cultivation can significantly contribute to soil carbon sequestration and promote sustainable agriculture (Andries et al., 2021). While challenges remain, the potential benefits underscore the importance of integrating these practices into mainstream farming systems.

Historically, the Green Revolution introduced high-yielding varieties and intensive input use, significantly boosting cereals production (Khush, 1999; Pingali, 2017). However, these methods often led to environmental concerns, including soil erosion, nutrient depletion, and increased greenhouse gas emissions. Consequently, contemporary agricultural research emphasizes integrated approaches that balance productivity with environmental stewardship. Key strategies for improving corn farming encompass: 1) Crop rotation and intercropping including implementing diverse cropping systems, such as maize-legume rotations, enhances soil fertility and reduces pest pressures; 2) Conservation tillage: adopting no-till practices preserves soil structure, mitigates erosion, and sequesters carbon; 3) Nutrient management with optimizing fertilizer application, including the use of green ammonia, reduces emissions and improves nutrient use efficiency; 4) Biological control and integrated pest management utilizinging biopesticides and IPM strategies curtails reliance on chemical inputs and promotes ecological balance. These practices not only bolster corn yields but also contribute to environmental sustainability (Nsabiyeze et al., 2024).

Establishing robust benchmarks for carbon sequestration in agriculture is crucial for evaluating the effectiveness of carbon farming practices and achieving climate mitigation goals. By integrating diverse methodologies and addressing existing uncertainties, the proposed framework offers a pathway towards reliable and scalable carbon quantification in agricultural systems (Avasiloaie et al., 2023). Monitoring and enhancing soil carbon levels are fundamental for assessing and improving soil health in agroecological systems (Oldfield et al., 2022). Practices that increase soil organic matter, such as agroforestry, cover cropping, and reduced tillage, not only sequester carbon but also bolster soil structure, fertility, and resilience (Rumpel et al., 2020; Smith et al., 2020; Tiefenbacher et al., 2021; Kyriakarakos et al., 2024). These benefits emphasize the significance of carbon as a central indicator of soil health and a fundamental element of sustainable agriculture.

MATERIAL AND METHODS

Field activity overview

In 2024, a series of field activities were undertaken to assess the effectiveness of different agricultural practices on soil health and carbon sequestration. The experimental setups included corn and sunflower crops. The field for each crop was divided into two sub-plots (0.25 hectares for conventional production and 0.25 hectares for carbon farming). Conventional corn production involved sowing it as a sole crop. The carbon farming plot used the sowing of beans between corn rows as an intercrop for natural nitrogen fixation. The sowing of corn, beans and sunflower, in both conventional and carbon production, was carried out on 12.04.2024. On sunflower plots, conventional production used sowing sunflower as a sole crop. For the carbon farming plot, sunflower sowing was carried out with the application of a cover crop. Before sowing and after the emergence of the sunflower, a biostimulator was applied using a drone.

A2 (Corn + Beans): This intercropping system aimed to enhance biodiversity and soil nutrient cycling. Regular irrigation was implemented to support early growth.

A3 (Corn): Conventional corn cultivation was practiced, focusing on standard agronomic practices. Irrigation was applied as needed to maintain optimal growth conditions.

A4 (Sunflower): Sunflower seeds were sown, with attention to spacing and depth to ensure uniform emergence. Irrigation was carefully managed to promote optimal plant growth and development.

A5 (Sunflower + Cover Crop): Sunflower planting was complemented with a cover crop (winter barley), to enhance soil organic matter and prevent erosion. Irrigation practices were adjusted to support both crops effectively.

Throughout this period, soil moisture levels were monitored, and necessary adjustments to irrigation schedules were made to accommodate the varying water requirements of each system. By August, all crops had reached full growth and midpoint assessments of soil agrochemical properties and carbon footprint estimates were conducted. After harvesting all crops in October, post-harvest residues were ploughed into the soil. The sunflower carbon production plot was sown with winter barley as a cover crop on 20.11.2024 and the winter barley was plowed into the soil on 26.03.2025, at the booting stage. Final assessments were conducted in May 2025.

A2 (Corn + Beans): Harvesting was performed for both corn and beans. Immediately after harvest, in October 2024, post-harvest residues were plowed, and in May 2025, soil samples were collected to analyze the impact of intercropping on soil carbon content.

A3 (Corn): Corn was harvested following standard procedures. After harvest, in October 2024, the post-harvest residues were plowed, and in May 2025, soil sampling was conducted to assess the effects of conventional practices on soil health.

A4 (Sunflower): Sunflowers were harvested, and after harvest in October 2024, post-harvest residues were plowed into the soil, and in May 2025, soil samples were taken to evaluate the influence of monoculture on soil properties.

A5 (Sunflower + Cover Crop): The sunflowers were harvested according to standard procedures and post-harvest residues were immediately plowed into the soil. The cover crop was sown on 20.11.2024, and incorporated into the soil on 26.03.2025. Soil samples were collected in May 2025, to determine the benefits of cover cropping on soil organic matter and carbon sequestration.

Soil samples were collected from multiple representative sites under agricultural land use. Sampling followed a stratified approach, accounting for variables such as crop type, fertilizer application history, inclusion of intercrops, cover crops and soil texture. At each site, composite samples were taken from the top 0-20 cm layer using a soil auger and stored in labeled polyethylene bags for laboratory analysis.

Table 1. General overview of the applied cultivation practices.

Setup	Crop(s)	Practice type	Start-point (April, 2024)	Mid-point (August, 2024)	End-point (May 2025)
A2	Corn + Beans	Carbon Farming	Sowing & Irrigation	Fertilizer (biostimulator)	Residue management, Intercrop
A3	Corn	Conventional	Sowing & Irrigation	Fertilizer (mineral)	Residue management
A4	Sunflower	Conventional	Sowing & Irrigation	Fertilizer (mineral)	Residue management
A5	Sunflower + Cover Crop	Carbon Farming	Sowing & Irrigation	Fertilizer (biostimulator)	Residue management, cover crop

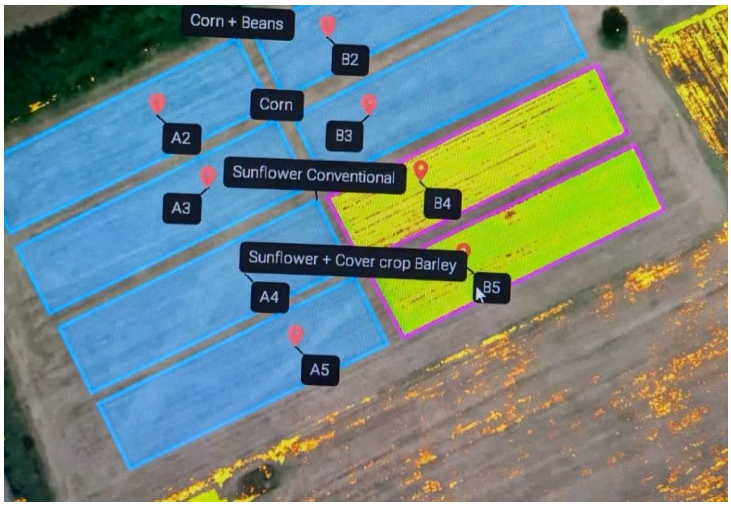

Figure 1. Field setup for the experiments: A2 (Corn + Beans) – Carbon farming; A3 (Corn) – Conventional; A4 (Sunflower) – Conventional; A5 (Sunflower + Cover Crop) – Carbon farming.

Agrochemical soil characterization

Agrochemical determination of soil typically outlines the procedures used to analyze key soil parameters related to fertility. To assess the agrochemical properties of the soil, a series of standardized laboratory analyses were conducted. Each soil sample was air-dried, ground, and passed through a 2 mm sieve prior to testing.

Soil pH and electrical conductivity (EC) were determined using aqueous soil suspensions. For pH measurement, a 1:2.5 soil-to-water ratio (weight/volume) was prepared and allowed to equilibrate before being analyzed with a calibrated digital pH meter. For EC, a separate suspension was made using a 1:5 soil-to-water ratio and measured using a conductivity meter to evaluate the soluble salt content of the soil.

Organic matter content was estimated using the Walkley-Black method, which involves the oxidation of organic carbon by potassium dichromate in the presence of sulfuric acid. The unreacted dichromate was then titrated to determine the amount of oxidized carbon, which was used to calculate the organic matter percentage.

Total nitrogen (N) content in the soil was determined by the Kjeldahl method. This procedure includes the digestion of the soil sample with concentrated sulfuric acid in the presence of a catalyst to convert organic nitrogen into ammonium. The digest was

then subjected to alkaline distillation, and the released ammonia was trapped and quantified through titration.

Available phosphorus (P) was measured based on soil reaction type. The Olsen method was applied for neutral to alkaline soils, utilizing a sodium bicarbonate extractant. For acidic soils, the Bray-1 method was used. In both methods, the phosphorus in the extract was quantified colorimetrically using a spectrophotometer, based on the formation of a phosphomolybdenum blue complex.

Exchangeable potassium (K) was extracted from the soil using 1M ammonium acetate solution. The potassium content in the extract was then quantified using either flame photometry or atomic absorption spectrophotometry (AAS), depending on the available instrumentation.

These methods collectively provided key insights into the fertility and chemical status of the soils under study, contributing to a comprehensive understanding of their suitability for agricultural use.

Soil carbon analysis – comparative validation

Soil organic matter was quantified using both the traditional Walkley-Black (WB) method and a modified spectrophotometric variant to improve sensitivity and analytical precision.

The Walkley-Black method is a classical wet oxidation technique that estimates soil organic carbon through chemical oxidation (Balabanova et al., 2024). In this method, a known amount of finely ground soil was treated with an excess of potassium dichromate ($K_2Cr_2O_7$) solution and concentrated sulfuric acid (H_2SO_4). The exothermic reaction generates sufficient heat to oxidize the organic carbon present in the soil sample. After a reaction period, the remaining unreacted dichromate was titrated with ferrous sulfate solution. The amount of dichromate reduced during the reaction is stoichiometrically related to the amount of oxidized organic carbon, which is then used to calculate organic matter content.

To improve sensitivity and detection, especially in soils with low organic content, the Modified Walkley and Black method was also employed. This variation follows the same principle of dichromate oxidation but replaces the titrimetric endpoint with spectrophotometric detection. After the oxidation reaction, the resulting chromate solution is analyzed using a UV-Vis spectrophotometer, typically at a wavelength around 600 nm, corresponding to the absorbance of the Cr^3 complex formed. This modification allows for more precise quantification of organic carbon, particularly in samples with low or variable organic matter, by reducing operator subjectivity and enhancing sensitivity.

By applying both methods, a comparative analysis of the accuracy and efficiency of classical versus modern detection techniques was achieved, ensuring robustness and reliability in the determination of soil organic matter.

Data collection and model application

To comprehensively evaluate the agrochemical profile of the soil and its associated environmental impact, a structured approach combining field data collection with model-based analysis was adopted. The study focused on characterizing key soil agrochemical parameters and estimating the carbon footprint resulting from agricultural inputs and practices. Integrating chemical characterization of soil with carbon footprint analysis, this approach provided a holistic view of both soil fertility and the environmental sustainability of agricultural practices, guiding more efficient and climate-conscious land management decisions. The key components included: Soil carbon fluxes, influenced by organic matter levels and land management practices.

RESULTS AND DISCUSSION

Climate variables such as temperature and precipitation significantly affect SOC dynamics. Warmer temperatures accelerate organic matter decomposition, potentially reducing SOC levels, while increased precipitation can enhance plant growth and organic matter input, promoting

SOC accumulation. These climatic factors interact with soil properties to influence overall soil health and carbon storage capacity. The field experiment aimed to compare the impacts of conventional farming and carbon farming practices on soil health and environmental performance, specifically for corn (*Zea mays*) and sunflower (*Helianthus annuus*) cultivation. The comparison was structured around two key analytical dimensions: soil agrochemical properties and carbon footprint assessments.

Agrochemical analysis outputs

The analysis of soil agrochemical parameters, specifically pH (KCl), pH (H_2O), total nitrogen (N), available phosphorus (P_2O_5), and potassium (K_2O), provided valuable insight into the impact of carbon farming practices compared to conventional agriculture for corn and sunflower cultivation during the 2024–2025 growing season. Measurements were taken at three key stages: the start of the experiment (April, 2024), mid-point (August, 2024), and end-point (May, 2025), allowing for a comparative temporal and treatment-based assessment.

Across both crops, soils managed under carbon farming practices showed notable trends in terms of nutrient retention and soil quality stabilization. For corn, carbon farming plots exhibited a gradual increase or maintenance of total nitrogen levels, in contrast to the more fluctuating and sometimes declining trends observed under conventional practices. This suggests that the application of organic amendments commonly associated with carbon farming may enhance nitrogen preservation through improved microbial activity and reduced leaching.

Phosphorus (P_2O_5) and potassium (K_2O) levels were generally higher or more stable in carbon farming systems over both years. In contrast, conventional plots showed greater year-to-year variability, likely due to standard fertilization regimes combined with increased nutrient runoff and lower organic matter retention. These findings are consistent with other studies emphasizing the nutrient buffering capacity of carbon-enriched soils.

Soil pH values remained within optimal agronomic ranges in all plots; however, a slight acidification trend was observed in conventional systems, particularly in sunflower cultivation, as reflected in decreasing pH (KCl) values from 2024 to 2025. This could be attributed to the continuous application of mineral fertilizers, which are known to gradually lower soil pH. In contrast, carbon farming plots maintained more stable pH values, suggesting a buffering effect from increased organic matter content and lower synthetic input intensity.

For sunflower, similar patterns were observed, with carbon farming systems showing a consistent or improved soil nutrient profile over time, particularly in nitrogen and potassium content. Notably, the mid-point measurements in 2024 captured an increase in nutrient availability in carbon farming plots, possibly reflecting cumulative improvements in soil structure and biological activity as a result of sustainable practices such as organic fertilization and the introduction of intercropping.

Total nitrogen (N) is a critical indicator of soil fertility, directly influencing plant growth and productivity. The monitoring of total nitrogen levels over the vegetation period under different agricultural management practices, carbon farming versus conventional farming, in corn and sunflower cropping systems provides important insight into how farming practices impact long-term soil nutrient dynamics and sustainability. At the start point of the measurement period, nitrogen content across both carbon farming and conventional plots was comparable, reflecting similar baseline soil fertility levels. However, divergent trends emerged over time. In the carbon farming plots, total nitrogen content showed a gradual increase or remained stable between mid-point and end-point of measurements. This trend can be attributed to the incorporation of organic matter and improved microbial activity, all of which promote nitrogen mineralization and retention in the root zone (Figures 2a and 2b).

By contrast, in conventional corn plots, nitrogen levels exhibited a declining trend, especially between the mid-point (2024) and end-point (2025) measurements. The decrease is likely due to the use of synthetic nitrogen fertilizers, which may lead to higher rates of nitrogen leaching or volatilization in the absence of sufficient organic matter to retain and buffer nutrients.

Table 2. Overall agrochemical soil characteristics.

Start-point	pH(KCl)	pH(H2O)	EC (mS/cm)	Total N mg/g	P2O5 (mg/100g)	K2O (mg/100g)	SOM (%)
A2	7.56	8.43	0.53	1.15	29.9	57.4	7.93
A3	7.66	8.43	0.50	1.36	47.8	71.3	7.59
A4	7.54	8.45	0.51	1.19	40.4	51.8	7.12
A5	7.55	8.46	0.51	1.34	33.5	47.2	7.02
Mid-point	**pH(KCl)**	**pH(H2O)**	**EC (mS/cm)**	**Total N (mg/g)**	**P2O5 (mg/100g)**	**K2O (mg/100g)**	**SOM (%)**
A2	8.55	7.74	0.54	1.31	41.59	99.64	1.95
A3	8.63	7.78	0.41	0.79	37.13	80.09	1.94
A4	8.57	7.76	0.46	0.82	39.39	68.82	1.71
A5	8.51	7.75	0.65	0.93	39.98	69.57	1.56
End-point	**pH(KCl)**	**pH(H2O)**	**EC (mS/cm)**	**Total N (mg/g)**	**P2O5 (mg/100g)**	**K2O (mg/100g)**	**SOM (%)**
A2	8.71	7.81	0.50	1.03	39.27	73.63	2.42
A3	8.79	7.85	0.45	0.86	39.08	67.85	2.32
A4	8.68	7.81	0.44	1.37	40.11	72.18	1.96
A5	8.77	7.79	0.43	1.77	48.88	73.19	2.05

In the sunflower plots, a similar pattern was observed. Carbon farming systems maintained relatively stable or slightly increasing total nitrogen content over the two-year period. The application of regenerative practices, such as the introduction of cover crops and organic inputs, likely contributed to enhanced nitrogen conservation, consistent with carbon sequestration goals. Conversely, conventional sunflower fields showed a more pronounced decrease in total nitrogen, particularly in mid-point assessments. This decline may be exacerbated by sunflower's relatively high nitrogen demand during flowering (Reproductive stage R5, according to the system of phenological phases according to Schneiter and Miller 1981), and - seed formation R6 (anthesis complete), which, under conventional systems, may not be replenished adequately through mineral fertilization alone. The absence of organic matter recycling and microbial support mechanisms further limits nitrogen retention.

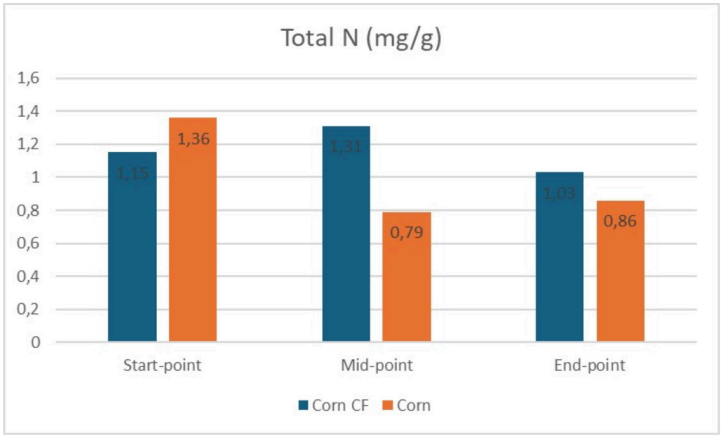

Figure 2a. Nitrogen content in soil, along the vegetation period Carbon farming vs. conventional practices (corn case study), CF – carbon farming.

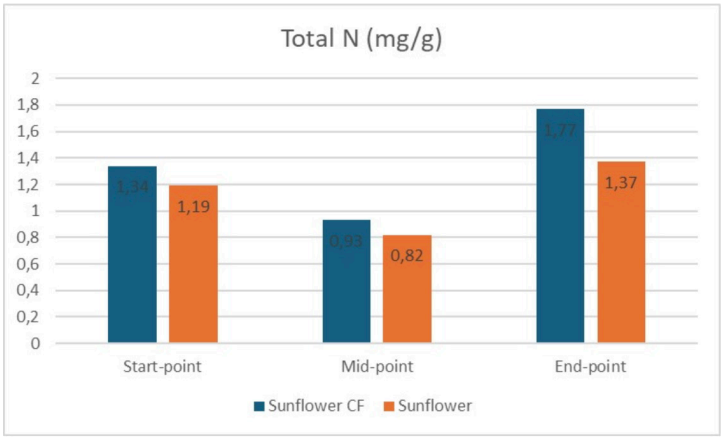

Figure 2b. Nitrogen content in soil, along the vegetation period Carbon farming vs. conventional practices (sunflower case study), CF – carbon farming.

The comparative trends clearly indicate that carbon farming practices are more effective in maintaining or improving soil nitrogen levels over time, compared to conventional systems that tend to deplete nitrogen reserves. The long-term retention of nitrogen in carbon farming systems is critical not only for maintaining crop yields but also for enhancing soil health, promoting microbial biodiversity, and reducing environmental impacts such as nitrate leaching into groundwater. These findings underscore the importance of integrating organic matter inputs and conservation management techniques in nitrogen-sensitive cropping systems such as corn and sunflower. Sustained improvements in total nitrogen under carbon farming contribute to the broader goals of increasing the level of agroecosystem resilience and climate-smart agriculture.

Carbon footprint and soil carbon measurements

Organic matter is the primary reservoir of soil organic carbon (SOC), and its accumulation is directly linked to the potential of soil to function as a long-term carbon sink. In this study, the temporal dynamics of OM were monitored across corn and sunflower systems under both carbon farming and conventional practices from start-point to end-point of measurement, providing valuable insight into the processes of soil carbon stabilization and loss. In the corn plots managed under carbon farming, total organic matter showed a steady increasing

trend across all three measurement points. This increase is a clear indication of successful carbon sequestration. The application of intercropping, cover crop and organic soil amendments contributed to the accumulation of plant residues and microbial biomass in the upper soil layers. These inputs not only directly add organic carbon but also foster a microenvironment that enhances humus formation and slows down organic matter decomposition.

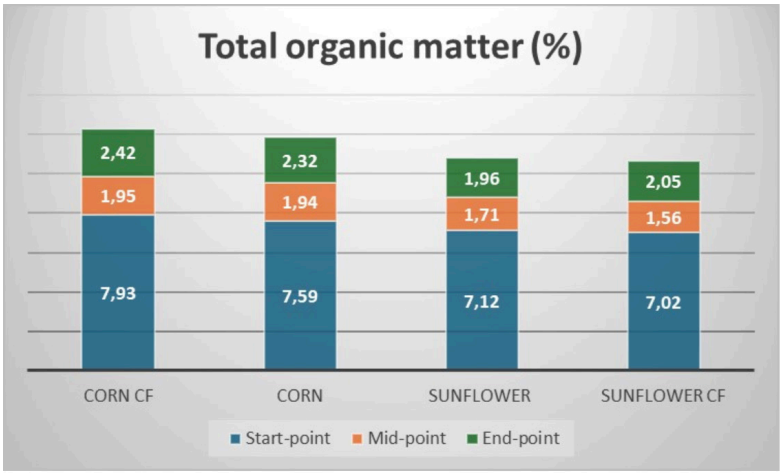

Figure 3. Total organic matter in experimental fields, carbon farming vs. conventional farming, CF – carbon farming.

In the plots under carbon farming, total organic matter initially decreased from April 2024 to August 2024, but then increased by the 2025 end-point (Figure 3). This initial decline could be attributed to several crop-specific and management-related factors. Sunflower is known for its deep rooting system and high nutrient demand, especially nitrogen and potassium, which can deplete the organic matter pool in the early growth stages. Additionally, decomposition of previously applied organic materials or transition from conventional to regenerative practices may result in a temporary imbalance between organic matter inputs and microbial decomposition rates.

By 2025, the observed increase in organic matter under carbon farming can be linked to improved soil structure and higher biomass return to the soil after harvest. The cumulative effect of residue retention and microbial activity leads to better humification of organic inputs, allowing the soil to recover and sequester more carbon over time.

In conventional plots, the organic matter trend remained flat or slightly declining, indicating limited capacity for carbon accumulation. The absence of systemic organic inputs and soil conservation practices hampers the replenishment of soil carbon, especially after high-demand crops like sunflower. The findings from both crop systems show a strong positive correlation between total organic matter and carbon sequestration potential. In carbon farming systems, increased TOM reflects enhanced biological activity, greater biomass input, and improved physical conditions that favor long-term carbon storage. Conversely, conventional practices tend to either deplete or stagnate organic matter levels, limiting the soil's ability to sequester carbon. As the primary component of soil organic matter, TOC is directly linked to soil fertility, microbial activity, and carbon sequestration potential. TOC was measured across corn and sunflower plots managed under both carbon farming and conventional practices. The temporal trends reveal important distinctions between the two management systems and their impact on carbon dynamics in agricultural soils. In corn plots managed with carbon farming practices, TOC levels showed a consistent and significant increase in 2024 and remained elevated through 2025. This trend reflects the cumulative benefits of regenerative practices such as organic residue incorporation, intercropping and cover cropping, all of which contribute to both the

addition and stabilization of organic carbon in the soil. Enhanced soil aggregation and microbial biomass formation under these systems likely contributed to the retention of newly added carbon, leading to a steady buildup of TOC. In contrast, conventional corn plots demonstrated either minimal increases or stagnant TOC levels, with minor fluctuations over the two years. This stagnation can be attributed to low organic input levels, and the reliance on synthetic fertilizers, which do not contribute organic carbon and may accelerate the mineralization of existing soil organic matter. As a result, the soil under conventional management exhibits limited capacity to sequester carbon in the long term.

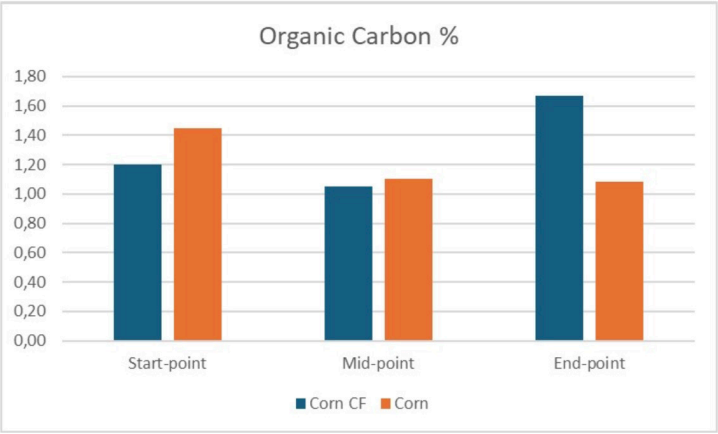

Figure 4. Total organic carbon for pilot corn cultivation, CF – carbon farming.

In sunflower plots under carbon farming, TOC levels also increased in 2024, and remained elevated in 2025, though the magnitude of change was slightly more gradual compared to corn. This pattern may be explained by the higher nutrient and carbon demands of sunflower, which can slow initial organic carbon accumulation, particularly in soils transitioning from conventional to regenerative practices. However, as the carbon farming system matures and stabilizes, the rate of carbon input from plant residues and microbial activity begins to exceed decomposition rates, leading to a net increase in TOC. On the other hand, conventional sunflower plots exhibited relatively static TOC levels with no significant upward trend. The absence of organic amendments and the lack of soil conservation practices reduce the opportunity for carbon input and stabilization, highlighting the limitations of conventional management in supporting carbon sequestration, particularly for high-demand crops like sunflower.

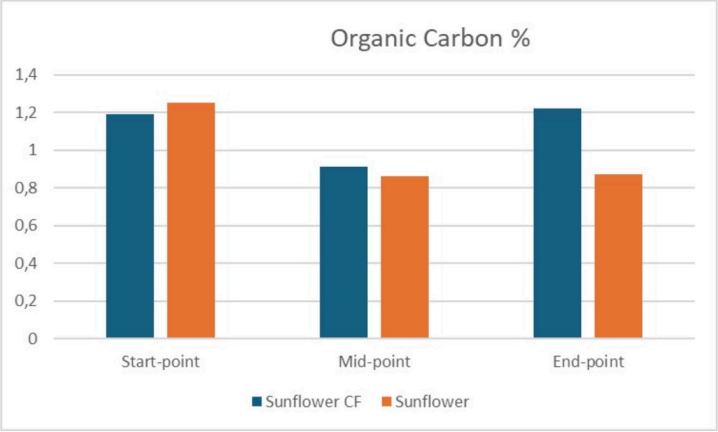

Figure 5. Total organic carbon for pilot sunflower cultivation, CF – carbon farming.

The experimental findings clearly demonstrate that carbon farming practices enhance total organic carbon content in agricultural soils for both corn and sunflower systems. The sustained increase in TOC over the two-year period under carbon farming reflects the effectiveness of these systems in promoting carbon storage, improving soil resilience, and mitigating climate change impacts. Importantly, the maintenance of elevated TOC levels at the end of the study (2025) suggests that carbon farming not only boosts carbon input but also creates the conditions necessary for long-term carbon stabilization, including increased microbial activity, better aggregation, and reduced soil erosion. These findings are in agreement with extensive literature that underscores the pivotal role of organic carbon in sustainable soil management. The distinction between the steady carbon gains in carbon farming and the stagnation in conventional systems underscores the need for widespread adoption of regenerative practices to enhance carbon sequestration and overall soil quality in intensive cropping systems.

CONCLUDING REMARKS

This study demonstrates the substantial benefits of carbon farming practices over conventional systems in enhancing soil health, improving agrochemical properties, and increasing carbon sequestration in corn and sunflower cropping systems. Over the 2024–2025 growing seasons, soils under carbon farming management exhibited more stable and favorable trends in total nitrogen, available phosphorus, and potassium content, alongside improved pH buffering capacity. In terms of carbon dynamics, carbon farming significantly increased total organic matter (TOM) and total organic carbon (TOC) across both crop systems. The observed gains in TOC reflect the cumulative effect of enhanced biomass input, improved soil structure, and greater microbial activity under regenerative management. Notably, while TOC and TOM levels in conventional plots remained static or declined, carbon farming plots showed sustained and measurable increases, indicating a superior capacity for long-term carbon storage and soil resilience.

The evidence from this field experiment underscores the crucial role of carbon farming in advancing climate-smart agriculture. By promoting nutrient stability, enhancing soil organic carbon stocks, and reducing environmental degradation, carbon farming practices offer a viable pathway to more sustainable and resilient agroecosystems. The findings support the broader adoption of regenerative techniques to mitigate soil degradation, support food security, and contribute meaningfully to climate change mitigation through agricultural carbon sequestration.

Overall, the results demonstrate that carbon farming has the potential to enhance soil fertility and stability over time, compared to conventional agriculture. The ability to maintain or improve agrochemical soil parameters over multiple growing seasons suggests not only environmental benefits but also agronomic sustainability. These findings highlight the critical importance of long-term soil monitoring for assessing the effectiveness of regenerative agricultural practices, especially within the framework of climate-smart and carbon-sequestering strategies.

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КОМПАРАТИВНА СТУДИЈА НА ПРАКТИКИ ЗА ЈАГЛЕРОДНО И КОНВЕНЦИОНАЛНО ОДГЛЕДУВАЊЕ НА ПЧЕНКА И СОНЧОГЛЕД: СТУДИЈА НА СЛУЧАЈ ВО СЕВЕРНА МАКЕДОНИЈА

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Резиме

Оваа студија претставува споредбена евалуација на јаглеродното земјоделство и конвенционалните агроеколошки системи при одгледување на пченка (Zea mays L.) и сончоглед (Helianthus annuus L.), со фокус на нивното влијание врз јаглеродната и азотната динамика во почвата, како и врз други почвени карактеристики во периодот 2024-2025. Почвени примероци беа земани на почетокот, на средината и на крајот од студијата, со цел да се процени вкупната органска материја (TOC) и вкупниот азот (TN) под различни системи на управување.

Резултатите покажаа доследно и значајно зголемување на содржината на јаглерод и азот во почвите третирани според принципите на јаглеродно земјоделство, во споредба со оние под конвенционално управување. Кај пченката, јаглеродното земјоделство доведе до прогресивно акумулирање на TOC и TN, што се припишува на внесот на органска материја и засилената микробна активност. Кај сончогледот исто така беше забележано зголемување на TOC и TN, иако со одложена реакција, најверојатно поради повисоките нутритивни потреби на културата и поголемото враќање на биомаса во почвата.

Наспроти тоа, конвенционалните системи покажаа стагнација или намалување на TOC и TN, што ги потенцира ограничувањата на зависноста од синтетички ѓубрива за одржување на долгорочната плодност на почвата. Овие наоди го нагласуваат потенцијалот на јаглеродното земјоделство како одржлива стратегија за подобрување на здравјето на почвата, зголемување на задржувањето на хранливи материи и придонесување кон климатски прилагодливо земјоделство во производството на житарки и маслодајни култури.

Клучни зборови: *јаглеродно земјоделство, конвенционално земјоделство, органски јаглерод во почвата, вкупен азот, пченка, сончоглед, агро-еколошки практики, почвена плодност, климатски прилагодливо земјоделство, одржливо управување со почвата.*

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CHEMICAL CHARACTERIZATION OF TOBACCO SOILS IN THE PRILEP REGION: ENVIRONMENTAL AND AGRICULTURAL PERSPECTIVES

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Abstract

The quality of the soil plays a fundamental role in agricultural productivity, particularly in tobacco cultivation, where both high yields and superior leaf quality are essential. The elemental composition of the soil, in particular the balance between essential nutrients and the presence of potentially toxic elements, plays a crucial role in shaping soil quality and influencing overall plant health and development. In this study, the soil quality in the Prilep region in North Macedonia, the main cultivation area for oriental tobacco, is investigated. During the 2021 and 2022 growing seasons, soil samples were collected from selected tobacco fields and analyzed using ICP-MS to determine the concentrations of selected macro and microelements (K, Mg, Fe and Na) as well as potentially toxic elements (As, Cd, Cr, Cu, Ni, Pb and Zn). In addition to elemental analysis, several key agrochemical properties were also assessed: organic matter content (ranging from low to moderate), total nitrogen content (0.03–0.14%), soil pH (mean 6.55, indicating slightly acidic to neutral conditions), the availability of essential nutrients (phosphorus and potassium), the physical structure of the soils (classified as medium loam), and clay content (20.6% to 58.7%). The content of macro- and microelements were closely related to the geological and pedological characteristics of the region. The concentration of potentially toxic elements remained below the internationally accepted thresholds for heavy metals in agricultural soils, indicating a low risk of contamination and confirming the suitability of these soils for sustainable tobacco cultivation.

Key words: *soil, tobacco fields, macroelements, microelements, potentially toxic elements, ICP-MS.*

INTRODUCTION

Agricultural soil is a vital natural resource that underpins plant growth and sustains ecological balance, making it indispensable for current and future agricultural productivity. As a dynamic and heterogeneous matrix, soil comprises varying proportions of inorganic particles — sand, silt, and clay — which determine its texture, structure, and water-holding capacity. In addition to these mineral components, soil contains a variety of organic substances, including humic substances (typically 10–15%), lipids, carbohydrates, lignin, flavonoids, pigments, resins, and fulvic acids. These organic constituents enhance nutrient availability, stimulate microbial activity, and contribute to overall soil health (Pinto et al., 2011). The complex interplay between organic and inorganic matter makes soil quality a cornerstone of sustainable agriculture, as it has a direct impact on soil fertility, crop productivity, and the long-term resilience of farming systems. Furthermore, soil is a fundamental component of the natural ecosystem, the health of which is a reliable

indicator of the well-being of the environment. Achieving sustainability in agriculture depends on maintaining soils with a balanced contents of trace elements and essential nutrients (He et al., 2005; Prasad, 2008; Kabata-Pendias, 2011).

Trace element contamination and accumulation in agricultural production systems is a potential threat to food quality, crop growth and has a direct impact on environmental health (McLaughlin at al., 2000; Micó et al., 2006; Peris et al., 2007). Since agricultural soils act as efficient sinks, they accumulate trace elements and pollutants quickly, while their removal occurs slower. Although metals are naturally occurring (Fadigas et al., 2010; Kabata-Pendias, 2011), agricultural soils are affected by anthropogenic influences, especially the application of sewage sludge, manure, pesticides, inorganic fertilizers and wastewater (Drury et al., 2009; Sheppard et al., 2009). The use of phosphate fertilizers is basic factor in pollution, as they contain a high concentration of heavy metals (Golia et al. 2009; Kabata & Pendias, 2011). Phosphate fertilizers, which may contain trace elements such as Cd and Se derived from the rock phosphates used in their manufacture, are a potential source of these elements (Singh, 1994; Prasad, 2008). Numerous of factors contribute to the mobility and availability of these elements, such as the bioecological characteristics of the plant species, the concentration and chemical forms of occurrence of the elements in the soil and, of course, ecopedological conditions (Alloway, 1999; Kabata-Pendias, 2011).

Tobacco growers strive for the highest possible yield and the best possible quality of their production. To achieve these goals, high-quality soil is essential, as it is the basis for optimal plant performance. The protection of tobacco plants involves a variety of measures, with chemical treatments being among the most reliable to ensure successful production. The Republic of North Macedonia is known for its high-quality oriental tobacco and produces 3% of the world's total oriental tobacco (Kabranova & Arsov, 2009). In order to achieve consistent yields, the use of metal-containing substances has increased significantly, with elements such as Cu, Zn, Fe, Mn commonly used in agricultural practices. These elements are frequently incorporated into various mineral fertilizers. in addition, many pesticides, fungicides, and herbicides contain Cu, Zn, Mn, Fe and even As. The group of elements that are considered potential pollutants in agriculture includes Ag, As, Ba, Be, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Zn, B, Sn, Co and V (Tóth et al., 2007). Among these, copper, zinc, lead and cadmium are the most widespread in agricultural areas (Alloway, 1999; He et al., 2005).

Deficiency and toxicity of trace elements in agricultural soils are closely related to various soil properties, such as organic matter content, type and amount of clay, pH, and cation exchange capacity (CEC), all of which are determined by the parent material of the soil (Fadigas et al., 2010; Kabata-Pendias, 2011). These properties influence the availability, mobilization, and sorption of trace elements and determine their concentration in the soil and their potential impact on plant growth (Chen et al., 1999; Golia, 2001; Kabata-Pendias, 2011). Understanding these interactions is essential for managing soil fertility and addressing both trace element deficiency and toxicity in agricultural systems, as they directly affect plant growth and productivity.

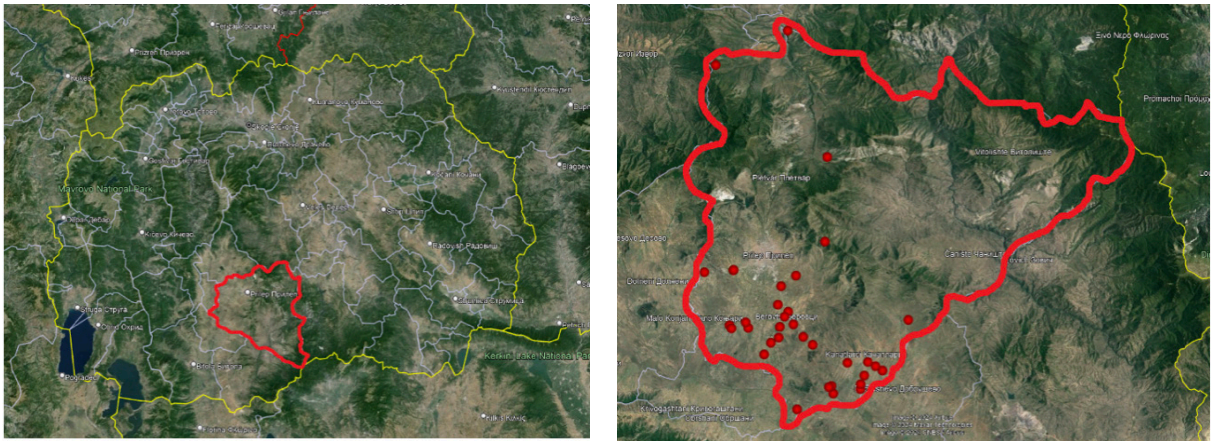
The main aim of our research was to monitor the condition of soils in tobacco cultivation, focusing in particular on the presence and distribution of important macro and microelements, including some potentially toxic element content, to assess their potential impact on plant health and productivity.

MATERIAL AND METHODS

Soil samples from arable land in the Prilep region were collected in 2021 and 2022. The samples were collected from pedological profiles at a standardized depth of 0-30 cm, from 31 locations in the following municipalities: Alinci, Kanatlarci, Topolčani, Berovci, Erekovci, Malo Konjari, Golemo Konjari, Mazučiste, Galičani, Kadino Selo, Varoš). The sampling locations are shown in Figure 1.

The samples were prepared according to the ISO 11464:2006 Soil quality — Pretreatment of samples for physico-chemical analysis. Soil samples were digested using the aqua regia extraction method using HCl and HNO_3 in a 3:1 ratio (U.S. EPA, 2007). A representative sample of up to 0.5 g was digested in a laboratory microwave system (CEM, USA). Two groups of elements were analyzed: macro and microelements (K, P, Mg,

Fe, and Na), and potentially toxic elements (As, Cd, Cr, Cu, Ni, Pb, and Zn). The ICP-MS analyses were performed at the Research Institute for Analytical Instrumentation, University of Cluj, Romania. The instrumentation included a SCIEX Perkin Elmer Elan DRC II mass spectrometer (Canada) equipped with an inductively coupled plasma source, a quadrupole and a single detector. The cadmium content was below the detection limit of 1 ppb and is therefore not listed in the following tables. Soil fertility was assessed based on the measured concentrations of organic matter (OM), total nitrogen, available phosphorus and potassium, carbonatesand, clay, and pH (Pelivanoska, 2011).



a b

Figure 1. (a) The Prilep region on the map of North Macedonia and (b) the locations of the soil sampling sites within the region.

RESULTS AND DISCUSSION

The basic soil properties from the Prilep region, which give an indication of the general soil quality, are presented in Table 1. The average organic matter content in the cultivated soil samples ranges from low to moderate. According to Filipovski (1990), soils with low humus content provide favorable conditions for the cultivation of high-quality oriental aromatic tobacco. The total nitrogen content in the analyzed soils varied between 0.03% and 0.14%. The mean soil pH was 6.55, and most samples were slightly acidic to neutral. All soil samples were non-calcareous and had a wide range of available macronutrients, with phosphorus content ranging from 2.38 to 149.9 mg P_2O_5/100 g and potassium content from 7.69 to 43.11 mg K_2O/100 g. The plant available nitrogen, potassium and phosphorus content of the topsoil varies according to land use and is monitored and corrected annually, which explains the high coefficient of variation for these macronutrients (Tab.1). The soil samples collected were predominantly medium loamy in texture, with clay content ranging from 20.6% to 58.7% (Tab. 1). All analyzed soil parameters exhibited similar values to those previously reported by Jordanoska et al. (2014), indicating consistent soil characteristics typical of tobacco growing soils in the Pelagonian region.

The descriptive statistics of the values of total K_2O, P_2O_5, Mg, Fe, and Na are presented in Table 1. The total potassium content in soils is typically between 0.5% and 3% (as K_2O), depending on soil texture and mineralogy (Lalitha & Dhakshinamoorthy, 2013; Firmano et al., 2020). The potassium content of 0.153% (as K_2O) determined in this study is below the average of 0.3% reported by Jordanoska Shishkoska (2014) for tobacco fields in the Pelagonian region.

The descriptive statistics of the values of total K_2O, P_2O_5, Mg, Fe and Na are presented in Table 1.

Table. 1. Basic soil properties and descriptive statistics of the analyzed parameters.

Parameter	Minimum	Maximum	Mean	Median	SD	CV (%)
Organic matter, %	0.56	2.98	1.62	1.53	0.61	38
Total nitrogen, %	0.03	0.14	0.08	0.07	0.03	38
pH (H_2O)	5.46	8.17	6.55	6.56	0.63	10
pH (KCl)	4.20	7.20	5.33	5.25	0.72	14
$CaCO_3$	0.00	5.09	0.20	0.00	0.93	465
av. P_2O_5, mg/100 g	2.38	149.9	17.62	6.59	34.52	196
av. K2O, mg/100 g	7.69	43.11	17.21	14.61	8.50	49
Clay, %	20.6	58.7	35.33	32.30	9.26	26
P_2O_5, %	0.04	0.848	0.356	0.328	0.163	46
K2O, %	0.040	0.698	0.153	0.115	0.152	99
Mg, %	0.019	0.477	0.091	0.064	0.108	119
Fe, %	0.256	3.152	0.843	0.651	0.600	71
Na, %	0.001	0.034	0.005	0.003	0.006	135

av. - available; SD - standard deviation; CV - coefficient of variation

The total potassium content in soils is typically between 0.5% and 3% (as K_2O), depending on soil texture and mineralogy (Lalitha & Dhakshinamoorthy, 2013; Firmano et al., 2020). The potassium content of 0.153% (as K_2O) determined in this study is below the average of 0.3% reported by Jordanoska Shishkoska (2014) for tobacco fields in the Pelagonian region. According to the Geochemical Atlas of Macedonia (Stafilov & Šajn, 2016), the average potassium content in soils of the Pelagonian region is 2.3% based on total digestion. Phosphorus is a vital nutrient for plant growth, and its content in agricultural soils, like potassium, varies greatly depending on soil type, mineral composition, and management practices. Phosphate fertilizers used in agriculture to replenish the amounts of this macroelement in the soil can contain significant concentrations of heavy metals, depending on the origin of the phosphorus and the appetites used in their production (Alkorta et al., 2004).

The Fe content in the agricultural soil samples from the Prilep region (Tab. 1) is significantly lower (0.843%) than the mean iron content of 3.1% in the soils of the Pelagonian region and 3.6% in the soils of the entire country according to the Geochemical Atlas of Macedonia (Stafilov & Šajn, 2016). In addition, the mean Fe content is also significantly lower than the European average for agricultural topsoil, which is 2.6% based on total digestion (Salminen et al., 2005; Soriano-Disla et al., 2013). The average sodium content in soils from tobacco fields in the Prilep region is 0.005%, which corresponds closely to the mean value of 0.004% (or 41 mg/kg) reported by Jordanoska Shishkoska (2014) for soils in the Pelagonian region. Table 2 summarizes the descriptive statistics of As, Cr, Cu, Ni, Pb, and Zn in soils of tobacco fields in the Prilep region. Contamination of agricultural soils with arsenic is a pressing global concern due to its toxicity and potential to enter the food chain. Both natural processes and human activities contribute to elevated arsenic levels in soils. Uncontaminated soils usually contain arsenic concentrations around 5 mg/kg (Gonga et al., 2020; Rahman et al., 2023). The average arsenic concentration in agricultural soils used for tobacco cultivation in the Prilep region is 5.45 mg/kg (Tab. 1) and thus corresponds exactly to the average levels found in uncultivated soils in the Pelagonian region (5.6 mg/kg), and the wider average for soils in North Macedonia (9.2 mg/kg) (Stafilov & Šajn, 2016).

Table 2. As, Cr, Cu, Ni, Pb and Zn content in tobacco-growing soils in the Prilep region (in mg/kg).

Element	Mean	Median	SD	Minimum	Maximum	CV
As	5.45	3.43	4.53	1.30	17.70	83
Cr	10.92	8.60	9.70	3.17	47.21	89
Cu	8.39	6.31	7.29	2.34	34.31	87
Ni	8.67	6.48	6.95	3.06	32.99	80
Pb	10.67	7.87	12.93	3.49	78.13	121
Zn	16.71	12.74	12.50	5.09	63.31	75

SD - standard deviation; CV - coefficient of variation

As shown in Table 2, the chromium content (10.92 mg/kg) in the soils of the Prilep region is significantly lower than the average value of 67 mg/kg reported for non-cultivable soils in the Pelagonian region (Stafilov & Šajn, 2016). Both values are below the target value for chromium in soils established by the Dutch standards (The New Dutch List), indicating that there is no potential contamination. Although the concentrations found are not critical, anthropogenic activities such as industrial emissions, improper waste disposal, and the intensive use of agrochemicals are considered to be the main sources of chromium accumulation in soil. Elevated levels of this element are of particular concern for the environment, as chromium is known to be highly toxic. It can impair soil microbial communities, reduce nutrient availability, and negatively impact plant growth and productivity (Zulfiqar et. al., 2023).

The copper content in agricultural soils typically between 1 and 50 mg/kg, depending on factors such as the parent material, the organic matter content, and the anthropogenic input (Hodges, 1995). The mean copper concentration in agricultural soils in the Prilep region (mean value of 8.39 mg/kg), as presented in Table 2, is significantly lower than the mean value of 21 mg/kg reported for non-cultivable soils in the Pelagonian region (Stafilov & Šajn, 2016). This difference can be attributed to lower anthropogenic pressure in the cultivated areas or to differences in the natural soil composition. Copper is an essential micronutrient for plant metabolism, involved in enzymatic activities and photosynthesis; however, both deficiency and excess can have adverse effects on plant health. The lower levels found in the soil from the Prilep region suggest adequate but not excessive availability, which is favorable for the cultivation of sensitive crops such as oriental tobacco.

Nickel concentrations in agricultural soils are typically between 3 and 1000 mg/kg (Kamboj et. al., 2018), depending on soil type, parent material, and anthropogenic influences. According to the data presented in Table 2, the mean nickel content in the soils of the Prilep region (8.67 mg/kg) is significantly lower than the average value of 30 mg/kg reported for non-cultivable soils in the Pelagonian region (Stafilov & Šajn, 2016). This suggests that the tobacco-growing soils in the study area are not impacted by elevated nickel levels and fall within the range considered typical for uncontaminated agricultural soils.

The lead content in soils is usually between 10 and 50 mg/kg in uncontaminated areas, but can exceed 100 mg/kg in regions affected by anthropogenic activities (Kabata-Pendias, 2011). The mobility of lead in plants is highly restricted, as only about 3% of the lead absorbed by the roots being translocated to the aerial parts, such as the stems (Collin et. al., 2022). According to the data presented in Table 2, the mean lead concentration in the tobacco-growing soils of the Prilep region (10.67 mg/kg) is lower than the average value of 70 mg/kg given in the Geochemical Atlas of Macedonia for non-cultivable soils in the Pelagonian region (Stafilov & Šajn, 2016).

The zinc content in soil, which ranges from 10 to 300 mg/kg (Kabata-Pendias, 2011), is also influenced by several factors including soil texture, organic matter content, and pH. Zinc concentrations in the soil of the Prilep region (16.71 mg/kg) were within the expected range for agricultural soils, indicating a balanced availability of this essential micronutrient for plant growth.

The mean values of all elements presented in Table 2 are comparable with the average concentrations reported in studies on agricultural soils throughout Europe (Salminen et al., 2005; Soriano-Disla et al., 2013).

CONCLUDING REMARKS

This study provides a comprehensive assessment of the agrochemical and elemental composition of soils used for oriental tobacco cultivation in the Prilep region, a major tobacco-growing area in North Macedonia. The results revealed considerable variability in the concentrations of macro- and microelements (K, Mg, Fe, Na), primarily influenced by differences in land management practices and the natural geological and pedological diversity of the region. Despite long-term and intensive tobacco production, this variability did not indicate contamination.

The evaluated soils were generally classified as having low to moderate organic matter content, low total nitrogen, slightly acidic to neutral pH, and variable phosphorus and potassium availability, reflecting a diversity of fertility levels across the region. The predominant soil texture was medium loam with moderate to high clay content, which is favorable for high-quality oriental tobacco cultivation.

Importantly, the concentrations of potentially toxic elements (As, Cd, Cr, Cu, Ni, Pb, Zn) remained below internationally recognized thresholds for heavy metals in agricultural soils. These findings confirm that the soils in the Prilep region are ecologically safe and agronomically suitable for sustainable oriental tobacco production.

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ХЕМИСКА КАРАКТЕРИЗАЦИЈА НА ТУТУНСКИ ПОЧВИ ВО ПРИЛЕПСКИОТ РЕГИОН: ЕКОЛОШКИ И ЗЕМЈОДЕЛСКИ АСПЕКТИ

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Резиме

Квалитетот на почвата игра суштинска улога во земјоделската продуктивност, особено при одгледување тутун, каде што се неопходни и висок принос и врвен квалитет. Елементниот состав на почвата, особено рамнотежата помеѓу есенцијалните хранливи материи и потенцијално токсичните елементи, значајно влијае врз квалитетот на почвата и развојот на растенијата. Оваа студија го оценува квалитетот на почвите во Прилепскиот Регион во Северна Македонија, главната област за производство на ориентален тутун. Почвените примероци беа земени од избрани тутунски површини за време на вегетациските сезони во 2021 и 2022 година и анализирани со индуктивно спрегната плазма масена спектрометрија (ICP-MS) за определување на концентрациите на избрани макро- и микроелементи (K, Mg, Fe и Na), како и на потенцијално токсични елементи (As, Cd, Cr, Cu, Ni, Pb и Zn). За целосна проценка беа анализирани и неколку агрохемиски параметри, вклучувајќи содржина на органска материја (од ниска до умерена), вкупен азот (0,03–0,14 %), pH на почвата (просек 6,55; слабо кисела до неутрална), достапен фосфор и калиум, физичка структура на почвата (средно глинеста текстура) и содржина на глина (од 20,6 % до 58,7 %). Концентрациите на макро- и микроелементи се тесно поврзани со геолошко-педолошките карактеристики на регионот, додека нивото на потенцијално токсични елементи е под меѓународно прифатените прагови за тешки метали, што укажува на низок ризик од загадување и потврдува погодност на овие почви за одржливо производство на тутун.

Клучни зборови: *почва, тутунски полиња, макроелементи, микроелементи, потенцијално токсични елементи, ICP-MS.*

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THE SUCCINATE DEHIDROGENASE INHIBITOR FUNGICIDES: FUNGAL RESISTANCE AND ITS MANAGEMENT

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Abstract

Effective disease management is essential to mitigate the rapid emergence of resistant pathogen populations. An important group of fungicides that play a pivotal role in the integrated management systems, among others, also because of their low environmental toxicity, are succinate dehydrogenase inhibitors, which act by binding to the mitochondrial complex II of the respiratory system. Unlike first-generation SDHIs (e.g., carboxin and oxycarboxin), which exhibit high efficacy against basidiomycetes, newer compounds in this class (e.g., cyclobutrifluram, furametpyr, and inpyrfluxam) demonstrate broad-spectrum activity against a wide range of fungal species. However, their repeated and inadequate application strategies, can exert strong selection pressure, favoring the development of resistant fungal genotypes, which may ultimately compromise fungicide efficacy. This review examines both historical and recent advancements in understanding the molecular mechanisms underlying SDHI resistance, as well as other factors influencing the evolution of resistance. In addition, it provides an insight into strategies for the effective use of newly developed SDHI molecules and highlights key research directions for combating resistance in the future.

Key words: *SDHI fungicides, resistance, plant protection, mode of action, fungicidal activity.*

INTRODUCTION

According to FRAC, fungicides that inhibit the succinate dehydrogenase (SDH) enzyme belong to the complex II inhibitors and are classified in group 7, which is comprised of 24 compounds that belong to 12 chemical groups (Tab. 1) (FRAC, 2024). Additionally, complex II inhibitors are also useful acaricides, insecticides, nematocides, and medicinal fungicides. Some of them are showing very high degrees of species selectivity (Earley, 2019). Shortly, after their introduction on the market, SDHI fungicides significantly impacted crop protection and by 2015 achieved nearly 8% of the total pesticide market, generating approximately €1 billion in turnover (Hermann & Stenzel, 2019). The first generation of fungicides that act as succinate dehydrogenase inhibitors (SDHI) belong to the chemical group of carboxamides and have a general structure of anilides (phenylamides) (R–C (=O)–N (–R')– C 6 H 5). The key factor enabling long-term research on these compounds is their relatively low toxicity, as most of them exhibit

LD_{50} values above 1500 mg/kg in land vertebrates. Another driver of sustained research interest in SDHI is the limited but promising fungicidal spectrum of carboxamide early compounds, and along with their structural flexibility, led to extensive exploration of both carbocyclic and heterocyclic scaffolds with various functional group substitutions. The advances in recognizing and understanding the enzyme target's structure and the mechanism of action of carboxylic amides have underscored the ongoing scientific and commercial focus on SDH inhibition. The earliest compounds in this class of fungicides were carboxin (1968) and oxycarboxin (1971), active mainly against basidiomycete pathogens (Von Schmeling & Kulka, 1966). In the next period (1971 – 1997), other SDHI such as benodanil, fenfuram, flutolanil, furametpyr, mepronil, and thifluzamide were introduced on the market. These SDHI fungicides possess limited activity against other pathogens except basidiomycetes. Following the first generation of SDHI molecules, the discovery of boscalid in 2003 with an increased spectrum of activity and potency heralded the age of synthesis of new SDHI molecules (Glättli et al., 2011). Novel SDHI fungicides (isofetamid, fluopyram, isoflucypram, pydiflumetofen, pyraziflumid, fluorine substituted pyrazol-4-yl-carboxamides, and phenyl-cyclobutyl-pyridineamide- cyclobutrifluram), has been established on the market, since 2008 are characterized with an extremely broad spectrum of activity not only against Basidiomycetes, but also against various Deuteromycetes and Ascomycetes and recognized by their application rates in many different crops (Tab. 1) (Stammler et al., 2007).

BIOLOGICAL ACTIVITY AND APPLICATION

The first discovered compound of SDHI fungicides, carboxin, was used predominantly for seed dressing against Rhizoctonia spp. in cereals and other crops, and it was also effective against smuts (*Ustilago spp.* and *Tilletia spp.*). Oxicarboxin, the structurally similar compound to carboxin, was used predominantly to control rust diseases, especially in cereals, ornamentals, and turf (Glättli et al., 2011). The next two compounds discovered, namely, benodanil and fenfuram, were shown to have similar activity and were also used for seed dressing. Mepronil and flutolanil are benzoic acid derivatives with very similar structures and activity that differ only by the fluorination of a methyl group in flutolanil. The activity of these compounds is similar to that of the previous ones, with application not only via seed treatment, but also with soil incorporation, or foliar spray (Stammler et al., 2015). The thiazole carboxamide–thifluzamide is still in use in some countries such as Asia and Latin America. It is used to control soil-borne and foliar fungal diseases caused by *Basidomycetes spp.*, particularly *Rhizoctonia solani*. One of its most important uses is to control Sheath blight in rice, Limb rot in peanuts, and Black scurf in potatoes. The largest subgroup of SDHIs, the pyrazole-4- carboxamides, comprises eleven active compounds. Their broad spectrum of activity is due to the presence of a pyrazole ring substituted at the 4-position with a carboxamide group. They are especially used in cereals to control *Septoria*, Rusts, Net blotch, Powdery mildew, and *Rhynchosporium*, and also can be effective against *Botrytis*, *Alternaria*, and *Sclerotinia* in vegetables and fruits (Dong et al., 2013). Fluopyram and cyclobutrifluram are unique among SDHIs with dual action as broad-spectrum fungicides and nematicides (Flemming et al., 2025; Schleker et al., 2022). Their fungicidal properties are documented against *Botrytis spp.*, *Alternaria spp., Sclerotinia spp.*, Powdery mildews, Anthracnoses, and Septorioses in grapes, apples, strawberries, cucurbits, tomatoes, and cereals (Flemming et al., 2025; MDA, 2012). In addition, they are also recognized for nematocide activity against the most important nematode genera of vegetables such as *Meloidogyne spp.*, *Pratylenchus spp.*, and *Heterodera spp.*, which attack soybean, sugar beets, and canola (Schleker et al., 2022). Isofetamid is a systemic fungicide used primarily to control *Botrytis*, *Monilinia*, and *Sclerotinia* diseases, in high-value horticultural crops such as grapes, strawberries, lettuce, tomatoes, beans, stone fruits, and ornamentals (Nishimi et al., 2024). Isoflucypram belongs to a novel subclass of SDHIs, characterized by an N-cyclopropyl substitution, which confers an altered binding mode at the ubiquinone binding site of the succinate dehydrogenase enzyme. This structural innovation contributes to its high intrinsic activity and broad-spectrum

effectiveness. It provides robust protection against key cereal diseases, including Septoria leaf blotch, Yellow and Brown rust, Eyespot, and Powdery mildew (Desbordes et al., 2020). Another SDHI, introduced by Syngenta in 1916, is pydiflumetofen, which has a unique chemical structure of N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide. In pydiflumetofen, the amide nitrogen (N–) is substituted with a methoxy group (–OCH_3) and a phenyl-ethyl side chain in the base structure represented by a pyrazole ring with a carboxamide group (-CONH-) at the 4-position (Padmathilake et al., 2022). The polarity and electronic effects of the N-methoxy group enhance lipophilicity and binding interactions, which enhance the interaction with the SDH enzyme and improve systemic movement in plant tissues (Walter, 2016). Pydiflumetofen, just as other novel SDHI fungicides, poses a broad-spectrum activity against powdery mildew, septoriosis, cercospora leaf spot; *Alternaria*, scab and grey mould in various crops such as soybeans, cereals, vegetables, including carrots, parsnip, corn, peanut, cucurbits, potato, grapes, melon, etc. (Padmathilake et al., 2022).

MODE OF ACTION

Succinate dehydrogenase inhibitors are a group of fungicides that target a crucial step in fungal energy metabolism. They exert their fungicidal activity by interfering with the mitochondrial respiratory chain, specifically targeting the succinate dehydrogenase (SDH) enzyme, which is also known as Complex II or succinate-ubiquinone oxidoreductase. It is the smallest complex in the mitochondrial respiratory chain and a functional part of the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, and also the mitochondrial electron transport chain (ETC) (Glättli et al., 2011). The complex comprises of four primary proteins (subunits): SdhA, SdhB, SdhC, and SdhD. The catalytic subunit SdhA is a flavoprotein responsible for succinate oxidation and contains a covalently bound FAD cofactor. An iron-sulfur protein SdhB contains three iron-sulfur clusters ([2Fe-2S], [4Fe-4S], and [3Fe-4S]) responsible for transferring electrons from $FADH_2$ to ubiquinone (Skinner et al., 1998). The membrane-bound subunits SdhC and SdhD form part of the cytochrome b and embed the complex in the inner mitochondrial membrane. These subunits contribute to the formation of the ubiquinone-binding site (Q-site), often in close proximity to the [3Fe-4S] cluster and a heme b prosthetic group. SDH act by catalyzing the oxidation of succinate to fumarate. This reaction also leads to reduction of flavin adenine dinucleotide (FAD), generating $FADH_2$ in the TCA cycle. In its second role, SDH transfers electrons from $FADH_2$ to ubiquinone (coenzyme Q), which is then reduced to ubiquinol. This transfer contributes to the proton motive force used by ATP synthase to generate ATP through oxidative phosphorylation (Cecchini, 2003). In fact, SDH represents a key link between substrate oxidation and energy generation (Keon et al., 1991). Modern SDHIs are defined by their capacity to interact with the ubiquinone-binding site (Q-site) located in the SdhB, SdhC, and SdhD subunits of the SDH enzyme complex. By blocking this site, SDHIs prevent the normal transfer of electrons from $FADH_2$ to ubiquinone and prevent the reduction of ubiquinone, disrupting the electron transport chain and leading to energy depletion in fungal cells. Normally, the oxidation of succinate, generates electrons that are passed through FAD and Fe-S clusters to ubiquinone. In the presence of SDHIs, electrons are unable to move through the electron transport chain because the SDHIs occupy the same site as ubiquinone, acting competitively or non-competitively, impairing the proton gradient across the mitochondrial membrane (Sierotzki and Scalliet. 2013). This leads to a failure in oxidative phosphorylation and a consequent decrease in ATP synthesis.

Table 1. Classification and representatives of SDHI fungicides (FRAC Code 7) according to the Fungicide Resistance Committee (FRAC, 2024).

Chemical or biological group	Common name	Company and year of first registration	Status in EU
phenyl-benzamides	benodanil	BASF, 1974	not approved
	flutolanil	Nihon Nohyaku Co., 1986	15/06/2025
	mepronil	Kumiai Chemical Industry Co., 1981	not approved
henyl-oxo-ethyl thiophene amide	isofetamid	ISK Biosciences, 2016	15/09/2026
pyridinyl-ethyl-benzamides	fluopyram	Bayer, 2012	30/06/2026
phenyl-cyclobutyl-pyridineamide	cyclobutrifluram	Syngenta, 2022	ni*
furan-carboxamides	fenfuram	Shell, 1974 (now Bayer CropScience)	not approved
oxathiin- carboxamides	carboxin	Uniroyal Chemical Co., 1968	not approved
	oxycarboxin	Uniroyal Chemical Co., 1971	not approved
thiazole- carboxamides	thifluzamide	Monsanto, 1997 (now Dow AgroSceince)	not approved
pyrazole-4-carboxamides	benzovindiflupyr	Syngenta, 2014	02/08/2026
	bixafen	Bayer, 2011	31/05/2025
	fluindapyr	FMC Corporation, 2019	pending
	fluxapyroxad	BASF, 2011	31/05/2025
	furametpyr	Sumitomo Chemicals, 1997 BASF, 2021	ni*
	inpyrfluxam	Sumitomo Chemical, 2020	pending
	isopyrazam	Syngenta,2010	not approved
	penflufen	Bayer, 2012	31/05/2025
	penthiopyrad	Mitsui, 2008	31/05/2025
	sedaxane	Syngenta, 2011	31/10/2027
N-cyclopropyl-N-benzyl-pyrazole-carboxamides	isoflucypram	Bayer, 2019	pending
N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides	pydiflumetofen	Syngenta, 2016	pending
pyridine- carboxamides	boscalid	BASF, 2003	15/04/2026
pyrazine-carboxamides	pyraziflumid	Nihon Nohyaku Co., Ltd.,2018	ni*

*ni – no information

So, fungal pathogens, which rely heavily on efficient mitochondrial respiration, such as the necrotrophic fungi *Botrytis cinerea*, *Alternaria spp., Fusarium spp., Sclerotinia sclerotiorum*, and hemibiotrophic *Zymoseptoria tritici*, some *Fusarium spp*., etc., are particularly vulnerable to this disruption. These fungi produce large amounts of cell wall-degrading enzymes, toxins, and secondary metabolites to kill host cells and feed on dead tissue. This requires high levels of energy (ATP) to support growth, sporulation, enzymatic degradation of plant tissues, and evasion of host defenses. High levels of energy are especially needed during spore germination and hyphal invasion, when glycolysis alone is insufficient for ATP production. Mitochondrial respiration is essential for these biosynthetic processes and for adapting to oxidative stress from plant defenses (Avenot & Michailides, 2010). The structural diversity among SDHIs allows for a broad spectrum of activity against various fungal pathogens.

MECHANISMS OF RESISTANCE

Fungal resistance to SDHI fungicides primarily arises through the target-site mutations in the succinate dehydrogenase (SDH) enzyme complex. These mutations alter the structure of the SDH complex in ways that reduce or prevent the binding of SDHI fungicides, while still allowing the enzyme to function in fungal respiration, and usually occur in SdhB, SdhC, and SdhD subunits. It is considered that the single-nucleotide polymorphisms (SNPs) in the genes encoding these subunits are responsible for amino acid substitutions (Broomfield & Hargreaves, 1992). Substitution of histidine (H), especially at position 272 is the most common mutation in the SdhB subunit associated with SDHI resistance (Tab. 2). Positively charged histidine acts as a proton donor or acceptor around physiological pH due to its pKa near 7, and it can be substituted with arginine (R), tyrosine (Y), leucine (L), vaniline (V), etc. Substitution with arginine (H272R) is the most frequently reported SdhB mutation. This mutation is commonly found in field isolates of Botrytis cinerea obtained from various crops (FRAC, 2021a). Substitution with leucine and tyrosine is also reported in *B. cinirea* (FRAC, 2021a). Similar histidine substitutions at position 272 have been observed in *Botrytis ecliptica, Stemphylium vesicarium, Podosphaera xanthii, Corynespora cassiicola, Didymella bryoniae,* and *Pyrenophora teres* (FRAC, 2015, 2021 a,b). Substitutions of asparagine (N) in the SdhB subunit, particularly at positions 225 or 230, have been documented in several fungal pathogens. The polar, uncharged amino acid asparagine is responsible for hydrogen bonding and structural stabilization in Sdh. Its substitution by more hydrophobic or charged residues can disrupt the SDHI binding pocket in SDH, leading to SDHI fungicide resistance. Aspargine substitutions at SdhB sections are identified at *B. cinirea* (N225T; N230I), *Zymoseptoria tritici* (N86S), *D. bryoniae* (N86S; N225S), and *Alternaria alternata* (N225S) (Tab. 3). Another well-documented mutation in SdhB subunit include proline substitutions, particularly at position 225 (Tab. 3). Proline has a rigid, cyclic structure that introduces kinks or turns in protein backbones (Hutchinson & Thornton, 1994). Substituting proline with a more flexible or chemically different amino acid (e.g., phenylalanine, leucine) can significantly alter the 3D structure of the SDH binding pocket. Such mutations are well documented in *B. cinerea* (P225H, P225T, P225L, P225F) and *D. bryoniae* (P225N) and are linked to multiple resistance against SDHIs such as boscalid, fluopyram, and isopyrazam (Bi et al., 2022; Liu et al., 2024). While mutations in SdhB are more frequently reported, SdhC mutations are increasingly recognized, especially in fungi like *Z. tritici, Pyrenophora teres, Alternaria spp., and B. cinerea*. Substitutions of histidine or serine with arginine (C-H134R; C-S135R) are among the most common mutations in Z. tritici. These mutations are well known to reduce binding of fluxapyroxad and bixafen and are frequently detected across Europe and other wheat-producing regions (Rehfus et al., 2017, 2018). Other frequently detected mutations in the SdhC subunit are recognized in *P. teres* (C-N75S, C-G79R, C-H134R, C-S135R) (Stammler et al., 2014). Mutations in SdhC in B. cinerea are less common but are documented on glicine (C-G84V, C-G79R), asparagine (C-N75S), and alanine (C-A85V) (Konstantinou et al., 2014; Leroux et al., 2010). Shao et al. (2022) conferred the C-A78V mutation in Fusarium. graminearum as precursor for pydiflumetofen resistance. These mutations are usually found in combination with SdhB mutations (Veloukas et al., 2014). C-H134R

mutation is documented in *A. alternata* isolated from pistachio (FRAC, 2021a) and SdhC-H151R in *Venturia inaequalis* (FRAC, 2021a). Mutations in SdhD subunit typically confer lower levels of resistance compared to SdhB and SdhC subunits, but they are still recognized to contribute to multi-side resistance when present alongside other mutations. Substitution of aspartic (D) with glutamic acid (E) in SdhD subunit is also frequently detected mutation in *Z. tritic*i, though its effect on resistance is usually moderate (Rehfus et al., 2018). Mutations in SdhD subunit are also documented in *P. teres (*SdhD-D124N/E, SdhD-H134R, SdhD-D145G), *Aspergilus orizae* (SdhD-D124E), B. cinerea (D-H132R), *A. alternata* (D-D123E, D-H133R), *A. solani* (D-H133R), *C. cassiicola* (D-G109V), *Sclerotinia sclerotiorum* (D-H132R) (FRAC, 2015; 2021a,b), etc. (Tab. 3).

While target-site mutations are the primary mechanism, evidence suggests the existence of additional or indirect resistance mechanisms that don't involve obvious genetic changes in SDH genes. These non-target site mutations include (i) Overexpression of Efflux Pumps and (ii) Metabolic Detoxification. Fungal cells have efflux pumps, which are proteins embedded in the cell membrane that actively transport toxic substances out of the cell. These pumps are part of the ATP-binding cassette (ABC) transporter family, and they use energy (ATP) to pump out harmful compounds, including fungicides. If a fungus produces more of these efflux pumps, it can remove SDHI fungicides from the cell before they reach the mitochondria, where their target (the SDH enzyme) is located (Sierotzki and Scalliet, 2013; Earley F., 2019). This reduces the effective concentration of the fungicide inside the cell, decreasing its toxicity. These mechanisms don't block SDHIs entirely but make them less effective because of the lower doses of SDHIs present in the fungal cell. Metabolic detoxification occurs in some fungi that produce detoxifying enzymes that can chemically modify or degrade fungicides, making them harmless before they can reach their site of action. These enzymes, for example, include cytochrome P450 monooxygenases, glutathione-S-transferases, or some other metabolizing enzymes (Sierotzki and Scalliet, 2013). Fungi with non-target resistance may survive low-dose treatments and typically provide low-level and partial resistance, eventually leading to more resistant populations. In addition, not all mutations are equally favorable. Some resistant mutants have reduced fitness, meaning they grow more slowly or are less competitive in the absence of the fungicide. However, some mutations confer resistance with little or no fitness cost, making them more likely to spread in field populations (Avenot & Michailides, 2010). Repeated use of SDHIs, especially as solo applications or with incomplete rotations, selects for resistant individuals. Once established, resistant strains can spread via spores, especially in polycyclic diseases like *Zymoseptoria tritici* (Rehfus et al., 2017). In some fungi, multiple mutations can occur in parallel, leading to a range of resistance levels depending on the specific SDHI used. For example, if non-target site mutations are combined with target-site mutations, they can amplify the resistance level. As it was mentioned before, even though all SDHI fungicides target the same site (ubiquinone-binding pocket) of the SDH enzyme, they don't all bind in exactly the same way. The main reason for this is because different SDHIs have different chemical structures. As a result, they interact with different amino acids within the Q-site or bind in slightly different orientations. When a mutation alters the shape or chemistry of the Q-site, some SDHIs may be more affected than others. For example, it is found that the mutation like SDHC-H134R greatly reduce the binding of boscalid, causing high resistance. The mutation also lightly affects the binding of fluopyram, leading to moderate or no resistance. This is because the new amino acid blocks or distorts only part of the binding pocket that boscalid needs, but not all SDHIs use the exact same part of the binding pocket (Avenot et al., 2011). As a result of this, the cross-resistance patterns in fungi can vary. Mutations in SDHC, especially at positions 84 and 134, are frequently associated with broad-spectrum resistance, which usually affects many or all SDHIs. The H134R mutations in *Alternaria alternata, Alternaria solani, and Didymella tanaceti,* have been linked to very high resistance levels to SDHI fungicides like boscalid and penthiopyrad (Förster et al., 2022; Bauske et al., 2017; Pearce et al., 2019). Some mutations may confer resistance to one SDHI (e.g., boscalid), but not necessarily to others (e.g., fluopyram or isopyrazam) (Tab. 2). Fluopyram often retains some activity even when other SDHIs fail due to a different binding conformation (Yamashita

& Fraaije, 2018). Cyclobutrifluram, the new-generation SDHI shows slightly different binding, but broad-spectrum mutations (A84V, H134R) and still confer high resistance (Li et al., 2023 a,b). Resistance mechanisms to SDHIs have been intensively studied in *B. cinerea*. The investigations confirmed that this ascomycetous pathogen developed serious resistance to multiple SDHIs such as boscalid, fluopyram, fluxapyroxad, and penthiopyrad in various crops (cucumber, grape, tomato, strawberry etc.).

Table 2. Common SDHI Resistance Mutations and Cross-Resistance Profiles to some SDHI fungicides (FRAC, 2023).

	SDHB-H267Y/L	SDHC-H134R/Y/Q	SDHC-S135R/N	SDHD-D123E/N	SDHC-A84V	SDHB-N225T	SDHC-G79R
Boscalid	High	High	High	Low–Moderate	High	High	High
Fluopyram	Moderate–Low	Variable	Moderate	Low	High	Low–Moderate	Moderate
Isopyrazam	Moderate	Moderate	High	Low	High	Low	High
Bixafen	Moderate	Moderate	High	Low	High	Moderate	High
Cyclobutrifluram	Moderate	High	High	Low	High	Low–Moderate	Moderate
Isofetamid	Low–Moderate	High	Moderate–High	Low	High	Low	High
Benzovindiflupyr	Moderate	High	High	Low	High	Moderate	High
Flutolanil	High	High	Moderate	Low	High	High	High

H – histidine; A -alanine; R – arginine; N – aspargine; D – aspartic acid; C – cysteine; E – glutamic acid; Q – glutamine; G – glycine; I – isoleucine; L – leucine; V – valine; Y – tyrosine, T – threonine; S – serine.

RESISTANCE MANAGEMENT

Fungal resistance development is significantly accelerated by the continuous use of fungicides with specific modes of action. However, using them occasionally alongside with unrelated fungicides reduces this risk. Resistance management strategies should balance long-term fungicide effectiveness with meeting farmers' needs and ensuring profitability for manufacturers. These strategies must be applied consistently over large areas, requiring cooperation from all involved supply companies and acceptance by farmers (Corkley et al., 2021). According to the specific measures related to SDHIs, they should always be applied preventively and in rotation with fungicides from different resistance groups. In this case, the total number of SDHI applications should not exceed three per year. If more than 12 fungicide applications per season are considered according to a specific protection program, SDHIs should comprise no more than one-third (33%) of the total applications. When combined

with other fungicides in a mixture, no more than two consecutive SDHI-containing applications should be applied. Also, they should include a pesticide or pesticides with a different mode of action and with proven efficacy against the target disease. When SDHIs are used alone and in mixtures throughout a season, the total number of applications containing SDHI fungicides should not exceed 50% of all fungicide applications for the season. Also, it is recommended foliar application in cereals to be in mixtures. When an SDHI fungicides are used as a seed treatment against low-risk foliar pathogens on cereals, there should be no implications regarding their use, while for pathogens with moderate or high resistance risk, application should be counted against the total number of applications (Tab. 2) (FRAC, 2022). When managing the resistance to SDHIs, field investigations and detection of the resistance level are of crucial importance. In case the field isolates show full or slightly decreased sensitivity and no impact on field efficacy is observed, the pathogen is considered as low-risk for the investigated area, and the general guidelines for the use of SDHI fungicides are considered sufficient (Tab. 3).

Table 3. List of fungal species with documented resistance to SDHI fungicides, the Sdh mutations identified, and the origin of the resistant isolates

Pathogen	**Sdh subunit**			**Area with high risk of resistance[1]**	**Reference**
	SdhB	**SdhC**	**SdhD**		
Alternaria alternata	H277Y/R, H277Y/R/L N235D/T/E/G, P230A/R/I/F/D	H134R, S135R	D123E, H133R H133P	Spain	Avenot et al.,2008
A. brassicae	ni	ni	ni	Germany	FRAC, 2024
A. brassicicola	ni	ni	ni	Germany	FRAC, 2024
Alternaria solani	H277Y/R, H278R/Y	H134R/Q	H133R D123E	Denmark, Belgium, Germany, the Netherlands Sweden	FRAC, 2021a
Aspergillus oryzae	H249Y/L/N,	T90I,	D124E	Lab	Shima et al., 2009
Aspergillus flavus		G91R			Yin et al., 2023
Botrytis cinerea	P225L/T/F, H272Y/R/L/V, N230I, K283N	A85V, A187F, G37S, G85A, I93V, M158V, P80H, V168I	H132R, V9A, I189L	Germany, Poland, Belgium, United Kingdom, Sweden, Portugal, Greece, Denmark, Norway	Yin,et al., 2011 Veloukas et al., 2011 Angelini et al., 2010 Samaras et al., 2016
Botrytis elliptica	H272Y/R			ni	FRAC, 2015
Blumeriella jaapii	H260R, I262V	S84L, N86S		ni	Yin et al., 2023
Corynespora cassiicola	H278Y/R, I280V	S73P, N75S	S89P, G109V V152I D95E, H105R	Brasil, China	Miyamoto et al., 2010 Yin et al., 2023 FRAC,2023
Clarireedia spp.	H267R	G91R, G150R		ni	Yin et al., 2023
Clarireedia homoeocarpa		G91R		ni	Yin et al., 2023
Didymella bryoniae	H277R/Y				Avenot et al., 2011

Didymella tanaceti	H277Y, I279V	S73P, G79R, H134R/Q, S135R	D112E, H122R	ni	Yin et al., 2023
Erysiphe necator	H242Y/R I244V B-H242R+C-G169S B-I244V+C-G169S	G169D/S A83V		Austria, France, Germany, Hungary, Portugal, Spain, Switzerland, Italy, Czech Republic, Slovakia, Greece, Croatia, Tuerkiye, Ukraine	Cherrad et al., 2018 FRAC, 2024
Fusarium graminearum		T73I, A78V, R86C		China	Yin et al., 2023
Monilinia spp.				No impact on field efficacy is reported	FRAC,2024
Phakopsora pachyrhizi		I86F N88S/D, H154R, G92R		Brasil, Paraguay	Yin et al., 2023 FRAC,2024
Podosphaera fusca		A86V, G151R, G172D	S121P, H137R	ni	Yin et al., 2023
Podosphaera xanthii	H272Y/R/L/V			France, Greece, Italy, the Netherlands, and Portugal	FRAC, 2021a
Pyrenophora teres	H277Y	N75S, G79R, H134R, S135R, R64K K49E	D124N/E, H134R, D145G; H134Y G138V	North-Western Europe (France, Germany, Ireland), United Kingdom	Stammler et al., 2014
Pyricularia oryzae	H245Y			ni	Yin et al., 2023
Puccinia hordei		I87F		No impact on field efficacy is reported	FRAC, 2024
Puccinia horiana		I88F		No impact on field efficacy is reported	Yin et al., 2023
Ramularia collo-cygni	T267I, N224T	H146R/L, H153R N164H, G167C, V184L N87S G91R G171D		France, Germany, Ireland, Slovenia, the Netherlands, UK	Yin et al., 2023 FRAC, 2024
Rhizoctonia solani	H249Y		F48L	ni	Yin et al., 2023
Sclerotinia sclerotiorum	H273Y,	H146R,	H132R	No impact on field efficacy is reported	Glättli et al., 2009
Sphaerotheca fuliginea	ni	ni	ni	France, Greece, Italy, the Netherlands and Portugal	FRAC, 2024
Stemphylium vesicarium	P225L, H272Y/R			Portugal, Italy	FRAC, 2021a,b
Ustilago maydis	H257L			No impact on field efficacy is reported	Keon et al., 1991 FRAC, 2024

Venturia inaequalis	T253I	H151R C-N85S		No impact on field efficacy is reported	FRAC, 2021a
Zymoseptoria tritici	N225T, N225I, H267Y/R/L, I269V,	T79N, W80S, N86S A84V, H152R, T79I, N86K, G90R,	H129E,	France, Germany, Ireland, Italy, United Kingdom	FRAC, 2021a Skinner et al., 1998; Scalliet et al., 2010; Scalliet et al., 2011; Fraaije et al., 2011

[1]according to FRAC; H – histidine; A -alanine; R – arginine; N – aspargine; D – aspartic acid; C – cysteine; E – glutamic acid; Q – glutamine; G – glycine; I – isoleucine; L – leucine; V – valine; Y – tyrosine, T – threonine; S – serine; ni – no information;

FUTURE PERSPECTIVES

Although routine monitoring and good agricultural practices can guide SDHI application strategies and delay the establishment of resistant populations, the issue is far more complex. The variation in sensitivity is common between species and among isolates from different geographic locations (Sierotzki & Scalliet 2013). Field and laboratory studies revealed the presence of naturally resistant fungal genotypes and cross-resistance patterns between SDHIs, as well as complex different t side mutations. Notably, in *Zymoseptoria tritici,* a major wheat pathogen, resistance to SDHIs is not solely limited to point mutations in the SDH subunits. Recent studies have identified two functionally redundant paralogs, SdhC and alt-SdhC, in certain field isolates (Steinhauer et al., 2019; Yin et al., 2023). These alternative subunits can be differentially expressed and are associated with variable sensitivity to SDHIs. A particularly striking example of this complexity involves high-level resistance phenotypes to the amide subclass of SDHIs, which have been linked to the insertion of transposable elements 182 base pairs upstream of the alt-SdhC start codon. This insertion appears to enhance the expression of *alt-SdhC,* which encodes a unique Qp-site residue that reduces SDHI binding efficacy (Stammler et al., 2015). Such genomic plasticity underscores the importance of considering both target-site and non-target-site resistance mechanisms in resistance management strategies. To better predict and counteract these evolving threats. Functional genomics plays a vital role in uncovering new resistance determinants and tracing their evolutionary trajectories. Insights from transcriptomic and epigenomic data can illuminate how regulatory changes, gene duplications, or horizontal gene transfers contribute to resistance. Moreover, this information feeds into predictive models of resistance emergence and spread, helping design more durable disease control strategies. In parallel, structure-guided design of next-generation SDHIs that can accommodate mutations or target alternative SDH configurations offers promising avenues for overcoming resistance. Continued development of advanced molecular diagnostics, including high-throughput sequencing, allele-specific PCR, and CRISPR-based detection systems, is critical for the early identification of resistant genotypes, particularly those carrying naturally occurring variants like *alt-SdhC.*

CONCLUSION

Succinate dehydrogenase inhibitors are frequently used in modern crop protection programs, valued for their broad-spectrum activity and relatively low environmental impact. However, their extensive and repeated use has led to the emergence and proliferation of resistant fungal populations, posing a growing threat to sustainable crop protection. Resistance is primarily driven by point mutations in the SdhB, SdhC, and SdhD subunits, which reduce fungicide binding affinity to the target site. The degree of resistance varies depending on the specific amino acid substitutions and their structural effects. Beyond these canonical mechanisms, other factors such as efflux pump overexpression, gene duplication, and regulatory

mutations, including transposon insertions near resistance-associated genes, make the situation more complex. Such resistance mechanisms have been documented in several economically significant pathogens, including *Zymoseptoria tritici, Botrytis cinerea,* and *Alternaria alternata*.

The continued reliance on SDHIs exerts strong selection pressure on pathogen populations, promoting the spread of resistance alleles at local, regional, and potentially global scales. The dynamics of resistance evolution are influenced by fungicide application strategies, pathogen biology, and the fitness costs associated with resistance mutations. Addressing this challenge requires a multidisciplinary approach. Integrating advanced molecular diagnostics, functional genomics, and population biology with agronomic practices and rational fungicide design will be essential. Improved resistance monitoring, deployment of integrated disease management strategies, and the development of next-generation SDHIs capable of overcoming existing resistance will help preserve the long-term efficacy of this important fungicide class. Sustained research and coordinated stewardship are vital to safeguarding crop yields and ensuring food security in the face of evolving fungal threats.

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ФУНГИЦИДИ ИНХИБИТОРИ НА НАДВОРЕШНИОТ КВИНОН, ПЕРСПЕКТИВНА ГРУПА НА ПРОИЗВОДИ ЗА ЗАШТИТА НА РАСТЕНИЈАТА

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Резиме

Ефикасното управување со растителните патогени е од суштинско значење за ублажување на појавата на популации од резистентни патогени. Значајна група на фунгициди кои играат клучна улога во интегрираните системи за управување со болестите кај растенијата, а, меѓу другото, и поради нивната ниска еколошка токсичност, се инхибиторите на сукцинат дехидрогеназа, кои делуваат на тој начин што се сврзуваат за митохондријалниот комплекс II од респираторниот систем кај габите. За разлика од првата генерација на инхибитори на сукцинат дехидрогеназа (на пр., карбоксин и оксикарбоксин), кои покажуваат висока ефикасност кон патогените од класата на базидиомицети, поновите соединенија од оваа група на фунгициди (на пр., циклобутрифлурам, фураметпир и инпирфлуксам) покажуваат широк спектар на активност кон различни видови на габи. Сепак, нивната несоодветна употреба може да го фаворизира развојот на резистентни генотипови, што ја намалува нивната ефикасност. Овој прегледен труд дава увид во молекуларните механизми на кои се должи отпорноста на габите кон SDHI, како и некои други фактори што влијаат на појавата на отпорност. Исто така, даден е увид во стратегиите за ефикасна употреба на новоразвиените SDHI молекули и предложени се клучните насоки на кои треба да се темелат идните истражувања за справување со резистентноста кон оваа група на фунгициди.

Клучни зборови: *инхибитори на сукцинат дехидрогеназа, резистентност, заштита на растенијата, механизам на делување, фунгицидно дејство.*

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AGROCHEMICAL CHARACTERIZATION OF SOILS FROM THE OVCHE POLE VINE DISTRICT: A CASE STUDY FROM TRI CHESHMI AND DOLNO TROGERCI

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Abstract

This study provides a physicochemical, and agrochemical characterization of vineyard soils in the Ovche Pole Vine District, located within the Povardarie Wine Region of North Macedonia. Two representative vineyard locations, Tri Cheshmi and Dolno Trogerci, were selected for comparative assessment based on their contrasting geological conditions. The analysis focused on key soil parameters, including pH, electrical conductivity (EC), organic matter (OM), organic carbon (OC), calcium carbonate ($CaCO_3$) content, texture, as well as available nitrogen (N), phosphorus (P) and potassium (K). The soils in Tri Cheshmi, developed over Neogene lacustrine sediments rich in marl and calcareous clay, showed alkaline pH, moderate carbonate levels and elevated EC, reflecting a strong pedogenic influence from the carbonate-rich parent material. In contrast, the soils in Dolno Trogerci, formed by colluvial-alluvial deposits with contributions from volcanic and metamorphic rocks from the Vardar zone, showed greater textural variability and higher levels of $CaCO_3$ content. The semi-arid climate of the region, characterized by hot, dry summers and moderately cold winters, further shapes soil development and fertility. This study provides a basic understanding of the physicochemical and nutrient-related soil properties in the Ovche Pole Vine District and supports the development of site-specific sustainable vineyard management practices.

Key words: *Ovche Pole Vine District, Povardarie Wine region, vineyard soils, physico-chemical properties.*

INTRODUCTION

Soil is a critical component of vineyard ecosystems, influencing vine development, grape quality and the sustainability of viticultural production. The success of vineyard management is largely dependent on understanding the physical, chemical and nutrient-related properties of soils, which affect water availability, nutrient uptake, root development and microbial activity. Parameters such as pH., electrical conductivity (EC), organic matter (OM), organic carbon (OC), calcium carbonate ($CaCO_3$), texture and essential nutrients like nitrogen (N), phosphorus (P) and potassium (K) are fundamental indicators of soil health and fertility.

The study area is located in the east-central part of North Macedonia and forms part of the Povardarie Wine region (Official Gazette of the Republic of Macedonia, No. 12, 1980; Official Gazette of the Republic of Macedonia, No. 74, 2024) This area is characterized by a semi-arid climate with hot summers and moderately cold winters, making it favorable for viticulture. A general geographical overview of the Ovche Pole Vine District and the vineyard sampling locations, Tri Cheshmi and Dolno Trogerci is presented in (Fig. 1).

Figure 1. Geographical location of the Ovche Pole Vine District within North Macedonia, including the vineyards sites Tri Chesmi and Dolno Trogerci in the Shtip Municipality.

Geologically, the Ovche Pole Vine District is part of the Vardar Zone, a major geotectonic unit in the region. Geological map of the Ovche Pole Wine District particularly around the city of Shtip, shows that this area belongs to the Vardar Zone (VZ) (Markoski & Mitkova, 2011), one of the main tectonic units in the Republic of North Macedonia, (Fig. 2).

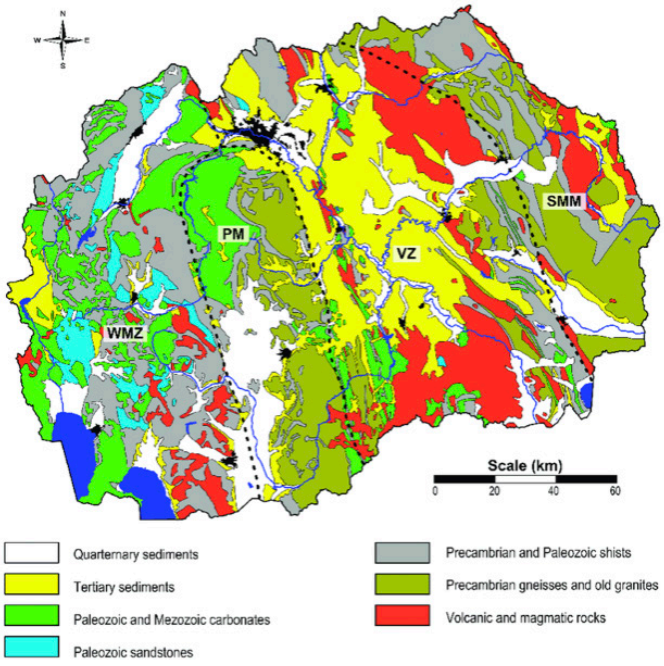

Figure 2. Lithological map of Macedonia, SMM - Serbo-Macedonian massif, VZ - Vardar zone, PM - Pelagonian massif, WMZ - West-Macedonian zone (Barandovski et al., 2012).

This zone is characterized by the presence of Tertiary volcanic rocks such as andesites and tuffs, as well as Neogene and Quaternary sedimentary formations, including marls and sandstones (Dumurdzanov et al., 2004; Dumurdzanov et al., 2005). These lithogenic formations have a significant impact on the soil composition in the region, which is crucial for agricultural activities, including viticulture. The geological structure of this area is dominated by a combination of volcanic (andesitic and pyroclastic) materials, as well as sedimentary (marl, sandstone and

conglomerates) and metamorphic rocks. The influence of these diverse geological formations contributes to the complex structure and composition of the soil. Volcanic materials enrich the soils with beneficial macroelements such as calcium, magnesium and iron. Sedimentary rocks affect the soil pH and nutrient retention capacity. The interaction between these base materials and pedogenic processes creates a heterogeneous soil profile that significantly affects soil fertility, and vine growth and productivity. The soil structure in the Ovche Pole Vine District is a product of the geological history, climate, and pedogenic processes that have shaped the area over time. The variety of volcanic, sedimentary, and metamorphic rocks present in this zone results in soils with distinct characteristics. This characteristics Influence viticulture, while also presenting certain challenges that require careful soil management.

Volcanic rocks, such as andesite, tuffs and pyroclastic, dominate the soil formation in parts of the Ovche Pole Vine District. These volcanic materials are rich in essential macroelements such as magnesium (Mg), calcium (Ca) and iron (Fe). When weathered, they contribute to clayey soils with high fertility, favorable for grapevine growth. These soils are usually characterized by good drainage, which is essential in regions with hot and dry summers. Well-drained soils encourage deep root penetration, promoting healthy vines and reducing the risk of root rot (Abad et al., 2021). The mineral content of volcanic soils also supports the health of the vine by providing a stable supply of nutrients that are essential for grape quality. In addition to macronutrients, volcanic soils often contain trace minerals and micronutrients such as zinc (Zn), copper (Cu), and boron (B), which are crucial for plant metabolic processes and disease resistance. These elements, although required in small quantities, can significantly affect vine growth, grape ripening, and the overall flavor of the wine (Pereira et al., 2021). In contrast, soils formed from sedimentary rocks, such as marls, sandstones, and conglomerates, are found in other parts of the Ovche Pole Vine District, particularly around Dolno Trogerci. These materials are more prone to weathering into finer-textured soils that tend to retain moisture and nutrients more effectively than volcanic soils. Soils rich in marl can lead to slightly alkaline conditions, which affect the availability of certain nutrients. The pH of the soil in Gorno Trogerci is typically higher, which can limit the availability of iron (Fe) and other micronutrients, creating a need for careful fertilization practices to ensure vine health (Markoski & Mitkova 2011). Sedimentary soils also tend to have a lower permeability compared to volcanic soils, meaning that water can be retained for longer periods, though excessive moisture can lead to reduced root aeration (Huggett, 2005) irrigation management in this region must take into account the retention of water in the soil, especially during the growing season, to avoid vine stress caused by over-saturation. However, the slightly higher fertility of these soils due to the nutrient retention properties supports grapevine growth, even if nutrient balance must be carefully monitored (Markoski et al., 2020).

The interaction between soil type and climatic conditions also plays a significant role in the Ovche Pole Vine District. The semi-arid climate, characterized by hot summers and cool winters, accelerates soil moisture evaporation in volcanic soils, leading to a requirement for irrigation during the growing season (Costa et al., 2023). However, the clay and silty characteristics of the soils at Gorno Trogerci retain moisture more efficiently, reducing irrigation needs but potentially increasing the risk of soil compaction if not properly manage (Mitkova et al., 2010). Thus, understanding both the soil characteristics and the geological makeup of the Ovche Pole Vine District is essential for optimizing vineyard management and ensuring high grape quality. The soil's texture, fertility, pH, and mineral composition are inextricably linked to the region's geological foundation, creating a complex yet fertile environment for grapevine cultivation that must be managed with precision to enhance vineyard productivity and grape quality.

Two representative vineyard locations within the Ovche Pole Vine District, Tri Cheshmi and Dolno Trogerci were selected for this study due to their contrasting lithological characteristic and landscape positions.

Tri Cheshmi is underlain by Neogene lacustrine sediments rich in marl and calcareous clays, which typically support the development of alkaline soils with moderately high calcium carbonate and elevated electrical conductivity. In contrast, Dolno Trogerci lies on colluvial-alluvial substrates with significant input from volcanic and metamorphic rocks originating from the Vardar Zone. Soils at this location are characterized by

higher $CaCO_3$ content, likely influenced by the accumulation and redistribution of carbonate material through slope processes and parent rock contributions.

While previous studies in the region have addressed broader geochemical frameworks and viticultural potential (Markoski et al., 2020), relatively few have focused on the fundamental physicochemical, pedological and agrochemical characteristics that are critical for evaluating the suitability of soils for grapevine cultivation (Mitkova & Mitrikeski, 2005). This study therefore aims to assess the key physical, chemical and nutrient related properties of vineyard soils from Tri Cheshmi and Dolno Trogerci. By establishing a detailed understanding of soil pH, EC, OM, OC, $CaCO_3$, texture and macronutrient levels (N, P, K), the study provides a valuable baseline for site-specific soil management of sustainable viticulture in the Ovche Pole Vine District.

MATERIAL AND METHODS

Soil sampling

A total of 18 representative soil samples were collected from three locations within the Ovche Pole Vine District. Sampling was performed in accordance with standardized procedures for soil collection in vineyard areas, as defined by ISO 18400-101:2017 and ISO 18400-104:2018. All samples were taken from a depth of 0–30 cm using a soil auger. The first two locations (L1 and L2) are situated near Tri Cheshmi, where samples were collected from two vineyard plots locally known as Ridot and Vucevi Livadi (Fig. 3a, b). The third location (L3) is in the vicinity of Dolno Trogerci, where sampling was conducted at three vineyard plots named Orman, Locva, and Bulin Dol (Fig. 3c). This sampling strategy was designed to capture the heterogeneity of vineyard soils influenced by variations in topography, vegetation cover, and geological conditions.

Figure 3. Location of soil sampling sites within the study area: a-Tri Cheshmi location, sampling site Ridot, b-Tri Cheshmi location, sampling site Vucevi Livadi, c-Dolno Trogerci location, sampling site Orman, Bulin Dol and Locva.

In vineyards block larger than 10 ha, the area was subdivided into smaller plots of approximately 1 ha. From each 1 ha plot, 15-20 individual soil cores were collected in a zig-zag pattern. This provides representative coverage of the field. These subsamples were thoroughly homogenized in the field. The quartering method was applied to reduce the volume of the composite sample. This resulted in representative samples with a mass of weighing between 1-1.5 kg. The exact locations of the sampling points were determined using GPS technology to ensure spatial accuracy and reproducibility. The coordinates and description of the sampling points are provided in (Tab. 1).

Sample preparation for analysis

The collected soil samples were dried in a laboratory oven at 40 °C for 48 hours. After drying, the samples were manually ground with a mortar and pestle. The samples were then sieved through a 2 mm mesh to remove coarse fragments and organic residues. For the analysis of organic matter (humus), the samples were sieved through a sieve with a mesh size of 0.25 mm. The prepared soil samples were stored in labeled paper bags and kept in a dry place until further laboratory analysis.

Used chemicals and reagents

All chemicals used for performing the chemical analyses of the soil samples were of analytical grade purity (p.a.). Sulfuric acid (H_2SO_4), lactic acid ($C_2H_4OHCOOH$), hydrochloric acid (HCl), nitric acid (HNO_3), oxalic acid (HCOOH), and orthophosphoric acid (H_3PO_4) were purchased from Sigma-Aldrich, Germany. Potassium permanganate ($KMnO_4$), potassium chloride (KCl), potassium sulfate (K_2SO_4), sodium hydroxide (NaOH), copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), potassium hydrogen phosphate ($KHPO_4$), potassium dichromate ($K_2Cr_2O_7$), iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$], tin (II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$), antimony potassium tartrate hemihydrate [$K(SbO)C_4H_4O_6 \cdot ½H_2O$], and ammonium acetate (CH_3COONH_4) were purchased from Merck (Darmstadt, Germany). The indicators phenolphthalein, diphenylamine, mixed indicator, as well as buffer solutions with pH 4, pH 7, and pH 10 were obtained from Alkaloid-Skopje. A standard soil sample with known content of the analyzed parameters (BIPEA Soil Terre 203-0115-0074) was also used during the chemical analyses of the soil samples.

Table 1. Labels, locations and coordinates of the unified soil samples.

Sample	Sample Label	Location	Place	Coordinates
1	L1S1	Tri Cheshmi	Ridot	41°46'29"N 22°07'23"E
2	L1S2	Tri Cheshmi	Ridot	41°46'33"N 22°07'39"E
3	L1S3	Tri Cheshmi	Ridot	41°46'18"N 22°07'32"E
4	L1S4	Tri Cheshmi	Ridot	41°46'23"N 22°07'47"E
5	L1S5	Tri Cheshmi	Ridot	41°46'10"N 22°07'40"E
6	L1S6	Tri Cheshmi	Ridot	41°46'13"N 22°07'54"E
7	L1S7	Tri Cheshmi	Ridot	41°46'03"N 22°07'46"E
8	L1S8	Tri Cheshmi	Ridot	41°46'04"N 22°08'00"E
9	L1S9	Tri Cheshmi	Ridot	41°45'54"N 22°07'49"E
10	L1S10	Tri Cheshmi	Ridot	41°45'56"N 22°08'04"E
11	L2S1	Tri Cheshmi	Vucevi Livadi	41°46'52"N 22°07'51"E
12	L2S2	Tri Cheshmi	Vucevi Livadi	41°46'52"N 22°07'46"E
13	L2S3	Tri Cheshmi	Vucevi Livadi	41°46'58"N 22°07'51"E
14	L2S4	Tri Cheshmi	Vucevi Livadi	41°46'57"N 22°07'31"E
15	L3S1	Dolno Trogerci	Locva	41°49'47"N 22°09'38"E
16	L3S2	Dolno Trogerci	Locva	41°49'35"N 22°09'34"E
17	L3S3	Dolno Trogerci	Orman	41°49'15"N 22°09'37"E
18	L3S4	Dolno Trogerci	Bulin Dol	41°49'19"N 22°10'23"E

Physico-chemical analysis of soil samples

Certified standard method were used to determine the physico-chemical and mechanical properties of the soil samples. These analyses included parameters such as pH, electrical conductivity, organic matter, organic carbon, available phosphorus, potassium and nitrogen, calcium carbonate content and soil texture classification. The following methods were applied:

- ISO 10390:2021 - soil quality, determination of pH in H_2O and KCl.
- ISO 11265:2024 - soil quality, determination of electrical conductivity (soil conductivity).
- ISO 10694:1995 - soil quality, determination of organic matter (humus).
- ISO 10693:1995 - soil quality, determination of carbonates, volumetric method.
- ISO 11261:1995 - soil quality, determination of total nitrogen by modified Kjeldahl method.
- ISO 11263:1994 - soil quality, determination of available phosphorus, spectrophotometric method with ammonium molybdate.
- ISO 11465:1993 - soil quality, determination of hygroscopic moisture and hygroscopic coefficient.
- ISO 11277:2020 - soil quality, determination of mechanical composition, pipette B method.
- ISO 11508:2017 - soil quality, determination of soil bulk density.

RESULTS AND DISCUSSION

Soil texture (mechanical composition)

The mechanical composition of the soil samples was determinate based on the relative proportions of sand, silt and clay. Soil texture classes were determined according to FAO/WRB classification system and USDA soil texture triangle (IUSS Working Group WRB, 2015; USDA, (1999). The results are presented in (Tab. 2).

The samples from the first location (L1) Tri Cheshmi, place Ridot, predominantly consist of sandy soils. The sand fraction varies between 68% to 91% with low clay content (1% to 6%). These soils are classified as sandy soils, which suggests that they are well-drained and may have lower nutrient retention. According to the literature (Kleber et al., 2021) sandy soils are typically low in organic matter but drain quickly, which could influence the irrigation and fertilization strategies for vineyards in the area. Soils from the second location (L2), Tri Cheshmi, place Vucevi Livadi, are characterized by a sandy loam texture, with sand content between 58% and 77%, clay content ranging from 2% to 3%, and silt between 21% to 27%. These soils are classified as sandy loam. Bulk density values range from 1.2 g/cm^3 to 1.5 g/cm^3, indicating that these soils are slightly denser that the ones from L1 location but steel exhibit good drainage properties. The higher clay content in location L2, compared to location (L1) suggests that these soils may have a better ability to retain nutrients and moisture, potentially leading to slightly higher fertility compared to the sandy soils from location (L1). The third location (L3), Dolno Trogerci has soils with significantly higher clay content, ranging from 17% to 20%, and much lower sand content between 18% and 31%. These soils are classified as silty clay loam (L3S1, L3S2, L3S3 and L3S4), with bulk density ranging from 1.1 g/cm^3 to 1.4 g/cm^3. The higher clay content and relatively lower sand fraction indicate that these soils have slower drainage rates and higher water and nutrient retention capacities (Filipovski, 2006). These properties are typically of clay-rich soils, which can retain more moisture but may also lead to drainage issues if not managed.The geological characteristics of the region play a crucial role in shaping the texture and overall fertility of vineyard soils. The Ovche Pole viticultural region is known for its ancient riverbed deposits, which contribute to the sandy texture of the soils. These soils tend to have lower clay and organic matter content, which can result in relatively lower water-holding capacity. The high sand content in location (L1), suggests that these location experiences good drainage, which is favorable for crops that require less water but can be challenging in terms of nutrient retention, requiring more frequent fertilization. On the other hand, location L3 has a more complex geological history with sedimentary deposits and clay-rich parent material.

This contribute to the high clay content in soils from Dolno Trogerci, making them

more fertile and capable of retaining water and nutrients. The clay-rich soils in L3, in particular, are more prone to waterlogging if not properly managed especially during heavy rains. These soils would benefit from proper drainage systems and soil amendments to optimize their structure for agricultural use (Jovanov et al., 2012).

Table 2. Texture classification of soil samples according to FAO/WRB classification system (2015) and USDA soil texture triangle (1999).

Sample	Coarse Sand, %	Clay and Silt, %	Clay, %	Fine Sand, %	Silt, %	Total Sand, %	Bulk Density, g/cm^3	Soil Type
L1S1	36	51	**4**	12	**47**	**48**	1.5	Sandy Soil
L1S2	34	16	**1**	50	**15**	**84**	1.6	Sandy Soil
L1S3	24	14	**1**	62	**13**	**86**	1.6	Sandy Soil
L1S4	21	9	**1**	70	**8**	**91**	1.7	Sandy Soil
L1S5	27	35	**6**	41	**29**	**68**	1.7	Sandy Soil
L1S6	30	14	**1**	56	**13**	**86**	1.5	Sandy Soil
L1S7	43	14	**1**	43	**13**	**86**	1.6	Sandy Soil
L1S8	35	12	**1**	53	**13**	**88**	1.5	Sandy Soil
L1S9	22	16	**2**	62	**14**	**84**	1.6	Sandy Soil
L1S10	32	18	**2**	50	**16**	**82**	1.6	Sandy Soil
L2S1	17	23	**2**	60	**21**	**77**	1.5	Sandy Loam
L2S2	17	24	**2**	41	**22**	**58**	1.4	Sandy Loam
L2S3	19	30	**3**	51	**27**	**70**	1.3	Sandy Loam
L2S4	16	25	**2**	59	**22**	**75**	1.2	Sandy Loam
L3S1	30	69	**20**	1	**49**	**31**	1.3	Powdery Clay Loam
L3S2	28	64	**20**	8	**44**	**29**	1.2	Powdery Clay Loam
L3S3	15	79	**17**	6	**62**	**21**	1.4	Powdery Clay Loam
L3S4	12	82	**10**	6	**72**	**18**	1.1	Powdery Clay Loam

L-location, S-sample

Chemical analysis

The chemical analysis of soil samples collected from Tri Cheshmi and Dolno Trogerci as part of Ovche Pole Vine District reveals a diversity in soil characterization influences by both anthropogenic processes and geological formations. The results from chemical analysis of soil samples are presented in (Tab. 3).

The studied locations are part of Ovche Pole Vine District, which is part of the Vardar Zone of North Macedonia. This region is characterized by Neogene-Quaternary sediments composed of marls, clays, sandstones, and occasional

volcanic materials. The soils in this region are typically alluvial and colluvial, developed under semi-arid to arid conditions, which is reflected in the calcareous nature of the parent material. The pronounced presence of calcium carbonate ($CaCO_3$), especially in Tri Cheshmi and Dolno Trogerci, is typical of soils derived from limestone-rich deposits. These conditions directly influence soil pH, nutrient retention and structure. The pH-H_2O across all samples ranged between 7.09 and 8.66, with most samples falling within the slightly to moderately alkaline range. This alkalinity is consistent with the calcareous composition of the soils, as corroborated by $CaCO_3$ content ranging from 0.31% to 25.4%. Samples from Dolno Trogerci (L3S3, L3S4 and L3S3) exhibit particularly high carbonate concentrations (>25%), likely due to the influence of lacustrine limestone sediments and reduced leaching in the semi-arid climate (Wilson, 1998). The high pH could limit the availability of micronutrients such as Fe, Zn and Mn, which is a typical concern for viticulture in alkaline soils. The electrical conductivity (EC) ranged from 250 to 481 µS/dm^3, suggesting low to moderate salinity across the sites. Although all values are within acceptable limits for grapevine cultivation, elevated EC in samples such as L3S3 and L3S4 may reflect the accumulation of soluble salts due to limited rainfall and poor drainage, common in regions with high evaporation rates and loamy-clay texture. Nitrogen levels were relatively low (0.98 to 1.22 mg/kg), aligning with semi-arid character and low organic matter content (OM ranging from 1.14% to 2.64%). The low organic carbon (OC ranging from 0.7% to 1.2%) further indicates limited organic input and microbial activity.

Table 3. Results from chemical analysis of soil samples.

Sample	pH-H_2O	pH-KCl	EC µS/cm	N g/kg	P_2O_5 mg/100g	K_2O mg/100g	OM %	OC %	$CaCO_3$ %	HM %	KH %
L1S1	7.86	7.46	394	0.99	19.2	24.8	2.13	1.16	1.22	0.50	1.005
L1S2	8.02	7.53	377	1.17	13.3	40.1	2.24	1.25	0.41	0.91	1.009
L1S3	7.48	6.79	323	0.98	24.8	65.5	1.74	1.05	7.13	0.85	1.008
L1S4	7.79	7.11	335	1.10	8.44	30.4	1.96	1.15	0.51	1.12	1.011
L1S5	8.09	7.41	326	1.15	42.9	50.1	2.22	1.25	0.31	1.16	1.011
L1S6	7.97	7.46	310	1.12	28.3	39.7	1.95	1.12	0.73	1.17	1.011
L1S7	8.02	7.51	298	1.01	19.9	41.3	1.83	1.11	0.75	1.21	1.012
L1S8	8.23	7.42	250	1.11	10.8	40.6	2.05	1.20	1.75	0.54	1.005
L1S9	7.82	7.46	442	1.01	9.06	42.7	1.71	0.95	2.11	0.54	1.005
L1S10	8.66	7.05	462	1.02	9.82	34.6	1.42	0.85	2.83	0.91	1.009
L2S1	7.74	7.42	314	1.17	26.6	30.1	1.24	0.72	7.79	1.21	1.012
L2S2	7.21	7.11	334	1.06	13.4	28.9	1.42	0.80	11.7	0.75	1.007
L2S3	8.16	7.41	341	1.07	28.5	43.6	1.96	1.16	10.5	2.82	1.029
L2S4	7.11	6.91	325	1.10	29.2	36.4	1.78	1.10	11.1	1.16	1.011
L3S1	7.78	7.41	377	1.22	24.6	46.5	2.46	1.45	8.69	0.95	1.008
L3S2	7.36	7.31	441	1.17	18.7	41.1	2.09	1.20	12.9	0.64	1.006
L3S3	7.62	7.42	481	1.12	9.99	45.2	2.04	1.20	25.4	0.56	1.005
L3S4	7.68	7.43	460	1.14	23.5	49.6	1.93	1.12	18.2	0.42	1.004

OM-organic matter, OC-organic carbon, HM- hygroscopic moisture, KH- coefficient of hydroscopicity

This suggests that nitrogen availability is likely to be a limiting factor for plant growth, especially in the absence of fertilization or cover cropping (Schleuss et al., 2020). Phosphorus (P_2O_5) was highly variable, ranging from 8.44 to 42.96 mg/100g. The highest levels were observed in sample (L1S5), possibly due to localized fertilization or natural phosphorus enrichment. However, due to the alkaline pH and high CaCO3, a significant portion of phosphorus may be present in forms less available to plan (Van Leeuwen & Seguin, 2006). Potassium (K2O) levels were generally high (24.52 to 65.51 mg/100g), particularly in samples from Tri Cheshmi (L1S3), potentially due to the presence of potassium rich minerals (feldspars, micas) in the parent rock or historical fertilization. High variability in $CaCO_3$ content (0.31% to 40.8%) further supports the geological heterogeneity of the study sites. Samples (L3S3, L3S4) suggest the influence of local calcareous bedrock or dust deposition from surrounding areas, consistent with aeolian input. The hygroscopic moisture (HM) and the coefficient of hydroscopicity (KH) values indicate that most soils have moderate moisture retention potential, thought the low organic content might reduce aggregation and structural stability.

Exploratory factor analysis (EFA) was performed on soil data collected from the Tri Cheshmi and Dolno Trogerci sites within the Ovche Pole Vine District. The analysis extracted four latent factors, collectively explaining 99.5% of the total variance (Tab. 4). This high cumulative variance suggests that the selected variables comprehensively capture the variability in the soil system influenced by both natural and anthropogenic factors. The four-factor analysis derived from the exploratory factor analysis (EFA) effectively summarized the multidimensional variability of the analyzed soil parameters from the Tri Cheshmi and Dolno Trogerci. Each factor captures distinct pedochemical and geogenic processes that influence soil formation and fertility in this semi-arid viticultural region.

Factor 1: This factor shows strong negative loadings for Organic Matter (OM = -0.95) and Organic Carbon (OC = -0.99) (Tab. 4). These variables are indicative of the biological activity and humification processes in soil. The high loadings suggest that this factor encapsulates the organic fertility status, likely influenced by both natural vegetation cover and anthropogenic inputs, such as vineyard practices. Soil richer in OM and OC typically show improved water retention, microbial activity and nutrient cycling (Reichenbach et al., 2021).

Factor 2: Dominated by Electrical Conductivity (EC = 0.78) and $CaCO_3$ ($CaCO_3$ = 0.81), this factor reflects the salinity and calcareous nature of the soils, which are strongly tied to the underlying geology (Tab. 4). The Tri Cheshmi region, in particular is characterized by Neogene-Quaternary lacustrine sediments rich in carbonates including marls, clays and sporadic limestone's. These substrates contribute to the accumulation of secondary carbonates and salts, resulting in the higher EC and $CaCO_3$ values. The soil exhibit low leaching potential due to the semi-arid climate, further favoring salt concentration. This factor is a geochemically significant indicator of pedogenesis in carbonate-rich, semi-arid environments.

Factor 3: This component is defined by pH-H2O and pH-KCl identifying the acid-base status and buffering capacity on the soil (Tab. 4). The positive loadings suggest that soils with higher pH values contribute more to this factor. The presence of carbonate minerals, particularly in the Tri Cheshmi area (L1, L2), loads to alkaline pH levels which affect nutrient solubility especially for phosphorus and micronutrients like Zn, Fe and Mn (Zhang, 2024). The buffering effects also stabilized pH across spatial and temporal scales. Geologically, this factor reflects the weathering of carbonate and parent materials, which are abundant in both study sites, though more prominent in Dolno Trogerci.

Factor 4: Factor 4 is positively associated with potassium (K_2O = 0.48) and negatively with nitrogen (N = -0.41) (Tab. 4). This suggests that may be governed by both soil mineralogy and anthropogenic inputs such as fertilization. Potassium is often present in primary minerals (feldspar and mica), while nitrogen is present in organic matter. The contrast between these nutrients may point to site-specific soil management practices, particularly in cultivated vineyards plots where fertilization regimes differ (Rashimi et al., 2020).

Table 4. Factor loading Matrix-Factor analysis (FA) of the analyzed soil samples.

Parameter	F1	F2	F3	F4	Comm.
pH-H_2O	-0.17	-0.26	**0.70**	0.15	0.62
pH-KCl	-0.56	0.01	**0.41**	-0.32	0.58
EC (µS/cm)	-010	**0.78**	0.32	0.14	0.74
N (g/kg)	-0.29	0.12	-0.06	**-0.41**	0.27
P_2O_5 (mg/100g)	-0.21	-0.29	-0.46	-0.04	0.35
K_2O (mg/100g)	-0.54	0.03	-0.22	**0.48**	0.57
OM (%)	**-0.95**	0.04	0.06	-0.04	0.91
OC (%)	**-0.98**	-0.01	-0.02	0.01	0.98
$CaCO_3$ (%)	-0.19	**0.81**	-0.21	0.09	0.75
E-Value	**0.98**	**0.84**	**0.70**	**0.55**	**0.64**
Variability (%)	**31.63**	**27.12**	**27.85**	**17.58**	

F1-loading of Factor 1, F2-loading of Factor 2, F3-loading of Factor 3, F4-loading of Factor 4, E-Eingene value, Comm-Communality, OM-organic matter, OC-organic carbon

Factor 4: Factor 4 is positively associated with potassium (K_2O = 0.48) and negatively with nitrogen (N = -0.41) (Tab. 4). This suggests that may be governed by both soil mineralogy and anthropogenic inputs such as fertilization. Potassium is often present in primary minerals (feldspar and mica), while nitrogen is present in organic matter. The contrast between these nutrients may point to site-specific soil management practices, particularly in cultivated vineyards plots where fertilization regimes differ (Rashimi et al., 2020).

The observed factor structure is consistent with the geological diversity of the studied area. The Tri Cheshmi location lies on carbonate-rich Neogene sediments contributing to elevated levels of $CaCO_3$ and electrical conductivity. The presence of marls, clay, lacustrine deposits, and sporadic tuffaceous material explains the buffering capacity and salinity of the soils. In Dolno Trogerci location includes soils developed on alluvial terraces, potentially with more heterogeneous mineral input, leading to more moderate levels of pH, EC and $CaCO_3$. These findings align with earlier studies on the Vardar Zone, where soils typically form over Proterozoic-Paleozoic granitites, and alluvial Quaternary sediments rich in base cations. The geogenic influences on Factor 2 and 3 loadings highlight the importance of substrate type in shaping soil chemistry especially in regions with limited precipitation and high evapotranspiration.

CONCLUDING REMARKS

The physicochemical, pedological and agrochemical assessment of vineyard soils from the Ovche Pole Vine District, specifically from Tri Cheshmi and Dolno Trogerci, reveals clear spatial variability shaped by underlying geology and semi-arid climatic conditions. Soils from Tri Cheshmi are predominantly sandy to sandy loam in texture, with high electrical conductivity, elevated calcium carbonate content and moderately low organic matter and nitrogen levels. These properties reflect the influence of Neogene-Quaternary lacustrine sediments rich in carbonates and marls, characteristic of Vardar Zone. In contrast, soils from Dolno Trogerci are fined-textured (silty clay loam), with higher clay content improved nutrient levels, shaped by colluvial-alluvial deposit influenced by metamorphic and volcanic rocks.

Soil pH in both locations ranged from slightly to moderate alkaline, consistent with the calcareous nature of the parent material, and may limit the bioavailability of certain micronutrients. The overall low nitrogen and organic carbon content across most samples suggest limited biological activity and a need for targeted organic matter enhancement. While phosphorus and potassium levels were more variable, their distribution appears influenced by both natural mineralogy and localized agricultural practices.

Factor analysis father emphasize the importance of organic matter, carbonate content, salinity and mineral buffering as key dimensions of soil variability in the region. These findings provide a critical baseline for the implementation of site-specific soil management practices, particularly in addressing water retention, nutrient supplementation and pH regulation. Such investigations are especially valuable for identifying micro-locational differences within vineyard sites, enabling precision viticulture adapted to the specific needs of each plot. Reorganizing the pedological and geological complexity of the Ovche Pole Vine District is essential for improving viticultural productivity and sustaining long-term soil health under semi-arid conditions.

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АГРОХЕМИСКА КАРАКТЕРИЗАЦИЈА НА ПОЧВИТЕ ОД ЛОЗАРСКИОТ РЕГИОН ОВЧЕ ПОЛЕ: СТУДИЈА НА СЛУЧАЈ ОД ТРИ ЧЕШМИ И ДОЛНО ТРОГЕРЦИ

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Резиме

Оваа студија обезбедува физичко-хемиска и агрохемиска карактеризација на почвите за винова лоза во лозарски регион Овче Поле, кој се наоѓа во рамките на Повардарскиот вински регион на Северна Македонија. Две репрезентативни лозја, Три Чешми и Долно Трогерци, беа избрани за компаративна проценка врз основа на нивните различни геолошки услови. Анализата се фокусираше на клучните параметри на почвата, вклучувајќи pH вредност, електрична спроводливост (EC), органска материја (OM), органски јаглерод (OC), содржина на калциум карбонат ($CaCO_3$), текстура, како и достапен азот (N), фосфор (P) и калиум (K). Почвите во Три Чешми, развиени над неогени езерски седименти богати со лапор и варовничка глина, покажаа алкална pH вредност, умерени нивоа на карбонат и покачена EC, што одразува силно педогено влијание од основниот материјал богат со карбонат. Спротивно на тоа, почвите во Долно Трогерци, формирани од колувијално-алувијални наслаги со придонеси од вулкански и метаморфни карпи од Вардарската зона, покажаа поголема текстурна варијабилност и повисоки нивоа на содржина на $CaCO_3$. Полусушната клима на регионот, карактеризирана со топли, суви лета и умерено ладни зими, дополнително го обликува развојот и плодноста на почвата. Оваа студија обезбедува основно разбирање за физичко-хемиските и нутритивните својства на почвите во лозовите насади од лозарскиот регион Овче Поле и поддржува развој на локациски специфични и одржливи практики за управување со лозјата.

Клучни зборови: *лозарски регион Овче Поле, вински регион Повардарие, почви за винова лоза, физичко-хемиски карактеристики.*

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DETERMINATION OF FREE HYDROCYANIC ACID IN HOMEMADE FRUIT BRANDIES

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Abstract

Fruit brandy is a traditional alcoholic beverage widely consumed in the Republic of N. Macedonia and other Balkan countries, produced by distillation of fermented fruits such as plum, apricot, quince and apple, using either homemade or industrial technology. This study aimed to evaluate the safety of 24 homemade fruit brandy samples by determining the content of free hydrocyanic acid (HCN), a potentially toxic compound. HCN is formed during alcoholic fermentation as a result of enzymatic hydrolysis of cyanogenic glycosides naturally present in fruit seeds. The quantification of free HCN was performed spectrophotometrically using König reaction, a colorimetric method based on the formation of cyanogen chloride, which reacts with pyridine and barbituric acid to form a stable pink complex with maximum absorbance at 580 nm. Results were recalculated to a 100% v/v ethanol basis to allowed comparison with the EU legal limit of 70 mg/L. All samples were within the permissible safety threshold. The highest HCN concentration was found in apricot and apple brandies (up to 9.81 mg/L), while plum and quince brandies contained significantly lower levels. A moderate correlation was observed between HCN levels and several chemical parameters, including methanol, aldehydes, ethanol, total esters, furfural and fusel alcohols. These results suggest that fruit type, fermentation conditions and the duration of seed contact during the preparation of the fruit mash before the fermentation play a critical role in HCN formation. This highlights the importance of controlled processing practices to ensure the safety of traditional fruit brandies.

Key words: *fruit brandies, free hydrocyanic acid, spectrophotometry.*

INTRODUCTION

The production of fruit brandies by distilling fermented fruit is a long-standing tradition throughout the Balkans, especially in North Macedonia, where it plays an important role in rural life and cultural identity (Petrova et al., 2024). In this study, analyses of homemade brandies made from different types of fruit were performed, including brandies produced from plum (*Prunus domestica*), apricot (*Prunus armeniaca*), apple (*Malus domestica*), and quince (*Cydonia oblonga*). Today, both homemade and commercial production continue to develop, helping to preserve traditional knowledge and support the local economy. However, homemade brandy production also comes with some safety concerns, especially when it comes to the final product.

One of the main risks of fruit brandies is the possible presence of hydrogen cyanide (HCN), a highly toxic and volatile compound. HCN is formed as a degradation by-product of cyanogenic glycosides, mainly amygdalin and prunasin, which are naturally present in seeds and stone fruits. During alcoholic fermentation, these glycosides are enzymatically hydrolyzed by *β-glucosidases* coming either from the fruit itself or from microorganisms involved in the fermentation process. The process releases

benzaldehyde, glucose and free HCN (Kuca et al., 2024). These reactions become more intense if the fruit seeds are damaged during processing or if the pulp remains in contact with the seeds for a long time (Ballhorn, 2005; Lee et al., 2021). Grinding or crushing the fruit activates certain natural enzymes such as linamarase or dhurrinase, which accelerate the degradation of these glycosides (Voldřich & Kyzlink, 1992). The steps of this process are shown in Figure. 1 (Nyirenda, 2020).

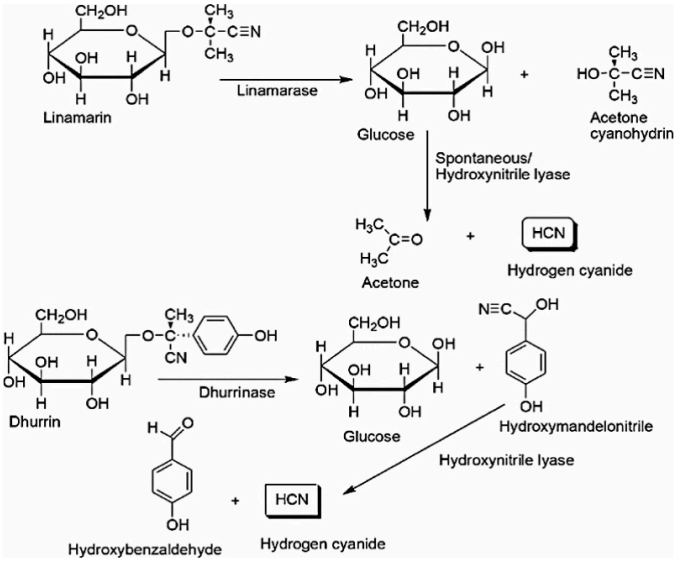

Figure 1. Mechanism of cyanogenic glycosides hydrolysis and spontaneous breakdown to hydrogen cyanide in stone fruit fermentation.

The process begins with enzymatic degradation of the sugar component, followed by degradation of the resulting nitrile compounds into free HCN. Aromatic aldehydes such as benzaldehyde are also formed as by-products (Novak et al., 2016). Considering the relatively low boiling point of HCN (26°C), it exhibits high volatility during distillation and can be co-distilled together with other volatile components (Liu et al., 2024). In domestic distillation, there are limited technological possibilities, the efficient separation of volatile fractions is at a low level and as a result there is a possibility of the appearance of toxic volatile components in the final distillate. One of these components is free HCN. From a health perspective, HCN is particularly dangerous because it blocks the activity of cytochrome oxidase in mitochondria, disrupting cellular respiration and causing oxygen deficiency at the tissue level (Claus & Berglund, 2005; Smith & Jernigan, 2019). The excess amount of HCN can lead to respiratory arrest, cardiovascular failure, and even death. Even at lower levels, long-term exposure to HCN has been associated with nervous system problems, hormonal imbalances that particularly affect thyroid function, and other cumulative toxic effects (Shmerling, 2025). In contrast, industrial distillation technologies typically involve fractionating columns and precise thermal control, which improves the removal of volatile contaminants and minimizes toxicological risk (Claus & Berglund, 2005; Bolarinwa et al., 2014). Regulatory frameworks, such as those of the European Commission, have subsequently imposed strict thresholds for the permissible concentration of HCN in alcoholic beverages. According to Regulation (EU) 2019/787 of the European Parliament and of the Council, the maximum permitted level of HCN in brandies produced from stone fruit is 7 g/hL (100% v/v) alcohol, which is equivalent to 70 mg/L. This regulation aims to protect consumers by limiting the concentration of free HCN. In view of the above risk, the analytical determination of free hydrogen cyanide in fruit distillates is of the utmost importance. Among the various available methodologies, spectrophotometric quantification using pyridine - barbituric acid reagent has emerged as the method of choice for

routine analysis due to its operational simplicity, cost-effectiveness and sufficient sensitivity (Sriprapat et al., 2014). This technique is based on the formation of a chromogenic complex with HCN, which gives a measurable absorption signal within the visible spectrum at 580 nm (Epstein, 1947). In this study, the content of free HCN was analyzed in 24 samples of home-made fruit brandies collected from the eastern region of the Republic of North Macedonia, including Berovo, Štip, Kočani and Veles and Kavadarci as part of the Tikveš region. The primary objectives were to determine the concentration of free HCN in these homemade fruit brandies and to assess their safety for human consumption based on the maximum permitted limits set by the EU. In addition to the determination of HCN, basic chemical analysis of fruit brandy samples was performed, including quantification of methanol, furfural, higher alcohols, total esters, sulfites, dry extract and ethyl acetate. The aim was not only to assess compliance with regulatory standards, but also to provide a comprehensive chemical characterization of the samples. Furthermore, this research aims to introduce a rapid and cost-effective spectrophotometric method for the routine determination of HCN, intended for use in both academic research and quality control laboratories.

MATERIAL AND METHODS

Sample collection

A total 24 samples of homemade fruit brandies were collected for the purpose of this study. All samples were traditionally produced through spontaneous fermentation of crushed fruit followed by classical distillation in copper pot stills. According to on-site interviews with local producers, the fermentation period lasted approximately 24 days and was carried out in plastic fermentation vessels under uncontrolled temperature conditions. In several cases, producers removing the stone and seeds prior the fermentation to reduce bitterness and potential cyanide release. However, other producers fermented the entire mush including pulp and seeds, which may have increased the probability of HCN formation due to enzymatic breakdown of cyanogenic glycosides. The collected samples originated from two district geographic regions from North Macedonia:

- The Eastern Region, which included samples from Štip, Kocani, and Berovo (Maleshevia region) – a region characterized by diverse microclimatic condition and traditional fruit-growing practices.
- The Tikveš Region, represented by Kavadarci and Veles, known for its rich viticultural heritage and long-standing tradition of fruit brandies production.

All brandy samples were transferred in the laboratory in 1L glass bottles, properly sealed and labeled with information regarding their geographical origin and fruit type. The samples were stored under appropriate conditions prior to analysis. Information on the location and type of fruit for each samples is provided in Table 1.

Sample preparation for analysis

Prior to analysis, the collected homemade fruit brandies sample were subjected to a redestilation step. This was necessary because several of the original samples were yellow colored, attributed to aging in oak barrels, a traditional practice used by local producers to enhance aroma and flavor characteristics. Coloration and matrix complexity could interfere spectrophotometric measurements too. A preliminary predestination was performed to obtain colorless distillates suitable for analytical determination. The redestilation was carried out using a standard simple distillation apparatus, without fractionation, to preserve the original volatile profile. For each 100 ml of sample was distilled, and 100 ml of distillate was collected for analysis. For the determination of free HCN, distillation was performed in the presence of H_3PO_4. The acidic environment facilitates release of HCN, as cyanogenic compounds and their anionic forms (such as CN-) are more readily converted to volatile molecular HCN under low pH conditions. This step is critical to ensure quantitative recovery of cyanide from the sample matrix during distillation (Claus & Böhme, 1980; Kawakami & Konno, 1980).

Table 1. Geographical location and fruit type of the analyzed homemade fruit brandy samples from North Macedonia.

Sample	Location (Municipality)	Fruit Type
S1	Berovo	Yellow Plum
S2	Berovo	Yellow Plum
S3	Berovo	Yellow Plum
S4	Berovo	Yellow Plum
S5	Štip	Yellow Plum
S6	Štip	Yellow Plum
S7	Berovo	Blue Plum
S8	Berovo	Blue Plum
S9	Štip	Blue Plum
S10	Kavadarci	Blue Plum
S11	Kavadarci	Blue Plum
S12	Veles	Blue Plum
S13	Kavadarci	Apricot
S14	Kavadarci	Apricot
S15	Kavadarci	Apricot
S16	Kavadarci	Apricot
S17	Berovo	Apricot
S18	Veles	Apricot
S19	Kavadarci	Quince
S20	Kavadarci	Quince
S21	Berovo	Quince
S22	Berovo	Apple
S23	Berovo	Apple
S24	Kavadarci	Apple

Chemical analysis

The chemical analysis of the fruit brandies samples was conducted in accordance with internationally recognized methods established by the International Organization of Vine (OIV). Methanol was determined spectrophotometrically using the chromotropic acid method, following preliminary oxidation of methanol to formaldehyde. This colorimetric method allows for quantitative detection based on chromogen formation, as described OIV-MA-AS312-03B Ethanol (alcoholic strength) was measured using the picnometric method in accordance with OIV-MA-AS312-01. Furfural content was determined according to OIV-MA-AS315-27. Total esters were quantified via acid-base titration, as described in OIV-MA-AS315-03. Ethyl acetate was determined spectrophotometrically, utilizing its absorption characteristics in the UV/Vis spectrum. The method complies with OIV-MA-AS315-03. Sulphur dioxide (SO_2) was determined using Iodometric titration method, in line with OIV-MA-AS323-04B, allowing quantification of total SO_2. Total dry extract was measured gravimetrically, based on the evaporation of a known sample volume and weighing of the residual non-volatile solids, according to OIV-MA-AS2-03.

Analysis of free HCN

The quantification of free hydrocyanic acid in the fruit brandies samples was performed using a spectrophotometric method based on the König reaction, in accordance with the standardized procedures described in OIV-MA-AS315-06A. This two-step colorimetric method involves the initial oxidation of cyanide ions (CN-) by Chloramine-T, forming cyanogen chloride (ClCN), which than reacts with pyridine and barbituric acid to form a stable pink-colored polymethine complex. The complex exhibits maximum absorbance at 580 nm, enabling sensitive and specific quantification of free HCN.

Each sample (100 mL of brandy) was redistilled in the presence of H_3PO_4 which provides an acidic environment to enhance the release of free HCN. A total of 100 mL of distillate was collected and used for the analisys. From the distillate, 25 mL was pipetted into a volumetric flask of 50 mL. Than 1 mL 3% Chloramine-T was added. After 1 min 10 mL of phosphate buffer (pH 7.6) was added, followed by 3 mL of barbituric reagent (3.65 g barbituric acid in 15 mL pyridine, diluted to 50 mL with distillated water). The mixture was left for 10 min at room temperature, diluted to 50 ml water and the absorbance was measured at 580 nm using UV/Vis spectrophotometer. A stock standard solution of KCN with concentration of 1000 mg/L CN- was used to prepare a series of working standards in the range of 1-20 mg/L. Each standard was processed identically to the samples. The obtained absorbance's values were used to construction of calibration curve, which used to linearity over the tested range and enabled the quantification of free HCN in the analyzed samples.

The free HCN concentrations obtained from the calibration curve were expressed as mg/L of the analyzed distillate. However, in accordance with EU Regulation 2019/787, which defines the maximum permitted HCN concentration as 70 mg/L of ethanol (100% v/v), it was necessary to standardize the results to enable accurate safety.

Therefore, for each sample, the measured HCN values was recalculated to its equivalent per (100% v/v) ethanol using the following formula:

HCN [100 % (v/v) EtOH] = HCN (measured mg/L) / Ethanol (% v/v) ×100%

- HCN (measured) represent the concentration determined from the calibration curve in mg/L of the sample
- Ethanol (% v/v) is the ethanol content of the sample, determined by picnometric analysis.

In addition, for each sample, the maximum allowed HCN concentration was calculated based on its ethanol strength using the following formula:

HCN (limit) = 70 mg/L × Ethanol (% v/v)/100%

These corrections allowed for precise comparison with regulatory thresholds and were used to assess whether the samples complied with legally permitted safety limits for free cyanide in fruit brandies.

RESULTS AND DISCUSSION

Basic chemical parameters

The overall chemical characteristics of the analyzed fruit brandy samples (S1-S24) revealed different compositional trends that were largely influenced by the type of fruit, as well as the specific technological practices applied during fermentation and distillation. The chemical composition of the analyzed fruit brandy samples is presented in Table 2. The chemical parameters were examined with a special emphasis on their impact on the aromatic quality, toxicological safety and overall sensory potential of the brandy. Among the analyzed samples, sample S1 showed the highest ethanol content (51.9% v/v). The higher ethanol content of this brandy sample is most likely because traditional producers prefer to produce brandy with a higher alcohol content. In contrast, sample S15 and sample S6 showed significantly lower ethanol levels (38.85 and 39.68% v/v respectively). This is potentially due to incomplete sugar conversion or premature termination of fermentation. Overall, yellow plum distillates (S1-S6) consistently showed increased alcohol levels compared to quince (S19-S21) and apple brandies (S22-S24). Methanol, a toxic by-product of pectin degradation (Lachenmeier et al., 2008), was present in varying concentrations across the samples. The highest value was detected in sample S3 (1.14% v/v) and S15 (1.12% v/v), most likely due to inclusion of crushed fruits stones during fermentation, rich in pectin substances.

Table 2. Chemical composition of the analyzed homemade fruit brandy samples.

Sample	Ethanol (% v/v)	Methanol (% v/v)	Aldehydes (mg/L)	Fusel Alcohols (mg/L)	Total Esters (mg/L)	Ethyl Acetate (mg/L)	Dry Extract (g/L)	Furfural (mg/L)	Free SO_2 (mg/L)
S1	51.91	0.99	305.2	3750.2	3119.1	1214.2	4.05	27.2	6.41
S2	42.25	0.98	147.9	2854.1	3420.3	1584.2	1.85	8.98	6.42
S3	41.32	1.14	197.4	3201.6	1711.3	920.11	1.12	40.6	6.44
S4	45.31	0.61	112.4	3017.6	1325.4	617.31	5.11	40.1	6.41
S5	40.28	0.36	395.1	2421.2	1668.2	826.12	4.21	90.7	6.41
S6	39.68	0.31	209.3	3750.4	739.81	202.11	4.11	2.51	12.8
S7	41.11	0.94	192.6	2692.8	3553.6	1820.2	1.92	9.62	7.68
S8	40.11	0.72	109.7	3252.3	1223.4	616.23	1.25	10.2	6.41
S9	42.51	0.44	222.1	2954.1	1428.3	512.42	1.15	7.65	7.41
S10	45.22	0.65	147.2	3214.2	2358.7	985.61	2.11	9.65	6.41
S11	40.55	0.32	154.7	1988.2	2012.2	1111.2	3.22	4.21	6.41
S12	41.25	0.62	241.7	2845.6	1325.2	625.32	2.22	5.21	7.68
S13	42.58	0.81	165.3	3428.6	3720.1	1860.2	2.12	13.9	6.41
S14	41.88	0.72	504.2	3780.9	2689.3	1344.5	2.11	16.1	6.41
S15	38.85	1.12	634.2	2187.1	235.51	100.21	1.17	4.62	7.41
S16	40.09	0.64	197.5	2861.1	1580.4	780.11	4.71	26.2	6.41
S17	42.61	0.52	144.6	1120.1	1115.4	626.21	1.07	12.2	6.41
S18	41.25	0.62	241.7	2845.6	1325.2	625.32	2.22	5.21	7.68
S19	43.52	0.81	283.1	1817.8	889.72	428.13	0.92	7.41	6.41
S20	40.12	0.31	354.2	2587.1	925.44	421.31	1.15	5.22	7.68
S21	40.25	0.22	428.7	3241.1	452.81	198.24	2.25	4.22	6.41
S22	41.22	0.32	444.1	1985.2	1369.2	624.54	1.11	8.99	6.41
S23	40.08	0.41	847.5	2477.7	2151.2	1111.8	2.25	10.4	6.41
S24	40.11	0.24	725.3	3547.2	1487.3	541.23	1.47	11.2	6.41

Samples S21 and S6 showed lower methanol levels 0.22–0.31% v/v respectively, likely reflecting de-seeding practices or milder fermentation conditions. These findings support the observation that apple brandies generally maintain a safer methanol concentration (Zhang et al., 2012). Fusel alcohols are crucial for mouthfeel and aromatic complexity, but excessive concentrations can impair sensory quality (Lachenmeier et al., 2008; Hazelwood et al., 2008). Samples such as S1 and S14 recorded exceptionally high levels (>3700 mg/L), indicating vigorous amino acid metabolism and uncontrolled distillation conditions. In contrast, sample S17 showed a significantly lower concentration (1120.1 mg/L), indicating a more controlled fermentation and improved distillation refinement. Quince samples (S19-S21) showed intermediate values for methanol, which consist of moderate metabolic activity and balanced fermentation profiles. Esters, especially ethyl acetate, are vital factors contributing to the fruity and floral aroma of brandies (Coldea et al., 2014). Sample S13 showed exceptionally high ester content (3720.1 mg/L) along with elevated ethyl acetate (1860.2 mg/L), indicating robust esterification processes and favorable enzymatic activity. In contrast, S21 showed minimal ester presence (452.8 mg/L) and low ethyl acetate (198.2 mg/L), reflecting the more neutral aromatic profile typical of fruits such as quince (Risticevic et al., 2001; Znang, 2019). Elevated levels of aldehydes, especially acetaldehyde, can result in harsh sensory attributes. The highest concentrations were found in S23 and S24 847.5–725.3 mg/L respectively, exceeding the usual sensory thresholds. In contrast, sample S4 and sample S8 contained significantly improved oxidative control and optimized distillation protocols. The formation of furfural, often associated with thermal degradation of sugars, serves as an indicator of the intensity of the distillation. Sample S5 showed the highest concentration of furfural (90.7 mg/L), indicating excessive thermal load during distillation. Conversely, samples S6 and S11 had minimal levels 2.51–4.22 mg/L respectively, indicating efficient temperature management during the distillation process. Dry extract reflects the concentration of non-volatile residual solids, including organic acids, sugars and polyphenols. Notably, sample S4 and S16 displayed high values (>4.7 g/L), possible due to partial carryover of non-volatile components or aging in oak barrels. In contrast, samples S19 and S23 had low extract levels (<1.2 g/L), suggesting cleaner distillation and minimal matrix interference. Sulfur dioxide concentrations remained within acceptable enological limits in all samples. The highest value was recorded in sample S6 (12.8 mg/L), while most other samples, such S1, S4 and S10, ranged between 6.4 and 7.6 mg/L. These values indicate minimal preservative use, consistent with traditional homemade production practices.

From the obtained results for the basic chemical analysis of fruit brandy samples, it could be concluded that the type of fruit, together with the accompanying fermentation and distillation technique, had a major impact on the chemical safety and sensory attributes of homemade fruit brandies. Stone fruits (plum and apricot) produce brandies with greater aromatic complexity, although accompanied by an increased risk of methanol and high alcohol (Zhao et al., 2014). In contrast, pome fruits (quince and apple) yield more natural and chemically stable distillates, sometimes at the expense of higher aldehyde content. These findings highlight the importance of raw materias selection, fermentation management and precise distillation control in the production of high-quality brandy.

Free HCN analysis

In addition to the general chemical profiling of the fruit brandy samples, particular attention was given to the determination and interpretation of free HCN concentrations, due to its toxicological relevance and regulatory importance. The quantitative results obtained from spectrophotometric analysis are presented in Table 3, along with ethanol-corrected values and legal threshold calculation. To better visualize the comparison between observed values and safety thresholds, Figure 2 illustrates both the measured and corrected HCN concentrations, alongside two reference lines: a fixed EU regulatory limit of 70 mg/L and an individualized limit calculated for each sample based on its ethanol content. The presence of free HCN

in fruit brandies is of significant toxicological concern, especially in brandies produced from stone and seeds fruits known to contain cyanogenic glycosides such amygdalin (Velíšek & Cepjek, 2012). In the present study, HCN concentration was determined using a validated spectrophotometric method, and the results ranged from 1.11 to 9.81 mg/L in the final product (Table 3). To ensure regulatory, all measured values were recalculated to their equivalent per liter of absolute ethanol (100% v/v), in accordance with the EU Regulation 2019/787, which imposes a maximum permitted limit of 70 mg/L free HCN per liter of pure alcohol. The recalculated HCN values ranged between 2.70 and 24.1 mg/L (Figure 2), with none of the samples exceeding the regulatory threshold. Furthermore, sample-specific thresholds were calculated based on individual ethanol content, ranging from 27.20 to 36.34 mg/L, depending on alcohol strength, and none of the samples exceeded their respective thresholds. Samples with highest absolute HCN concentrations S22 (Apple, 9.81 mg/L), S15 (Apricot, 9.37 mg/L) and S23 (Apple, 8.11 mg/L) remained well below both standardized and ethanol-adjusted limits. These findings demonstrate that, despite the use of traditional, non-standardized fermentation and distillation processes, the analyzed brandies are toxicologically safe under current European regulations.

Fruit type was identified as a major factor influencing HCN concentration. Samples produced from apricot and apple (S13-S17 and S22-S24) exhibited consistently higher levels of HCN averaging above 7.5 mg/L. These fruit types are known for their high amygdalin content, particularly in seeds and stones (Balarinwa et al., 2014). In traditional brandy production, whole fruits are often fermented without seed removal, allowing enzymatic hydrolysis of cyanogenic glycosides, by endogenous β-glucosidases. In contrast, plum-based distillates (S1-S12) had markedly lower HCN values, mostly under 2.5 mg/L regardless of ethanol content or region. The lower values may be attribute to lower glycoside content in plum seeds, or to more frequent removal of pits before fermentation, a common practice in homemade distillates. Quince-based samples (S19-S21) showed intermediate values, consistent with their limited but present cyanogenic potential. This fruit-specific distribution is in line with previous studies, and confirms that raw material selection and seed treatment are primary contributes to cyanide risk in traditional fruit brandies

To better understand the technological and biochemical dynamics of cyanide formation, HCN levels were evaluated in relation to a set of basic chemical quality parameters. This includes comparison with the basic chemical parameters of the fruit brandy samples such as methanol, aldehydes, fusel alcohols, furfural, dry extract and SO2. Methanol, a by-product of pectin hydrolysis, shares a metabolic pathway with cyanide release and was found to moderately correlate with HCN levels. Fruits rich in pectin and processed with extended maceration may release both methanol and cyanogenic glycosides from seeds and skin of the fruit. Samples S23 (0.41% v/v) and S14 (0.72% v/v) (Table 2) also exhibited high HCN values 8.11 mg/L and 7.88 mg/L respectively (Table 3). This suggests that a processing method involving prolonged contact with the skin and seeds may induce the formation of both toxic alcohols and cyanide. An inverse relationship was generally observed between ethanol concentration and HCN levels. Samples with lower ethanol content (S15: 38.85% v/v) tended to have higher HCN concentrations (9.37 mg/L), indicating that uncontrolled distillation allowed toxic compounds from early distillation, such as HCN, to remain in the final product. Conversely, high ethanol concentrations often indicate better distillation control, leading to reduced HCN formation. This correlation highlights the role of distillation efficiency as a critical factor in limiting cyanide content. Total aldehydes, which reflect oxidative processes during fermentation and storage, also showed a significant relationship with HCN content. High aldehyde concentration were observed in several samples with elevated HCN levels (S23: 847.5 mg/L and 8.11 mg/L HCN), suggesting that oxidative stress and microbial imbalance during fermentation may promote both aldehyde formation and enzymatic hydrolysis of cyanogenic glucosides (Niedźwiedź-Siegień, 1998).

Table 3. Free HCN concentration, ethanol content and ethanol-corrected HCN values in homemade fruit brandies.

Sample	Free HCN mg/L	Ethanol % v/v	HCN mg/L 100% v/v alcohol	Legal Limit mg/L
S1	1.76	51.91	3.39	36.34
S2	1.14	42.25	2.70	29.58
S3	1.32	41.32	3.19	28.92
S4	1,57	45.31	3.47	31.72
S5	1,41	40.28	3.50	28.20
S6	2.21	39.68	3.57	27.78
S7	1.18	41.11	2.87	28.78
S8	1.45	40.11	3.62	28.08
S9	2.14	42.51	5.03	29.76
S10	1.84	45.22	4.07	31.65
S11	1.11	40.55	2.74	28.39
S12	1.12	41.25	2.71	28.88
S13	8.78	42.58	20.6	29.81
S14	7.88	41.88	18.8	29.32
S15	9.37	38.85	24.1	27.20
S16	7.88	40.09	19.6	28.06
S17	2.46	42.61	5.77	29.83
S18	1.12	41.25	2.71	28.88
S19	1.91	43.52	4.39	30.46
S20	2.01	40.12	5.01	28.08
S21	1.54	40.25	3.83	28.18
S22	9.81	41.22	23.8	28.85
S23	8.11	40.08	20.2	28.06

The table shows the measurement HCN concentrations (mg/L) the ethanol content (v/v %) and the HCN values recalculated to 100% alcohol for comparability. The legal limit for HCN was individually adjusted for each sample based on ethanol content using the formula (Limit = 70 mg/L × Ethanol (% v/v)/100 %.

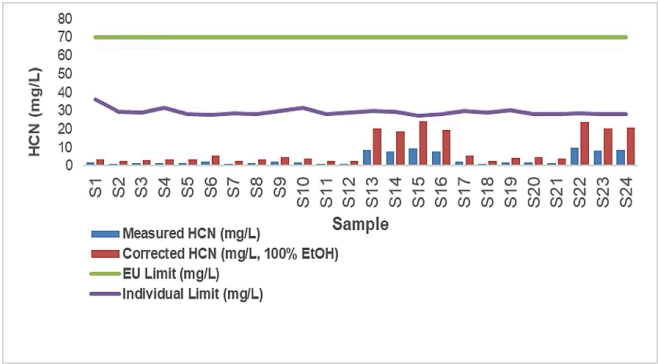

Figure 2. Measured and ethanol-corrected HCN concentration in 24 homemade fruit brandy samples. The green line indicates the EU regulatory limit (70 mg/L of Absolut ethanol), while the blue line represents individualized limits based on the ethanol content of each sample.

This indicate that oxidative fermentation conditions could be a co-factor in cyanide formation. The presence of fusel alcohols and total esters was more variable but occasionally consistent with elevated HCN levels. These compounds are often associate with fermentation intensity and microbial activity (Ough et al., 1998). For example, samples with intense esterification reactions, such S13 and S14, exhibited both high ester levels and increased cyanide. This could be due to enhanced enzymatic activity under vigorous fermentation, indirectly favoring the breakdown of cyanogenic precursors (Kósnáčová et al., 2009). High levels of ethyl acetate, a marker of fermentation stress and volatile acidity, were higher HCN concentration. Samples S13 and S23 not only had high content of ethyl acetate (1860.2 and 111.8 mg/L respectively), but also high HCN values. This indicated a fermentation imbalances or contamination, which may favor enzymatic release of cyanide. Furfural, derived from the decomposition of sugar under heat and the dry extract content representing non-volatile solids, did not show a strong statistical correlation with HCN levels. However, the elevated furfural in some samples with high HCN content suggests that the higher distillation temperature and use of direct fire may be associated with the increased retention or formation of volatile cyanides. This observation requires further controlled investigation.

A factor analysis was performed to investigate the underlying relationships between HCN levels and other chemical parameters in the brandy samples. This multivariate statistical method was employed to reduce the dimensionality of the dataset and to identify latent structures (factors) that explain shared variability among the measured parameters. Based on the E-values and the Kaiser criterion (E-value >1), four factors were extracted. Together, these four factors explain over 85 % of the total variance in the dataset, indicating a strong and comprehensive representation of the chemical relationships among the variables. The results of the factor analisys are presented in Table 4.

Factor 1: This factor is moderately influenced by methanol (loading = 0.40) and ethanol (loading = 0.36), with a smaller contribution from fusel alcohols (loading = 0.25) (Table.4; Figure 3). These compounds are typically formed during alcoholic fermentation. Factor 1 reflects the general alcohol production potential of the row material and fermentation conditions. While HCN does not load significant here, this factor describes the baseline ethanol-methanol profile that may indirectly affect the behavior of cyanogenic precursors in fruit brandies.

Factor 2: This factor is strongly defined by ethanol (loading = 0.85), with supporting influence from fusel alcohols (loading = 0.34) and methanol (loading = 0.14) (Table 4; Figure 3). This factor likely reflects the distillation efficiency, particularly the effect of rectification and dilution on volatile compounds. A high ethanol score indicates well purified distillates, while the presence of fusel alcohols hints at retention of some fermentation volatiles. HCN shows no meaning loading here, suggesting it is less influenced by distillation strength than by precursor breakdown.

Factor 3: Factor 3 shows strong loadings for HCN (loadings = 0.65) and aldehydes (loadings = 0.70) (Figure 4). This is key factor for toxicological and technological concern. It suggests that HCN and aldehydes are co-related or co-formed possibly due to cyanogenic glucoside hydrolysis, thermal degradation of sugars or suboptimal fermentation and distillation. The strong coupling of HCN and aldehydes indicate that this factor represents risky bio product formation, relevant for both flavor deterioration and safety monitoring.

Factor 4: This factor has moderate positive loading for fusel alcohols (loadings = 0.41), negative loadings for HCN (loadings = -0.37) and aldehydes (loadings = - 0.33). This factor likely represents a counterbalance between favorable aroma active compounds and toxic by-products. Higher fusel alcohols are often associate with aromatic complexity, while negative contributions from HCN and aldehydes suggest that more aromatic distillates may also be cleaner in terms of toxic content. This factor may reflect quality perception and aromatic profile development.

Table 4. Factors loadings, Communalities, E-values and explained variance from the factor analisys of brandy samples parameters.

Parameter	F 1	F 2	F 3	F 4	Comm.
HCN (mg/L)	0.04	-0.29	0.65	-0.37	0.65
Ethanol (% v/v)	0.36	0.85	-0.16	-0.21	0.91
Methanol (% v/v)	0.40	0.14	-0.20	-0.15	0.25
Aldehydes (mg/L)	-0.20	-0.21	0.70	-0.33	0.68
Fusel Alcohols (mg/L)	0.25	0.34	0.37	0.41	0.48
Total Esters (mg/L)	0.99	0.08	0.05	0.03	1.00
Ethyl Acetate (mg/L)	0.99	-0.10	-0.04	-0.02	1.00
Furfural (mg/L)	0.11	0.05	-0.15	-0.10	0.05
Eigenvalue (E-value)	2.38	0.99	1.15	0.49	0.75
Explained Variance %	47.5	19.8	22.9	9.79	

F1-loading of Factor 1, F2-loading of Factor 2, F3-loading of Factor 3, F4-loading of Factor 4, E-Eingene value, Comm-Communality.

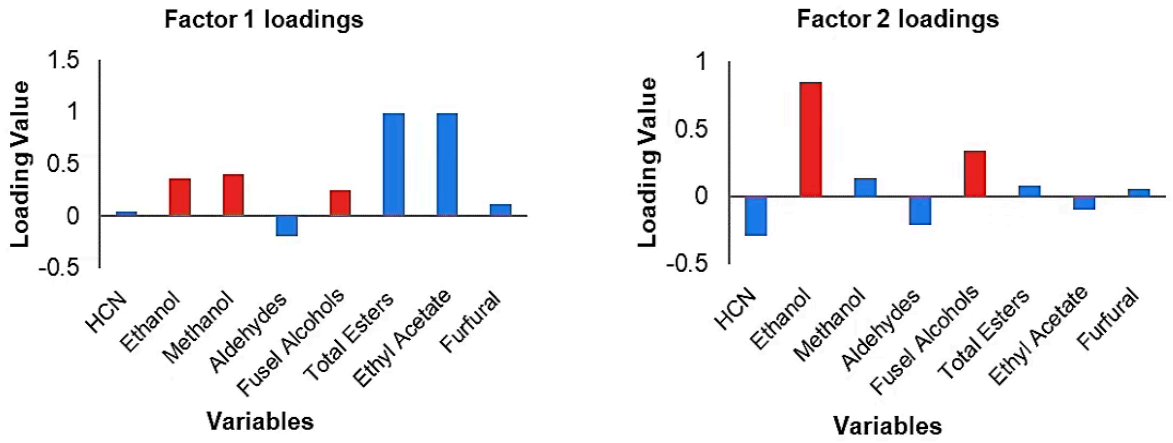

Figure 3. Variable loadings of Factor 1 and Factor 2.

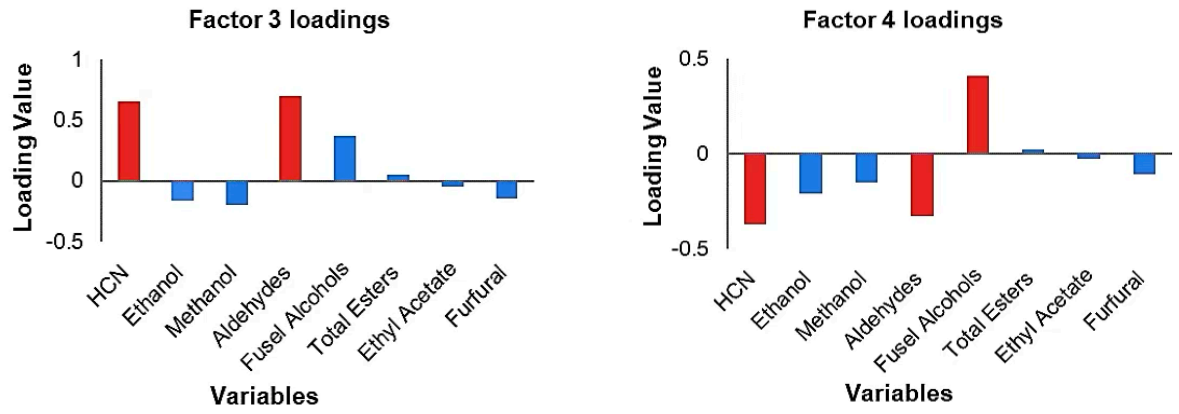

Figure 4. Variable loadings of Factor 3 and Factor 4.

Sulfur dioxide (SO_2) was not included in the factor analysis due to its very low concentrations in the analyzed samples and the absence of common practice for its intentional addition in traditional fruit brandy production. Furthermore, dry extract was excluded as it represents a non-volatile stable residue that is chemically and functionally distinct from the volatile and reactive compounds examined in this study. Its inclusion could have introduced statistical heterogeneity and reduced the interpretability of the extracted factors related to aroma, fermentation and toxicological parameters.

CONCLUDING REMARKS

The results of this study showed that all analyzed samples of homemade fruit brandies met the regulatory threshold for free hydrogen cyanide (HCN), as established by EU Regulation 2019/787 (70 mg/L absolute alcohol). Even in the samples with relatively high HCN content, especially those obtained from apricot and apple, the ethanol-corrected values were within the permitted limits, confirming the toxicological safety of the distillates. The obtained results showed that the type of fruit has a major influence on the free HCN content in fruit brandies. Apricot and apple brandies show a higher cyanogenic potential. This is attributed to the enzymatic degradation of cyanogenic glycosides present in the seeds and peel of the fruit, especially when the seeds were not removed before fermentation. In contrast, plum and quince brandies showed a lower cyanogenic potential. This was due to the practice of removing seeds from the fruit before fermentation.

Given the observed variability, the study highlights the need for routine analytical monitoring and targeted education of domestic producers. Particular emphasis should be placed on the impact of seed management, fermentation duration and distillation efficiency on HCN formation and its transfer to the final distillate. To ensure the safety and quality of traditional fruit spirits, it is recommended that small-scale producers be provided with access to accredited laboratories and receive practical training in safe and hygienic distillation techniques. These interventions are essential to protect public health and promote responsible production practices within the domestic distillation.

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ОПРЕДЕЛУВАЊЕ НА СЛОБОДНА ЦИЈАНОВОДОРОДНА КИСЕЛИНА ВО ДОМАШНИ ОВОШНИ РАКИИ

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Резиме

Ракијата од овошје прерставува традиционален алкхолен пијалак што широко се консумира во Република С. Македонија и другите балкански земји, а се произведува со дестилација на ферментирано овошје како слива, кајсија, дуња и јаболко, користејќи традиционални (домашни) или индустриски методи. Целта на ова истражување е да се процени безбедноста на 24 домашно произведени примероци на овошна ракија преку определување на содржината на слободна цијановодородна киселина (HCN), потенцијално токсично соединение. HCN се формира за време на алкохолната ферментација како резултат на ензимска хидролиза на цијаногените гликозиди кои природно се присутни во семките на овошјето. Определувањето на слободната HCN беше извршено користејќи ја Кениговата реакција, колориметриска метода базирана на формирање на цијаноген хлорид, кој реагира со пиридин и барбитурна киселина и формира стабилен розов комплекс со максимална апсорпција од 580 nm. Вредностите беа пресметани на основа на 100% v/v етанол за да се овозможи споредба со законскиот лимит на ЕУ од 70 mg/L. Највисока концентрации беа измерени во ракија од кајсија и јаболко (до 9,81 mg/L), додека оние од слива и дуња покажаа пониски нивоа на слободна HCN. Умерена корелација беше забележана помеѓу HCN и неколку хемиски параметри како што се метанол, алдехиди, етанол, вкупни естри, фурфурал и фузелни алкохоли. Резултатите укажуваат дека видот на овошје, условите на ферментацијата и степенот на контакт со семките при подготовката на овошната каша пред ферментацијата играат клучна улога во формирањето на слободна HCN.

Клучни зборови: *овошни ракии, слободна цијановодородна киселина, спектрофотометрија.*

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USING MINERALS AS TRACERS FOR FUNCTIONAL VEGETABLES AND FRUITS

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Abstract

This study investigates the elemental composition of a diverse selection of fruits and vegetables collected from the Vinica region in eastern North Macedonia, aiming to evaluate the use of minerals as tracers for identifying functional properties in plant-based foods. A total of 26 plant species were analyzed, including commonly consumed fruits (orange, grapes, melon, banana, apple, kiwi, pomegranate and others) and vegetables (carrot, spinach, broccoli, beetroot, arugula, ginger and others), using inductively coupled plasma mass spectrometry (ICP-MS). Concentrations of 34 elements, from essential nutrients (K, Ca, Mg, Fe, Zn, Se) to potentially toxic trace elements (Pb, Cd, As, Hg), were quantified. Descriptive statistics and multivariate techniques, such as factor and cluster analysis, were applied to explore patterns of mineral association, inter- and intra-species variability, and differentiation between fruit and vegetable groups. The results revealed clear differences in mineral content, with leafy and root vegetables showing higher levels of macro-elements and trace metals, while fruits were richer in elements linked to reproductive and metabolic functions. Mineral clustering revealed co-association trends influenced by botanical, physiological, and environmental factors. These findings highlight the utility of elemental composition as a reliable indicator for evaluating nutritional value, functional potential, and geographic provenance of plant-based foods.

Key words: *fruits and vegetables, trace elements, food traceability, elemental profiling, mineral composition, ICP-MS, multivariate analysis.*

INTRODUCTION

The application of minerals as tracers in functional vegetables and fruits represents a growing area of research in food science and nutrition. Functional plant foods defined by their ability to provide health benefits beyond basic nutrition, are increasingly studied for their bioactive compounds, such as antioxidants and anti-inflammatory agents (Temple, 2022; Calleja-Gómez et al., 2024; Xue & Yin, 2024). Minerals can serve as natural geochemical markers or nutritional indicators, offering insights into the origin, quality, and functional properties of these crops (Wang et al., 2021). Elements such as strontium, lithium, germanium, palladium, beryllium and some of the rare earth elements (REEs) have been employed to trace geographical origin due to their stable and region-specific profiles linked to soil composition (Kabata-Pendias, 2011; Kopačková et al., 2015; Miller, 2017)., Additionally, essential minerals like magnesium, calcium, potassium, zinc and selenium are investigated for their roles in plant metabolism and their correlation with bioactive compound synthesis (Bhat et al., 2024; Hossain et al., 2024; Singh, 2024). Minerals are essential inorganic micronutrients that play a vital role in maintaining human physiological and biochemical functions (Jing et al., 2024). They are involved in structural and regulatory processes such as bone formation, enzymatic

activity regulation, maintenance of osmotic balance, muscle contraction, and nerve impulse transmission (Kabata-Pendias, 2011; Wanget al., 2025). Although required in small amounts, their deficiency can lead to serious health disorders.

In recent years, researchers have begun to investigate how specific mineral profiles can act as indicators of bioactivity or nutritional functionality in plant foods. For example, higher levels of Zn and Se in certain vegetables have been associated with increased antioxidant activity, as these elements are cofactors in antioxidant enzymes like superoxide dismutase and glutathione peroxidase (Ríos et al., 2008; Dai et al., 2019; Wang et al., 2022). Moreover, mineral content can reflect the metabolic state of a plant, as the synthesis of bioactive compounds often involves mineral-dependent enzymatic pathways (Szerement et al., 2022). This opens the possibility of using mineral composition as a biochemical fingerprint to predict or validate the health-promoting potential of functional produce (Sharma et al., 2020; Liu et al., 2022.

Mineral's concentration varies depending on plant species, soil composition, pH, irrigation and agronomic practices (Gupta & Gupta, 2021), which also affect the uptake of potentially toxic elements such as Cd, Pb, As and Hg. Mineral tracers also play a role in biofortification strategies, where crops are enriched with essential minerals to combat micronutrient deficiencies in human populations (Afzal et al., 2020). Tracking the uptake and accumulation of biofortified minerals using trace analysis supports the development of functional produce with enhanced health benefits. Additionally, understanding mineral dynamics can inform sustainable agricultural practices, as soil health and fertilization regimes directly influence the mineral and bioactive content of crops (Ram & Govindan, et al., 2020; Gauliya et al., 2025). These findings align with prior studies that used mineral composition as a classification tool. Similar mineral-based classification approaches have been discussed in previous studies (Taranova & Kochubey, 2018; Zhou et al., 2023), supporting the potential of certain fruits and vegetables to be considered as functional foods.

In recent years, there has been a growing interest in monitoring and utilizing mineral indicators to characterize the functional properties of plant-based foods. These efforts reflect an increasing recognition of the role that trace elements and mineral composition play in the nutritional and therapeutic value of plants. According to Balabanova, et al., (2016) and Balabanova & Fan (2024) plant food cultivated in Macedonia possesses a distinctive lithogenic background, which contributes to its enrichment with specific minerals that enhance its functional properties. This geochemical uniqueness offers valuable insights into the relationship between soil composition, mineral uptake, and the health-promoting potential of local agricultural products. The primary aim of this study is to investigate the use of mineral elements as tracers for identifying and characterizing functional properties of vegetables and fruits. By analyzing the mineral composition and its correlation with specific lithogenic backgrounds, the study seeks to determine how trace elements can serve as indicators of the nutritional quality, geographic origin, and potential health benefits of plant-based foods. This approach aims to support the development of more precise methods for evaluating functional food attributes and contribute to the promotion of region-specific agricultural products with enhanced mineral functionality.

The objective of this study was to assess the total content of macro-elements, microelements, and potentially toxic metals in commonly consumed fresh fruit and vegetable products in the Republic of North Macedonia. This approach provides a foundation for improved dietary planning, supports biofortification strategies, and enables the assessment of nutritional and toxicological risks for consumers.

MATERIAL AND METHODS

General overview of the research goals

In this study, a total of 26 fresh fruit and vegetable products were analyzed in this study, including: orange, black grapes, white grapes, melon, tomatoes, banana, peach, pear, kiwi, apple, pomegranate, cucumber, blueberry, pepper, carrot, pumpkin, arugula, parsnip, broccoli, beetroot, kohlrabi, potato, spinach, lettuce, ginger, and lemon. These samples

were selected based on their prevalence and availability in local markets, to provide a representative overview of plant-based dietary intake in the Republic of North Macedonia.

A total of 26 samples were collected during the autumn period (October-November 2024) from local markets and households. Among the samples analyzed, 19 were locally grown in the Republic of North Macedonia, while 7 were imported from regional and international markets. The imported samples included tropical fruits and vegetables such as banana, peach, ginger, lemon, pomegranate, as well as orange and arugula. The remaining samples were locally grown. The samples were homogenized, stored under laboratory conditions, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) following a standardized wet acid digestion procedure regarding already validated methodology (Balabanova et al., 2015). Comparative analysis indicated minor variations in elemental composition between imported and local produce, though no statistically significant differences were observed for the majority of the evaluated elements. Special attention was given to Ca, K, Na, P, S, Mg, Fe, Zn, Cr and Se due to their key nutritional value, as well as to Pb, Cd, Hg and As due to their toxicological potential. The obtained results are expected to serve as a basis for improved dietary planning and the development of regional biofortification strategies.

In addition, Principal Component Analysis (PCA) was applied as a statistical method for identifying patterns and grouping the samples based on their elemental similarities. This approach enables a better interpretation of the differences between fruit and vegetable samples and the identification of elements dominant in specific samples.

Sample selection and preparation

For the purposes of this study, seasonally available fruits and vegetables were selected and purchased from local markets and households across various regions of the Republic of North Macedonia during the period from October to November 2024. The selection was based on the frequency of consumption and the availability of the products in the local diet during the autumn season.

To ensure consistency of results, only the edible parts of each plant (flesh of fruits, leaves of leafy greens, roots of root vegetables) sample were used in the preparation process, in order to reflect actual consumer exposure to the mineral composition. Each sample was thoroughly washed with distilled water to remove any residual soil, dust, and potential surface contaminants. After cleaning, the samples were subjected to homogenization in order to obtain a representative and uniform sample mass suitable for further analysis. The homogenized samples were stored at -20°C until the time of processing and laboratory analysis.

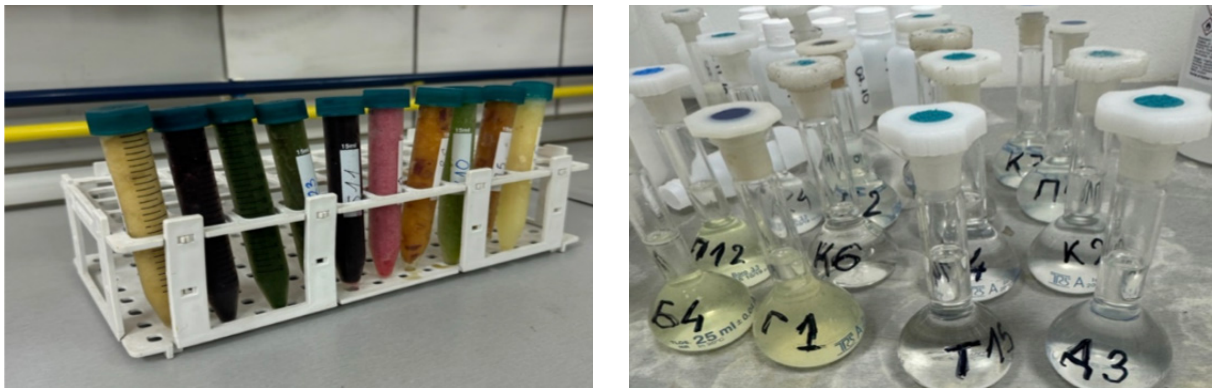

Figure 1. Sample preparation and digestion.

Acid digestion for mineral extraction

For mineral content analysis, 1-2 grams of each homogenized plant sample were accurately weighed and transferred into Teflon digestion vessels (Fig. 1). A wet digestion protocol was applied using a mixture of 7 ml concentrated nitric acid (HNO_3, 65%) and 5 ml hydrogen peroxide (H_2O_2, 30%).

The digestion process was performed in a microwave digestion system under controlled temperature and pressure conditions, with the temperature ramped to 180°C over 15 minutes and held for 30 minutes to ensure complete mineralization of the organic matrix.

After digestion, the resulting clear solutions were cooled to room temperature and diluted to a final volume of 25 ml with ultrapure deionized water (18.2 MΩ·cm). Before analysis, all solutions were filtered through 0.45 µm membrane filters to remove any remaining particulates.

Analysis by ICP-MS

The determination of macro-elements, microelements, and potentially toxic metals was performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A state-of-the-art ICP-MS system was employed, enabling multi-element detection with high sensitivity and analytical precision. The instrument was calibrated using certified reference standards prepared in the same matrix as the analyzed samples. A five-point calibration curve was constructed, covering the expected concentration range for each element. To ensure analytical accuracy and precision, blank samples, a quality control standard, and sample duplicates were analyzed in parallel. Limits of detection (LOD) and quantification (LOQ) were calculated individually for each element.

A total of 31 elements were analyzed, covering essential macro-elements, trace microelements, and toxic heavy metals. The elements included Li, Be, B, Na, Mg, K, Ca, S, P, Ni, Cr, Mn, Co, Cu, Fe, Ga, Ge, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb, and Bi.

This wide elemental panel was selected to evaluate both nutritional and toxicological profiles of the studied samples.

None of the analyzed samples exceeded the WHO/FAO recommended safety thresholds for toxic elements such as Cd, Pb, As, and Hg, indicating that all products were within acceptable limits for human consumption.

Table 1. Instrumental conditions for ICP-MS analysis (Agilent technologies, series 7850).

Parameter	Condition
ICP-MS Instrument	Agilent 7850
Plasma gas flow rate (argon)	15.0 L/min
Auxiliary gas flow rate	1.0 L/min
Nebulizer gas flow rate	1.05 L/min
RF power	1550 W
Nebulizer type	Micromist
Sample uptake rate	0.4 mL/min
Spray chamber temperature	2–5°C
Integration time per mass	0.3–1.0 s
Collision/reaction gas (He cell gas, 4.5 mL/min)	Helium mode
Internal standards	Sc, Y, Rh, In
Detection mode collision mode	Standard and He

Statistical data processing

Statistical analysis of the data was performed using Statistica 13.0 software. Basic descriptive statistics, including mean, minimum, and maximum values, were calculated for each element across all analyzed samples. To assess differences between sample groups, analysis of variance (ANOVA) was conducted with a significance level of $p < 0.05$. To explore patterns of association and clustering among the samples and elements, factor analysis was employed to identify groups of elements with strong correlations. Additionally, Principal Component Analysis (PCA) was used to examine inter-species variation. This multivariate technique enabled the visualization of dominant factors contributing to variability and facilitated the identification of samples with similar mineral profiles.

Limit of detection (LOD) and limit of quantification (LOQ)

Limits of detection (LOD) and quantification (LOQ) were determined according to the standard deviation of the blank signal (σ) and the slope (S) of the calibration curve, using the equations: $LOD = 3 \cdot \sigma/S$, $LOQ = 10 \cdot \sigma/S$. For most elements, LOD values ranged from 0.0001 to 0.001 mg/kg, and LOQ values ranged from 0.0005 to 0.005 mg/kg, depending on the element and matrix. These thresholds ensured reliable detection of even low-abundance trace elements such as selenium (Se) and cadmium (Cd), when present.

Quality control and assurance

To ensure accuracy and precision, a comprehensive quality control protocol was implemented throughout the analytical workflow. All analytical batches included the certified reference material NIST 1573a (Tomato leaves) and equivalent plant-based standards Herba- mix (herbal plants dry mixture, Bipea, 32-f-8-Elements traces – herbs and medicinal plants cod 5032). Recovery rates for all measured elements ranged from 92.3% to 119%, demonstrating method accuracy. Reagent blanks and digestion blanks were included in each batch to monitor background contamination. All blank values were below LOD, confirming the absence of significant contamination. Selected samples were spiked with known concentrations of multi-element standards. Mean recovery values ranged between 88.6-112%, and relative standard deviations (RSDs) for replicate measurements were consistently below 5%, confirming the precision of the method. Calibration curves were constructed using multi-point standards ($R^2 > 0.999$ for all elements), and internal standards (Rhodium) were used to correct for instrumental drift and matrix suppression/enhancement effects. Together, these measures ensured the robustness, reliability, and reproducibility of the elemental data generated from fruit and vegetable samples.

RESULTS AND DISCUSSION

Overview of elemental composition

Total of 31 elements were analyzed in collected samples of fruits and vegetable species. The overall mineral profile showed dominance of macroelements such as sodium in range of 0,69 – 42,5 mg/100 g, magnesium (5,12 – 88,3 mg/100 g), and potassium (143,5 – 822,1 mg/100g), phosphorus from 13,6 – 71,4 mg/100 g, with significantly higher median values than trace elements. Micronutrients like Fe, Zn, B, Cu, Zn and Mn were present at nutritionally relevant levels, confirming their biological importance across plant types (Tab. 2). Toxic elements such as Cd, Pb, Hg, As, Tl, Bi, Sb and Sn were found in very low or undetectable concentrations, indicating a high safety level of the analyzed samples.

Variation in Li, Be, Al, B, V, Mn, Cr, Ni, Co, Ga, Ge and Mo levels suggests influence from species differences and soil conditions, supporting the need for further categorization and comparative analysis.

Table 2. Descriptive data base for the total element content.
Abbreviations: Min. – Minimum; Max. – Maximum; Med. – Median.

Element	Isotope	Unit	Min.	Max.	Med.
Li	7	mg/100g	0,042	2,33	0,15
Be	9	mg/100g	<0,001	0,0045	0,0017
B	11	mg/100g	0,0028	0,985	0,018
Na	23	mg/100g	0,69	42,5	37,0
Mg	34	mg/100g	5,12	88,3	39,8
Al	27	mg/100g	0,013	2,19	1,07
P	31	mg/100g	13,6	71,4	39,2
S	34	mg/100g	0,82	133,7	71,9
K	39	mg/100g	143,5	822,1	578,7
Ca	44	mg/100g	12	155	83,5
V	51	mg/100g	0,0062	0,023	0,0146
Cr	52	mg/100g	0,0029	0,61	0,31
Mn	55	mg/100g	<0,001	0,25	0,12
Fe	56/57	mg/100g	0,049	3,18	1,61
Co	59	mg/100g	<0,001	0,029	0,015
Ni	60	mg/100g	0,049	0,33	0,18
Cu	63	mg/100g	0,058	0,92	0,489
Zn	65	mg/100g	0,26	4,88	2,57
Ga	71	mg/100g	<0,001	0,0074	0,0040
Ge	72	mg/100g	<0,001	0,0056	0,0031
As	75	mg/kg	<0,001		
Se	78	mg/100g	<0,001	0,0024	0,0015
Sr	88	mg/100g	0,29	3,18	1,74
Mo	95	mg/100g	0,0026	0,035	0,0188
Pd	105	mg/kg	<0,001		
Ag	107	mg/kg	<0,001		
Cd	111	mg/kg	<0,001		
Sn	118	mg/kg	<0,001		
Sb	121	mg/kg	<0,001		
Ba	137	mg/kg	<0,001		
Hg	201	mg/kg	<0,001		
Tl	205	mg/kg	<0,001		
Pb	206/207/208	mg/kg	<0,001		
Bi	209	mg/kg	<0,001		

The factor analysis conducted on the concentrations of 31 elements in fruit and vegetable samples resulted in the extraction of four dominant factors (Table 3), cumulatively explaining a significant proportion of the total variance in the dataset. These factors represent distinct elemental associations that likely reflect underlying geochemical and biological processes influencing mineral uptake in plants. Ten elements (Pd, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and Bi) were excluded from the FA due to the determined contents below the LOQ. These results reaffirm the safety of the analyzed produce and emphasize the relatively low risk of exposure to heavy metals through local fruits and vegetables.

Factor 1 accounted for the largest portion of the variance and was characterized by strong loadings of lithogenic elements such as Al, Fe, Li, Be, Co, Cr, Ge and Mn. These elements are typically associated with soil-derived inputs and reflect the influence of the geological substrate on plant mineral composition. The presence of these elements in high concentrations across samples suggests a strong environmental and edaphic control on mineral accumulation, particularly in plants grown in regions with metal-rich soils.

Factor 2 showed high loadings of essential nutrients such as K, Na, B, Mg, Ca, Sr and P, which are biologically regulated and play crucial roles in plant metabolism and growth. This factor likely represents physiological uptake processes and highlights the nutritional functionality of the samples. The clustering of these macroelements indicates their coordinated regulation in plant tissues and supports their use as indicators of plant health and food quality.

Factor 3 was dominated by trace elements including Zn, Cu, Ni, Co, Cr, S, and Mo, which are also essential micronutrients but required in smaller amounts. Their co-association in this factor suggests a pattern of trace element accumulation influenced by both soil availability and species-specific uptake mechanisms. This factor may be useful in distinguishing functional properties related to antioxidant potential and enzymatic activity in fruits and vegetables.

Factor 4 comprised elements such as V, Se, S, Co, and Al, which are considered toxic in higher content and of anthropogenic origin. The grouping of these elements points to possible environmental contamination sources, such as industrial activity or agrochemical use. Although present at lower concentrations, their association in a distinct factor underscores the need for monitoring food safety and evaluating potential health risks associated with long-term exposure.

Overall, the extracted factors provide meaningful insights into the elemental composition of plant-based foods. They reflect the combined effects of environmental, physiological, and anthropogenic influences on mineral accumulation. Understanding these patterns not only aids in characterizing the functional properties of fruits and vegetables but also supports the development of targeted strategies for agricultural management, food quality assessment, and nutritional profiling (Table 3).

Comparison of mineral composition between fruits and vegetables

The comparative analysis of nine macronutrients: Na, Mg, P, K, Ca, Cr, Fe, Cu and Zn between fruits and vegetables revealed statistically relevant patterns associated with plant physiology and nutritional profiles (Figure 2).

Sodium contents were notably higher in fruits, potentially reflecting its physiological role in osmotic regulation and transport in fleshy fruits such as citrus and melons. Magnesium and iron concentrations were elevated in vegetables, particularly in leafy and root varieties, supporting their established roles in chlorophyll structure and redox metabolism, respectively.

Phosphorus was abundant in both groups but showed slightly higher concentrations in fruits. This may be linked to its involvement in sugar transport and energy metabolism during fruit development. Potassium was the most prevalent element in all samples, with higher average values in fruits, underscoring its importance in cell turgor and enzymatic activation during ripening. The consistent presence of P and K in the samples supports their contribution to bioactivity, as these elements are involved in energy transfer and osmoregulation in plants, and serve as indicators of functional food potential.

Calcium was present at slightly elevated levels in vegetables, aligning with its function

in cell wall stability and signal transduction, especially in structural tissues. Chromium, while typically present at trace levels, showed marginally higher concentrations in vegetables, though its essentiality in plant metabolism remains less clearly defined. Copper and zinc exhibited relatively balanced concentrations across both categories. These trace elements are involved in enzymatic catalysis and antioxidant defense, and their consistent presence reflects the basic metabolic needs shared across plant species.

Collectively, the data emphasize distinct nutrient accumulation patterns governed by plant function, anatomy, and environmental interactions. These findings reinforce the value of dietary diversity and the role of fruits and vegetables in providing complementary mineral profiles essential for human nutrition, particularly in regions like North Macedonia.

Table 3. Extraction of dominant element's correlation – factor analysis.

Element	Factor 1	Factor 2	Factor 3	Factor 4
Li	**0.66**	0.04	-0.07	0.14
Be	**0.70**	0.07	-0.24	-0.16
B	0.38	**-0.56**	-0.12	-0.07
Na	0.36	**-0.38**	-0.27	0.21
Mg	0.22	**-0.74**	**0.13**	0.34
Al	**0.72**	0.1	0.01	**0.47**
Cu	0.09	-0.25	**-0.51**	0.11
P	0.14	**-0.74**	-0.5	0.43
S	0.32	-0.09	**-0.76**	**0.48**
K	0.46	**-0.53**	-0.45	0.38
Sr	0.47	**-0.72**	-0.19	0.47
Ca	0.45	**-0.83**	-0.39	0.36
V	0.03	-0.38	-0.27	**0.52**
Cr	**0.51**	-0.24	**-0.66**	0.47
Mn	**-0.78**	0.02	-0.18	-0.12
Fe	**0.55**	-0.08	-0.28	0.36
Co	**0.48**	-0.37	**-0.58**	**0.47**
Ni	0.33	-0.32	**-0.63**	0.29
Se	0.03	-0.31	-0.34	**0.42**
Zn	0.38	0.17	**-0.86**	0.15
Ge	**0.67**	-0.22	-0.03	-0.01
Mo	-0.06	-0.16	**-0.69**	0.25
Variance (%)	54,7	10,5	6,16	5,54
Eigenvalue	13,6	2.63	1.54	1.38

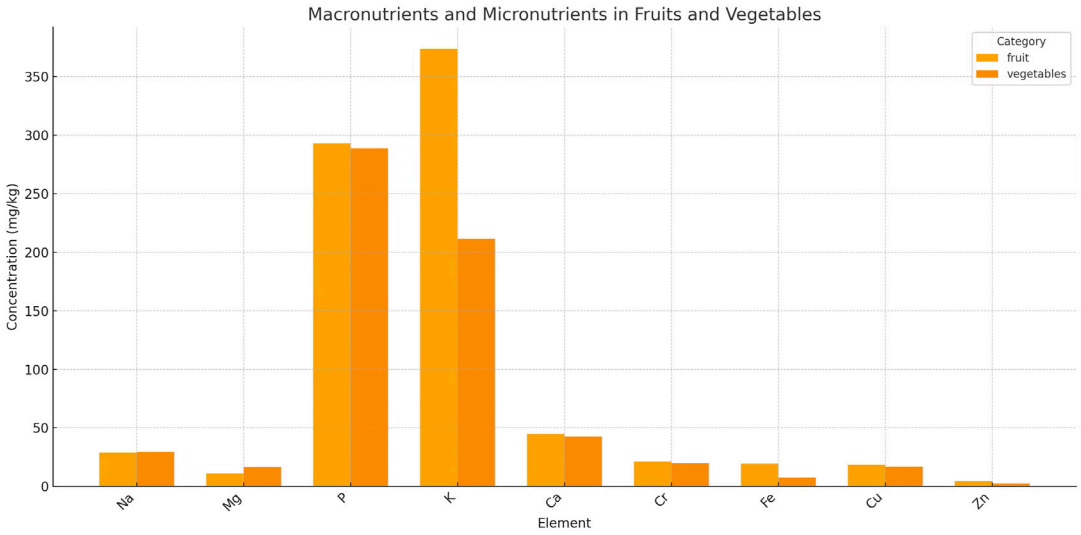

Figure 2. Elemental distribution of key plant-pased macronutrients across fruit and vegetable groups.

Inter- and Intra-species variability

Principal component analysis (PCA) revealed clear grouping of samples based on elemental composition, distinguishing between fruits and vegetables, as well as between leafy and root vegetables. The first two components explained over 70% of total variance. Strong positive correlations were observed between calcium and magnesium (r = 0.81), and between iron and manganese (r = 0.74), suggesting shared uptake pathways. A weak negative correlation between potassium and calcium in fruits may indicate competitive absorption. These patterns confirm that mineral accumulation is both species- and environment-dependent, supporting the need for integrated nutritional and agronomic planning.The PCA results indicate clear elemental variability both between and within plant species. The separation reflects species-specific differences in mineral profiles (Figure 3).

Leafy vegetables such as spinach and arugula cluster closely, reflecting elevated levels of Fe and Mg, whereas citrus fruits and grapes form separate clusters influenced by higher Na and B concentrations.

These patterns are likely driven by a combination of genetic traits and environmental influences such as soil composition, irrigation practices, and pH. Although geographic origin was not explicitly analyzed, the distinct groupings suggest that growing conditions play a crucial role in elemental uptake and distribution.

The most variation in elemental composition was observed in leafy vegetables (e.g., spinach, arugula) and root vegetables (e.g., beetroot), likely due to differences in soil conditions and agricultural practices.

The hierarchical clustering method revealed distinct groupings that reflect shared mineral uptake profiles, which are influenced by plant species, botanical family, physiological functions, and potential environmental factors such as soil composition and agricultural practices. The dendrogram produced from the cluster analysis distinguished two primary clusters: one predominantly comprising fruits, and the other comprising vegetables (Figure 4).

This division is consistent with the physiological and metabolic differences between these groups, which influence their elemental uptake and accumulation patterns. Citrus and tropical fruits (orange, lemon, banana, kiwi) formed a distinct group, characterized by elevated concentrations of potassium, magnesium, and phosphorus, elements essential for fruit ripening and sugar metabolism. Grapes (black and white), melon, and pomegranate were grouped together, indicating similar profiles in terms of trace elements such as Fe, Mn and Zn, likely due to similarities in their reproductive biology and climacteric ripening behavior. Apple, pear, and peach, all members of the *Rosaceae* family, clustered closely, showing a shared mineral profile influenced by genetic traits and similar cultivation conditions.

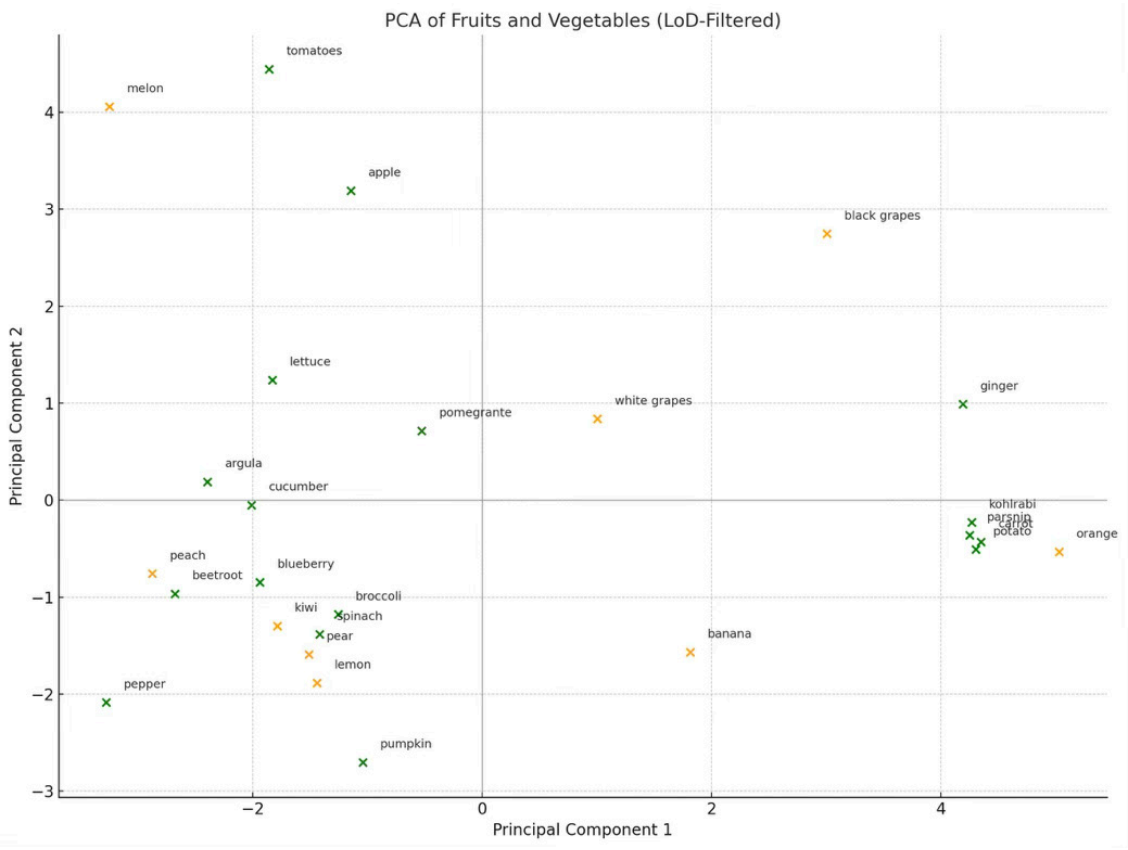

Figure 3. PCA plot of fruits (orange) and vegetables (green) based on elemental composition.

Root vegetables (carrot, beetroot, parsnip, potato) grouped together, displaying higher accumulations of elements like Ca and Mg, and certain trace elements (Cu and Zn), which are known to be concentrated in subterranean tissues. Leafy greens such as spinach, lettuce, and arugula formed a tight cluster, characterized by elevated levels of Fe, Cu, and Cd, the latter possibly due to enhanced absorption capacity of leafy tissues for both essential and non-essential elements. *Brassica* vegetables including broccoli and kohlrabi showed a distinct association, likely due to their known ability to bioaccumulate selenium and sulfur-containing compounds, as well as elements like molybdenum involved in enzymatic defense mechanisms. Cucumber and pumpkin, both members of the *Cucurbitaceae* family, clustered together, reflecting similar profiles in macroelements like K and Ca. Pepper and tomato, although botanically fruits, clustered with vegetables, likely due to their culinary use and similar mineral content influenced by intensive cultivation under greenhouse conditions. Interestingly, ginger, a rhizome with distinct secondary metabolism, appeared as an outlier, forming a separate cluster. This reflects its unique accumulation of elements such as Fe, Mn, and possibly heavy metals, consistent with its medicinal properties and subterranean growth habit.

The cluster analysis highlights how mineral composition can serve as a discriminant feature for plant-based foods classification. It also reinforces the role of plant taxonomy, growth habit, and environmental conditions in shaping elemental profiles. Such insights are valuable for food authentication, nutritional profiling, and developing strategies for the biofortification of essential minerals while minimizing the uptake of potentially harmful elements.

This study highlights the nutritional importance of fruits and vegetables commonly consumed in North Macedonia. Crops like spinach and arugula were rich in Fe and Mg, while melon and orange showed higher Na and B, reflecting crop-specific mineral profiles.

These results support targeted dietary planning and emphasize the role of soil factors in mineral uptake. Practices such as biofortification and soil improvement can enhance crop quality.

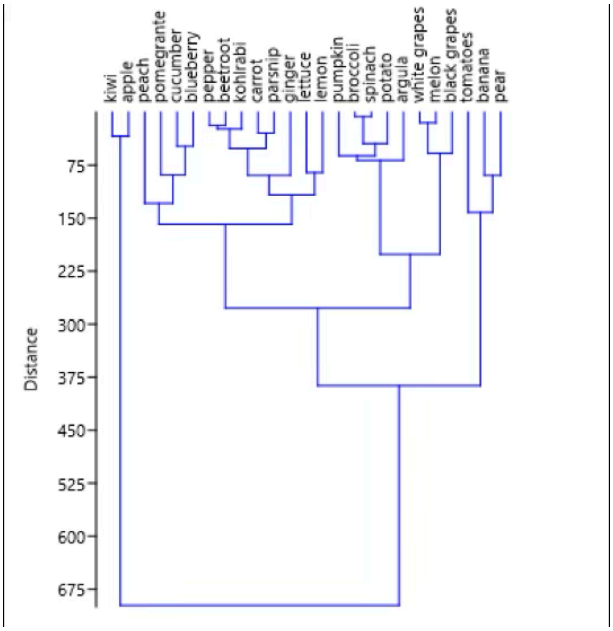

Figure 4. Cluster analysis for the interspecies correlation.

CONCLUDING REMARKS

The findings highlight distinct differences in mineral profiles between fruits and vegetables, as well as notable inter- and intra-species variability. Leafy and root vegetables exhibited higher concentrations of macro- and trace elements, while fruits tended to accumulate elements related to reproductive development and sugar metabolism. Multivariate statistical approaches, including factor and cluster analysis, successfully revealed underlying patterns of elemental associations, offering insight into both biological and environmental influences on mineral uptake. These patterns suggest that mineral profiling can serve not only as a tool for evaluating the nutritional and functional properties of plant-based foods but also as a tracer for geographic origin and agricultural conditions. The results support the potential application of mineral elements as biomarkers for the classification, traceability, and quality assessment of fruits and vegetables. This approach contributes to the broader field of functional food research and reinforces the importance of integrating geochemical and botanical data for advancing food safety, authenticity, and nutritional evaluation. The mineral profiling approach described here can be integrated into national strategies for food traceability, quality control, and dietary planning in agricultural policy. Future work should explore seasonal, soil, and cultivation influences in greater detail and expand the database to include additional regions and plant species for more robust comparative analyses.

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ПРИМЕНА НА МИНЕРАЛИТЕ КАКО ИНДИКАТОРИ ЗА ФУНКЦИОНАЛНИ СВОЈСТВА НА ОВОШЈЕ И ЗЕЛЕНЧУК

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Резиме

Во овој труд се истражува елементарниот состав на разновиден избор на овошје и зеленчук собрани од Виничкиот регион во источниот дел на Северна Македонија, со цел да се процени употребата на минерали како трасери за идентификување на функционални својства во храната базирана на растенија. Анализирани се вкупно 26 растителни видови, во примероци на најчесто консумирано овошје и зеленчук, со примена на масена спектрометрија со индуктивно сврзана плазма (ICP-MS). Одредени се концентрациите на 34 елементи, вклучувајќи есенцијални макро- и микронутриенти (K, Ca, Mg, Fe, Zn, Se) до потенцијално токсични елементи во траги (Pb, Cd, As, Hg). Една од главните цели на ова истражување е да се направи сеопфатен елементарен профил на испитуваната растителна храна. Дескриптивна статистичка анализа и мултиваријантни техники, вклучувајќи факторна и кластерска анализа, беа применети за да се евалуираат моделите на минерална асоцијација, да се процени варијабилноста помеѓу, но и во рамките на видовите и да се направи разлика помеѓу групите овошје и зеленчук врз основа на нивните елементни асоцијации. Резултатите укажаа на специфични разлики во минералниот состав помеѓу овошјето и зеленчукот, при што лиснатиот и коренестиот зеленчук генерално покажуваат повисоки концентрации на макроелементи и метали во траги, додека овошјето е побогато со елементи поврзани со метаболичките процеси. Идентификувани се неколку минерални кластери, што укажува на силна ко-асоцијација на елементи под влијание на физиолошки и еколошки фактори. Резултатите укажуваат на потенцијалната употреба на минералната содржина како сигнификантен индикатор за процена на функционалниот квалитет, хранливата вредност и можното географско потекло на прехранбените производи со растително потекло.

Клучни зборови: *овошје и зеленчук, елементи во траги, следливост на храна, елементарно профилирање, минерален состав, ICP-MS, мултиваријантна анализа.*

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