JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 23, No. 1, 2025

Manuscript received 25.06.2025
Accepted: 15.07.2025

In print: ISSN 2545-4447
On line: ISSN 2545-4455
doi:
Original scientific paper

USING MINERALS AS TRACERS FOR FUNCTIONAL VEGETABLES AND FRUITS

Lolita Spirova[1*], Biljana Balabanova[1]
[1]*Faculty of Agriculture, Goce Delcev University, Stip, Krste Misirkov 10A, 2000 Stip, Republic of North Macedonia*

**Corresponding author: lolita.209128@student.ugd.edu.mk*

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

IN PRESS

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

IN PRESS

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.

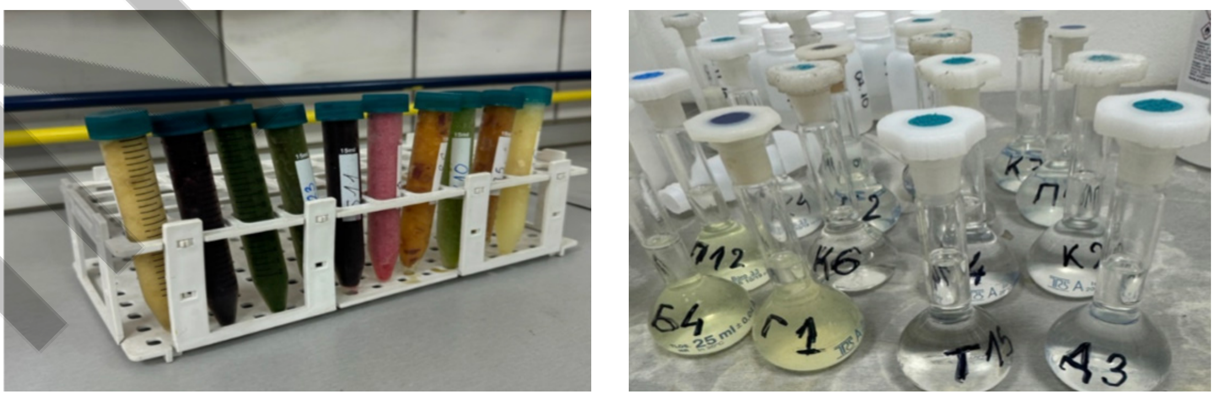

Figure 1. Sample preparation and digestion.

IN PRESS

IN PRESS

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 (Figure 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 (Table 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.

IN PRESS

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		

IN PRESS

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 PRESS

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

IN PRESS

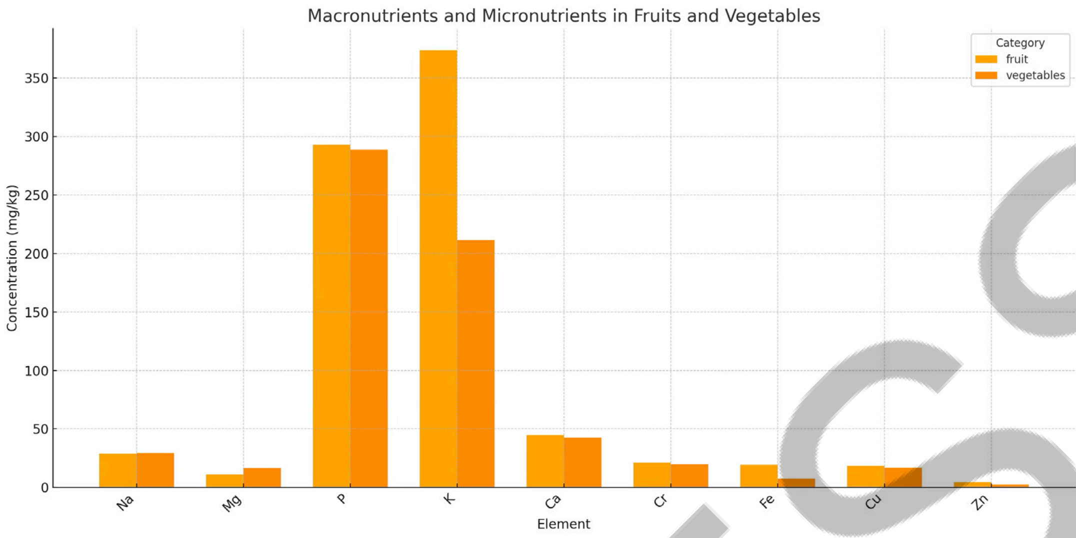

Figure 2. Elemental distribution of key plant-pased macronutrients across fruit and vegetable groups.

IN PRESS

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.

IN PRESS

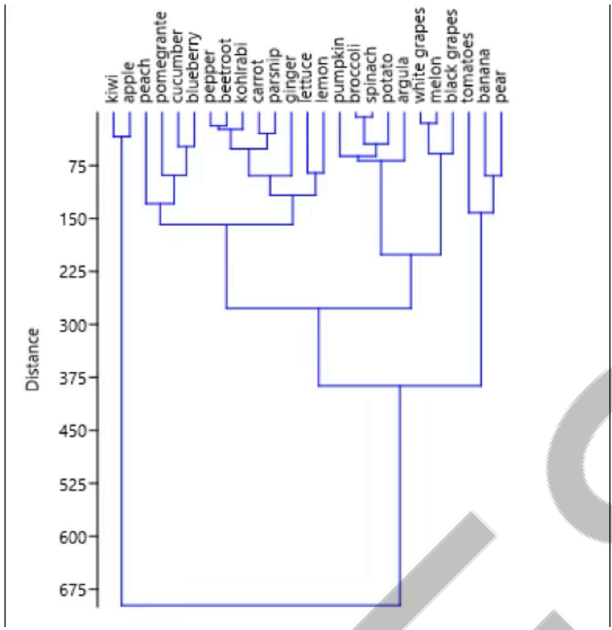

Figure 4. Cluster analysis for the interspecies correlation.

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.

ACKNOWLEDGEMENT

Authors express their acknowledgment to UNILAB, Faculty of Agriculture, Goce Delcev University, Stip, North Macedonia (https://unilab.ugd.edu.mk) where the analytical procedures have been conducted.

REFERENCES

Afzal, S., Sirohi, P., Sharma, D., & Singh, N. K. (2020). Micronutrient movement and signalling in plants from a biofortification perspective. P*lant micronutrients: deficiency and toxicity management*, 129-171.

Balabanova, B., & Fan, L. (2024). Lead and strontium isotope evidence for local herbal varieties due to the elemental soil chemistry. International *Journal of Agriculture and Environmental Research*, 10(2), 287-300.

IN PRESS

Balabanova, B., Boev, B., Mitrev, S., & Ivanova-Petropulos, V. (2015). Method for determination of 35 elements content in various samples with application of microwave digestion and inductively coupled plasma with mass spectrometry (ICP-MS). *Journal of Agriculture and Plant Sciences,* 13(1), 99-113.

Balabanova, B., Karov, I., & Mitrev, S. (2016). Comparative analysis for macro and trace elements content in goji berries between varieties from China and R. Macedonia. *Agricultural Sciences and Technologies,* 79-84.

Bhat, M. A., Mishra, A. K., Shah, S. N., Bhat, M. A., Jan, S., Rahman, S., ... & Jan, A. T. (2024). Soil and mineral nutrients in plant health: A prospective study of iron and phosphorus in the growth and development of plants. *Current issues in molecular biology,* 46(6), 5194-5222.

Calleja-Gómez, M., Roig, P., Pateiro, M., Dominguez-Valencia, R., Lorenzo, J. M., Fernández-López, J., ... & Carrillo, C. (2024). Health-promoting benefits of plant-based by-product extracts obtained by innovative technologies. *Current Opinion in Food Science,* 57, 101161.

Dai, H., Wei, S., Skuza, L., & Jia, G. (2019). Selenium spiked in soil promoted zinc accumulation of Chinese cabbage and improved its antioxidant system and lipid peroxidation. *Ecotoxicology and environmental safety,* 180, 179-184.

Gauliya, K., Pathak, A., Mandal, N., Manjhi, M. K., Upadhyaya, D. C., Raj, A., & Upadhyaya, C. P. (2025). Fostering Nutritional Equity: Biofortification Strategies, Socioeconomic Implications, and Regulatory Policies for Developing Biofortified Staple Crop. *Journal of Soil Science and Plant Nutrition,* 1-23.

Gupta, U. C., & Gupta, S. C. (2021). Trace element toxicity in soils and plants.

Hossain, M. S., Garcia Caparros, P., & Mühling, K. H. (2024). Mineral nutrition and plant stress tolerance. *Frontiers in plant science,* 15, 1461651.

Jing, T., Li, J., He, Y., Shankar, A., Saxena, A., Tiwari, A., ... & Awasthi, M. K. (2024). Role of calcium nutrition in plant Physiology: Advances in research and insights into acidic soil conditions-A comprehensive review. *Plant Physiology and Biochemistry,* 108602.

Kabata-Pendias, A. (2011). *Trace Elements in Soils and Plants.* CRC Press.

Kopačková, V., Lhotáková, Z., Oulehle, F., & Albrechtová, J. (2015). Assessing forest health via linking the geochemical properties of a soil profile with the biochemical parameters of vegetation. International *Journal of Environmental Science and Technology,* 12, 1987-2002.

Liu, X., Mu, J., Tan, D., Mao, K., Zhang, J., Sadiq, F. A., ... & Zhang, A. (2022). Application of stable isotopic and mineral elemental fingerprints in identifying the geographical origin of concentrated apple juice in China. *Food Chemistry,* 391, 133269.

Miller, D. D. (2017). Minerals. In *Fennema's food chemistry* (pp. 627-679). CRC Press.

Ram, S., & Govindan, V. (2020). Improving wheat nutritional quality through biofortification. In *Wheat quality for improving processing and human health* (pp. 205-224). Cham: Springer International Publishing.

Ríos, J. J., Rosales, M. A., Blasco, B., Cervilla, L. M., Romero, L., & Ruiz, J. M. (2008). Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulturae,* 116(3), 248-255.

Sharma, S., Singh, A. K., Tiwari, M. K., & Uttam, K. N. (2020). Prompt screening of the alterations in biochemical and mineral profile of wheat plants treated with chromium using attenuated total reflectance fourier transform infrared spectroscopy and X-ray fluorescence excited by synchrotron radiation. *Analytical Letters,* 53(3), 482-508.

Singh, A. K. (2024). *Impacts of Minerals on the Plant's Growth and Metabolism*. Addition Publishing House.

Szerement, J., Szatanik-Kloc, A., Mokrzycki, J., & Mierzwa-Hersztek, M. (2022). Agronomic biofortification with Se, Zn, and Fe: An effective strategy to enhance crop nutritional quality and stress defense—A review. *Journal of Soil Science and Plant Nutrition,* 22(1), 1129-1159.

Temple, N. J. (2022). A rational definition for functional foods: A perspective. *Frontiers in nutrition,* 9, 957516.

Wang, J., Liu, Z., Dou, J., Lv, J., Jin, N., Jin, L., ... & Yu, J. (2022). A comparative study on the nutrients, mineral elements, and antioxidant compounds in different types of cruciferous vegetables. *Agronomy,* 12(12), 3121.

Wang, L., Ju, C., Han, C., Yu, Z., Bai, M. Y., & Wang, C. (2025). The interaction of nutrient uptake with biotic and abiotic stresses in plantsFA. *Journal of Integrative Plant Biology.*

Wang, X., He, Y., Gao, Q., Yang, D., & Liang, J. (2021). Approaches to evaluate nutrition of minerals in food. *Food Science and Human Wellness,* 10(2), 141-148.

Xue, J., & Yin, Y. (2024). Plant-Based Foods: From Nutritional Value to Health Benefits. *Foods,* 13(22), 3595.

Taranova, M., & Kochubey, S. (2018). Trace elements in fruits and vegetables and their significance. In *Functional Food* (pp. 81–102).

Zhou, Y., Gao, W., Liu, X., et al. (2023). Mineral element profiles and their variation in fruit crops: Insights into breeding and traceability. *Scientia Horticulturae*, 322, 112157.

IN PRESS

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

Лолита Спирова[1], Биљана Балабанова[1]
[1]Земјоделски факултет, Универзитет „Гоце Делчев",
ул. „Крсте Мисирков" 10-А, 2000 Штип, Република Северна Македонија
10-А, 2000 Штип, Република С. Македонија
**Контакт автор:* lolita.209128@student.ugd.edu.mk

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.*

IN PRESS