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A screening study of elemental composition in legume (*Fabaceae* sp.) cultivar from Serbia: Nutrient accumulation and risk assessment

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ABSTRACT

The study is the first analytical approach to evaluate thirteen elements' profiles of 4 different species (*Phaseolus* spp., *Vicia* spp., *Pisum* spp. and *Lathyrus* spp.) comprising 38 varieties of legumes cultivated in Serbia. The inductively coupled plasma with an optical emission spectrometer (ICP-OES) was used to determine the levels of macro-, micro- and trace elemental contents, namely, P, K, Ca, Mg, Fe, Cu, Zn, Mn, Cd, Pb, Ni, Cr and As, after microwave-assisted digestion. MANOVA was utilized to reveal significant differences in elemental composition within and between groups, while PCA to reveal the underlying patterns. Among the macroelements, the most abundant was K (8980.7–14177.4 mg kg^{-1}), followed by P, Mg and Ca, being the highest in *Phaseolus* spp. The data revealed that the studied legumes generally contained a high amount of Zn and Fe, with *Lathyrus* spp. being the richest in Zn. The mean concentration of trace elements in the analyzed legume samples was in the following order: Ni (24.2–57 mg kg^{-1}) > Cr (0.8–4.1 mg kg^{-1}) > Pb (0.07–1.2 mg kg^{-1}) > Cd (0–0.07 mg kg^{-1}). The determined Pb and Cd contents in all cultivars exceeded the set maximum limits by European and Serbian legislation, having a potential for human health risk. Pattern recognition techniques applied to the data did not distinguish among the species, revealing a similar elemental profile. In conclusion, this study highlights legumes as an extremely valuable source of macro- and microelements, but also the importance of monitoring the level of heavy metals in this commonly consumed foodstuff.

1. Introduction

The legumes comprise the bulk of human food resources, occupy 12–15% of arable land worldwide, account for 27% of the world's primary food production, and have substantial economic relevance in the Serbian region (Chaudhary et al., 2020). Increased demand for plant-based products and awareness about the health benefits of legumes are the key drivers enhancing the market growth. Additionally, there is the global shift toward plant-based diets thus increasing the importance of legumes as alternative protein sources. In the forecast period of 2023–2032, the legumes market is projected to witness growth at a Compound Annual Growth Rate (CAGR) of around 5.30% (Market Research Future, 2023). In addition, the role of legumes in addressing malnutrition, nutrition security, smallholder incomes, and sustainable and resilient agrosystems with a low environmental footprint (climate, land use and nitrogen fertilization), is becoming in the limelight not only to the rural and urban poor areas, but also to developed countries, following the Sustainable Development Goal 2 set out by the Food and Agriculture Organization of the United Nations (FAO).

Fortification of staple and processed foods with protein- and micronutrients-rich ingredients is one of the main strategies used to enrich their nutritional profile and improve the quality of nutrition of

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the world population. Some of the commonly consumed legumes include navy beans, faba beans, pigeon pea, cowpeas, soybeans, chickpeas, lentils, and lupines. These legumes have a very rich nutritional profile, being high in protein, dietary fiber, unsaturated fatty acids, carbohydrates, vitamins, and bioactive phytochemicals. Epidemiological studies have associated their consumption with proper gut functioning and reduced risk of several chronic diseases, including cancers and heart disease (Didinger et al., 2022). Low glycemic index diets containing legume as an ingredient had promising effects in preventing or halting the progression of type 2 diabetes (Bajka et al., 2021). As a gluten-free food source, legumes are one of the mainstays in treating celiac disease. Legumes also provide essential minerals, such as K, P, Mg, Ca, Fe, Zn, and Mn. Minerals perform several functions in metabolic processes; they are an integral part of many enzyme systems, including DNA polymerase, prevent stunted growth and delayed sexual development, therefore fractures and osteoporosis, reduce antioxidant stress; they are involved in the cellular production of adenosine triphosphate, as well as in the transport of ions across membranes in all human tissues (Sinkovič et al., 2022). High Fe and Zn content makes legumes an efficacious food source for preventing anemia in children and women of reproductive age (Bouhlal et al., 2019). These attributes of legumes make a plant-based diet more health beneficial, via in-creasing the per capita daily availability of legumes (100 g day^{-1} for optimal health outcomes) above global intake levels (21 g day^{-1}) (Willett et al., 2019; Semba et al., 2021) and thereby reducing the nutritional problem of inadequate micronutrient intake, especially in developing countries where dietary diversity is limited. According to Stevens et al. (2022), from 2003 to 2019, 372 million preschool-aged children and 1·2 billion non-pregnant women of reproductive age suffered from "hidden hunger", a multiple micronutrient deficiency condition. It is important to mention here that, while lead, cadmium, mercury, and arsenic are inherently toxic in small quantities and lack essentiality for human health, the excessive intake of essential elements like copper, potassium, magnesium, and zinc can also lead to toxicity, causing harmful effects (Hassan et al., 2023).

Industries, agriculture, and domestic activities are primary contributors to the rise of potentially toxic elements in the environment. Wastes from these sources, including pesticides, fertilizers, and toxic chemicals, accumulate in the ecosystem. Chromium, widely used in various industries, especially leather and pharmaceuticals, is a notable toxic element. Inadequate waste disposal directly impacts water bodies, causing metal pollution. Pesticides and herbicides introduce metals like arsenic, cobalt, chromium, nickel, lead, iron, manganese, and more into the environment. Agricultural practices intensify contamination in soil, with fertilizers and wastewater irrigation elevating levels of cadmium, zinc, copper, and arsenic. Mining, vehicle emissions, waste incineration, and natural sources also contribute to pollution. Transportation-related activities, such as corrosion and brake abrasion, induce metal toxicity. Road dust and roof runoff further introduce heavy metals into the environment. Therefore, human activities and natural processes play significant roles in the widespread contamination of the environment by potentially toxic elements (Modabberi et al., 2018; Qian et al., 2020; Singh et al., 2022; Zhang et al., 2023). The level of heavy metals in plants and food products is influenced by the increased anthropogenic load, which has significantly elevated human exposure to these metals due to rapid urbanization and industrial developments (Sajjadi et al., 2022; Peirovi-Minaee et al., 2023). Humans are exposed via different pathways, including inhalation, ingestion of water or food, and dermal contact, of which daily exposure to elements from diet accounts for 90–95% (Marti-Cid et al., 2009). However, oral intake of contaminated foods is considered the main route of heavy metal exposure (Sajjadi et al., 2022; Peirovi-Minaee et al., 2023). Arsenic, cadmium, and lead are heavy metals of particular concern because they tend to accumulate in tissues and organs being toxic for living ecosystems and human health (Scaeteanu et al., 2021). Therefore, it is imperative to control plant-origin food concerning its content of heavy elements for a risk assessment strategy for human health. It should comply with the EU/national/international limits, or in the absence of limits include constant monitoring activities and comparison with the available data in literature.

Several analytical methods were performed to analyze the elemental composition such as inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectroscopy (ICP-MS), and atomic absorption spectroscopy (AAS). ICP-OES provides a holistic approach for simultaneous multi-elemental analysis in a single assay for rapid and accurate data collection. In comparison to other spectroscopic techniques such as AAS which is commonly used for trace element analysis, the high-lighted advantages of ICP are high sensitivity, long-term stability, good reproducibility, accuracy, low matrix effect, a wide variety of analytical tasks, calibration function with wide dynamic range and a high number of measurable elements. This technique uses radiofrequency-induced argon plasma for atomization, while samples introduced in plasma must be liquid, requiring sample digestion before injection into the instrument (Scaeteanu et al., 2021). Momen et al., (2006) successfully developed an analytical method involving digestion procedure for determination with ICP-OES of toxic and nutrient elements in legumes. They evaluated different digestion methods to develop and recommend an analytical procedure for the digestion of legume samples. The microwave digestion method in which the sample is heated up by microwaves and destroyed or dissolved using concentrated acids or mixtures thereof and only the metals remain in solution, is usually preferred to alternative digestion methods for beans as it is fast and reliable (Dos Santos et al., 2013).

This integrated approach that includes microwave-assisted acid digestion and ICP-OES has been used for the first time for multi-elemental analysis of 38 varieties of legumes cultivated in Serbia to ensure data on their quality and safety. Thirteen elements including macroelements, namely P, K, Mg, and Ca, microelements (Fe, Cu, Mn, and Zn) and trace elements (Cd, Pb, Ni, Cr and As) were analyzed. In order to allow for a more comprehensive understanding of the data, principal component analysis (PCA) was performed. This analysis gained insights into the patterns, relationships, groupings, and variable importance within the legume dataset. To the best of the authors' knowledge, there was a notable gap in the existing literature regarding elemental compositions of these specific legume species cultivated in Serbia. This dearth of information prompted the researchers to undertake a comprehensive analysis to contribute novel insights into the nutritional profiles of these legumes within the local context.

2. Materials and methods

2.1. Chemicals and reagents

Single-element standard solutions (1000 mg L^{-1}) were purchased from Sigma Chem (St. Louis, MO, USA). The elements studied are as follows: microelements Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn), macroelements Phosphorus (P), Potassium (K), Magnesium (Mg) and Calcium (Ca), and trace elements Cadmium (Cd), Lead (Pb), Nickel (Ni), Chromium (Cr), and Arsenic (As). Nitric acid and hydrogen peroxide were obtained from PanreacQuimica SA (Barcelona, Spain). Ultra-pure water was utilized for the preparation of solutions. All lab containers used were properly washed first with nitric acid (10%) and then with ultra-pure water. This is done to avoid cross-contamination of samples.

2.2. Sample material

The experiments were carried out on the legume population grown conventionally during 2019 across experimental fields of the Institute of Field and Vegetable Crops, from the area of RimskiŠančevi (45°20'N, 19°51'E, 87 m a.s.l.), Pannonian lowland, Vojvodina Province, Serbia. The long-term data (1981–2015), show that the mean annual temperature is 11.3 °C, annual precipitation sum is 610.3 mm, the mean temperature for the growing period (April–September) is 18.2 °C and the precipitation sum for the growing period (April–September) is 359.6 mm. Collected crops were milled into flour using a laboratory mill (Laboratory Mill LM 3100, Perkin Elmer), packed in plastic bottles and stored at refrigeration temperatures between 4 and 10 °C. These included the following species and varieties: *Phaseolus* spp. (*Phaseolus vulgaris* ssp. *nanus* or beans L1 – L9; *Phaseolus vulgaris* ssp. *volubilis* or green beans L10 – L13; *Phaseolus lunatus* var. *compresus* or lima beans L14; *Phaseolus coccineus* or mountain (turkish) beans L15 – L17); *Vicia* spp. (*Vicia faba* or faba (fava, broad) beans L18 – L25); *Pisum* spp. (*Pisum sativum* or peas L26 – L32); and *Lathyrus* spp. (*Lathyrus sativus* or grass (white) pea L33 – L38).

2.3. Microwave digestion

The legume flours were digested in a microwave oven (Speedwave two microwave digestion system S/N00386, Berghof, Eningen, Germany), following a slightly modified and shortened methodology recommended by the manufacturer for cereal grain samples. About 0.5 g (± 2%) of flour sample was put into a teflon vessel of the instrument and then added 5 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide were added. The following temperature program was applied: step 1 – 160 °C was reached in 5 min and held for 15 min; step 2 – 170 °C was reached in 3 min and held for 10 min; step 3 – 75 °C was reached in 2 min and held for 5 min. The total digestion time was 40 min at 800 W, with an additional 20 min for cooling down the Teflon vessels to the room temperature of 25 °C. After digestion, samples were filtered and clear liquids were obtained, after which each sample was properly diluted with 2% nitric acid and analyzed on an ICP-OES device. A method blank consisting of 2% nitric acid was carried through the digesting procedure. The Teflon vessels and all the other vessels were properly cleaned ensuring negligible blank values in ICP-OES.

2.4. Determination of elemental composition of legumes with ICP-OES

Elemental composition of samples was determined on an ICP–OES device (Optima 8300, Perkin Elmer, Inc.). Operating conditions are reported in the following table (Table 1).

The following element classes were screened: macroelements (P at 213.617 nm; K at 766.490 nm; Ca at 317.933 nm; Mg at 285.213 nm); microelements (Fe at 238.204 nm; Cu at 327.393 nm; Zn at 206.200 nm; Mn at 257.610 nm); and trace elements (Cd at 228.802 nm; Pb at 220.353 nm; Ni at 231.604 nm; Cr at 267.716 nm; As at 188.979 nm).

IUPAC guidelines were followed for method development (IUPAC, 1995). Calibration of the device was done using standard solutions and a blank. A very high linearity of the calibration curves ($R^2 > 0.9999$) was observed for all analytes tested. More detailed information about the procedures followed to perform calibration, reproducibility and accuracy of the method is reported by Skendi et al. (2020). The obtained calibration curve for each studied element, the linearity range and the correlation coefficient are given in Table 2. Matrix effect was studied by spiking a sample (mixture of each legume in equal proportions) with different amounts of standard solutions. Matrix-matched solutions obtained after digestion were analyzed in ICP-OES. This resulted in satisfactory repeatability (RSD less than 5.6%) and recovery (accuracy) (88–106%) for each element studied.

The detection limit (LOD) and the quantification limit (LOQ) value are the concentrations that are equal to three and ten times, respectively, the standard deviation of the responses of the blank solution versus the slope of the calibration curve. The limit of detection and quantification (in parenthesis, in mg L^{-1}) were as follows: P 0.0760 (0.232), K 0.0010 (0.0030), Ca 0.010 (0.030), Mg 0.0016 (0.0048), Fe 0.0046 (0.014), Cu 0.0097 (0.0294), Zn 0.0059 (0.0178), Mn 0.0014 (0.0042), Cd 0.0027 (0.0082), Pb 0.042 (0.126), Ni 0.015 (0.046), Cr 0.0071 (0.0213), As 0.001 (0.003). The analytical procedures were performed in triplicate, and the average values are herein reported. The contents of analyzed elements obtained as mg L^{-1} was converted into mg kg^{-1} of flour. The method blank (2% nitric acid) was subtracted from the results obtained for each sample in order to correct the background contributions associated with matrix effects. Each element was expressed as total content encompassing all oxidation states. Calibration blank was analyzed after each batch of 10 measurements to ensure that there is no contamination followed by a mixture of standards to confirm calibration.

Table 1
Parameters and operation conditions for the ICP-OES instrument.

Parameter	Value
Nebulizer Flow (Cross-Flow type)	0.6 L min^{-1}
RF Generator	40-MHz free-running solid state
RF power	1500 watts
Auxiliary Flow	0.2 L min^{-1}
Plasma Flow	8 L min^{-1}
Sample Flow Rate	1.5–2.5 mL min^{-1}
Equilibration Time	8 s

Table 2
Calibration curves for ICP-OES analysis of macro-, micro- and trace-elements.

	Equation	Correlation coefficient (R^2)	n	Wavelength (nm)	Linearity Range (mg/L)
Macroelements					
P	Intensity = 1537.1 × *C*	0.9999	4	213.617	0.232–100.
K	Intensity = 272603 × *C*	0.9997	4	766.490	0.00300–100.
Ca	Intensity = 80377 × *C*	0.9997	4	317.933	0.0300–100.
Mg	Intensity = 129541 × *C*	1.000	4	285.213	0.00480–100.
Microelements					
Fe	Intensity = 101268 × *C*	1.000	4	238.204	0.0140–20.0
Mn	Intensity = 527362 × *C*	1.000	4	257.610	0.00420–20.0
Cu	Intensity = 53876 × *C*	1.000	4	327.393	0.0294–20.0
Zn	Intensity = 48856 × *C*	0.9995	4	206.200	0.0178–20.0
Trace elements					
Cd	Intensity = 44951 × *C*	0.9994	4	228.802	0.00820–5.00
Pb	Intensity = 4783.3 × *C*	0.9998	4	220.353	0.126–5.00
Ni	Intensity = 19545 × *C*	0.9995	4	231.604	0.0460–5.00
Cr	Intensity = 44496 × *C*	0.9999	4	267.716	0.0213–5.00
As	Intensity = 914.2 × *C*	0.9998	4	188.979	0.00300–5.00

2.5. Metal pollution index (MPI) calculation

To assess the overall heavy metal impact of analyzed legume crop species, the metal pollution index (MPI) was calculated by employing mean quantified heavy metal concentrations using the formula below. This method follows the approach outlined by Usero et al. (1997) and Orecchio et al. (2014):

$$MPI = (C_1 \times C_2 \times \dots C_n)^{\frac{1}{n}} \quad (1)$$

where Cn in the mean concentration of a single metal in each legume species.

According to the United States Environmental Protection Agency (US

EPA United States Environmental Protection Agency, 2023), metals that commonly cause toxic effects comprise As, Cd, Cr, Cu, Pb, Ni and Zn. These were therefore included in MPI calculations.

2.6. Data processing

Statistical analyses (descriptive and inferential) were performed using the Statistical Package for the Social Sciences (SPSS version 26.0) software (IBM Corporation, Armonk, New York, United States). The Shapiro–Wilk test was used to estimate the normality of distribution for continuous random variables. For non-normally distributed data, a log transformation has been applied to produce a reasonably symmetric distribution, account for curvature in a linear model and stabilize variations within groups. A multivariate analysis of variance (MANOVA) was employed to determine if an independent variable effects on multiple dependent variables. For further analysis, Wilks' lambda, and Scheffe post-hoc tests were applied. The significance level for calculated differences was set at 0.05.

Principal component analysis (PCA) was performed to explore the underlying patterns, relationships, and variability, further helping to identify the most significant components or dimensions of variation in a multivariate dataset. Bi-plots with legume samples as scores and macro-, micro- and trace element contents as loadings, were obtained using an open-source Paleontological Statistics software PAST v4.13 (Natural History Museum, University of Oslo, Norway). By performing PCA and generating bi-plots, the following objectives can be achieved. PCA allows the reduction of the high-dimensional dataset into a smaller number of principal components, which capture most of the data's variability while retaining important information. The bi-plots generated from PCA visually represent the relationships between the legume samples and the element contents. Positioning of the samples and loadings on the bi-plot can reveal patterns and associations among the variables. Furthermore, bi-plots help identify grouping patterns in legume samples, where proximity suggests similar elemental profiles. Loadings on bi-plots indicate the significance of each element in explaining dataset variance; larger loadings signify greater influence in sample differentiation (Psodorov et al., 2015; Horvat et al., 2021; Habuš et al., 2022).

3. Results

3.1. Multi-elemental analysis of legumes

The determined elements were categorized as macro- (P, K, Ca and Mg), micro- (Fe, Cu, Zn and Mn), and trace elements (Cd, Pb, Ni, Cr and As). Their means/medians, standard deviations, minimum and maximum values are represented in Table 3. Regarding the latter group, some of the elements have been considered toxic to humans and maximum permitted levels have been set for legume vegetables (Pb 0.10 mg kg^{-1} wet weight, Cd 0.020 mg kg^{-1} wet weight) (Commission Regulation 2023/915). The nutritional value of legumes was proved by the high presence of different metals specifically Zn, Fe, Cu and M (Bouhlal et al., 2019; Sinkovič et al., 2022).

The metal pollution index values for different legume species reveal varying degrees of metal contamination. Among these species, *Vicia* group exhibited the highest MPI at 2.39, indicating a relatively elevated level of metal pollution. *Lathyrus* group also displayed a significant metal pollution index of 2.31, indicating a notable level of metal contamination. In contrast, *Phaseolus* and *Pisum* groups had lower metal pollution index values of 2.02 and 1.84, respectively, suggesting relatively lower levels of metal pollution.

The results of MANOVA analysis using the Wilks' lambda and Scheffe post-hoc tests, and setting the *p*-value to 0.05, are shown in Table 4. This allowed us to test whether there are overall differences among groups – species, across all dependent variables – element concentrations. In other words, it assessed whether the group means differ in a multivariate

Table 3
Macro-, micro-, and trace elements levels in the different legume species, expressed in mg kg^{-1} of flour in wet basis. Results are expressed as the average of n analyses.

	***Phaseolus* spp. (n=17)** *	***Vicia* spp. (n=8)**	***Pisum* spp. (n=7)**	***Lathyrus* spp. (n=6)**
Macroelements				
P	4000 (3100 – 4540)	3390 (2920 – 4860)	2310 (2060 – 3860)	4320 (3910 – 4520)
K	14200 (11400 – 17000)	13700 (10900 – 16500)	8980 (7060 – 10900)	11800 (10700 – 12900)
Ca	1400 (1070 – 1800)	1000 (940. – 1130)	938. (822. – 1330)	1000 (956. – 1220)
Mg	1280 (1190 – 1450)	1160 (982. – 1280)	961. (854. – 1080)	1140. (1080 – 1200)
Microelements				
Fe	35.9 (31.7 – 50.4)	26.0 (22.5 – 29.9)	41.4 (31.5 – 47.4)	26.5 (22.8 – 28.4)
Cu	4.30 (3.00 – 6.00)	3.00 (2.70 – 3.80)	3.20 (2.00 – 4.80)	3.00 (2.50 – 3.30)
Zn	43.0 (25.4 – 67.0)	25.2 (18.1 – 48.9)	18.4 (13.6 – 34.4)	79.0 (21.0 – 148.)
Mn	10.1 (7.80 – 11.7)	7.90 (5.50 – 9.30)	7.20 (5.70 – 9.50)	10.1 (8.60 – 10.7)
Trace elements				
Cd	0.0200 (0.00 – 0.0500)	0.0300 (0.0200 – 0.0700)	0.0200 (0.00 – 0.0500)	0.0200 (0.00 – 0.0300)
Pb	0.470 (0.0700 – 0.870)	1.00 (0.500 – 1.50)	0.700 (0.200 – 1.20)	0.550 (0.180 – 0.920)
Ni	32.3 (28.7 – 42.5)	35.4 (26.9 – 39.8)	31.7 (24.2 – 32.8)	34.4 (30.1 – 57.0)
Cr	1.20 (0.800 – 2.30)	2.30 (1.00 – 4.10)	1.50 (0.900 – 1.80)	1.70 (1.00 – 4.00)
MPI	2.02	2.39	1.84	2.31

[*] The values are numbers (normally distributed data: mean ± standard deviation; non-normally distributed data: median (interquartile range; Q1 – Q3)). MPI – metal pollution index.

Table 4
Between-subject effects (MANOVA).

	Sum of squares	**Mean squares**	**F-test**	***p*-values***	**Partial eta squared**
Macroelements					
P	0.217	0.0720	3.83	**0.0180**[a,b]	0.253
K	0.212	0.0710	11.0	**<0.0010**[a,b,c]	0.493
Ca	0.862	0.621	3.64	**0.022**[a]	0.243
Mg	0.116	0.0390	4.64	**0.0080**[a]	0.291
Microelements					
Fe	0.467	0.156	5.10	**0.0050**[b,c]	0.311
Cu	0.297	0.0990	2.78	0.056	0.197
Zn	1.40	0.466	3.45	**0.027**[c]	0.233
Mn	0.129	0.0430	2.57	0.070	0.185
Trace elements					
Cd	1.29	0.430	0.850	0.48	0.0700
Pb	1.58	0.526	2.68	0.062	0.191
Ni	0.0660	0.0220	1.47	0.24	0.115
Cr	0.118	0.0390	0.267	0.85	0.0230

[*] One-way ANOVA. Post-hoc comparisons using the Scheffe test; a - statistical significance between *Phaseolus* and *Pisum* groups; b – statistical significance between *Pisum* and *Lathyrus* groups; c – statistical significance between *Vicia* and *Pisum* groups. Values in bold are statistically significant.

sense, considering the combined variation in all dependent variables. MANOVA thus showed significant differences between *Pisum* and *Lathyrus* groups in the contents of all analyzed macroelements: P (which was also significant between *Pisum* and *Lathyrus* groups), K, Ca and Mg. Furthermore, the differences in K and Fe contents seemed to be significant both between *Pisum* and *Lathyrus*, and *Vicia* and *Pisum* groups. The Zn content showed to be significantly different only between species Vicia and Pisum. However, there were no significant differences in the trace element contents among groups.

3.2. Principal component analysis

Diagrams obtained after performing PCA are represented in Fig. 1. PCA correlation bi-plots describe the total variance of the processed datasets in the amounts of 90.51% for macroelements (Figs. 1a), 74.28% for microelements (Figs. 1b) and 69.01% for trace elements (Fig. 1c).

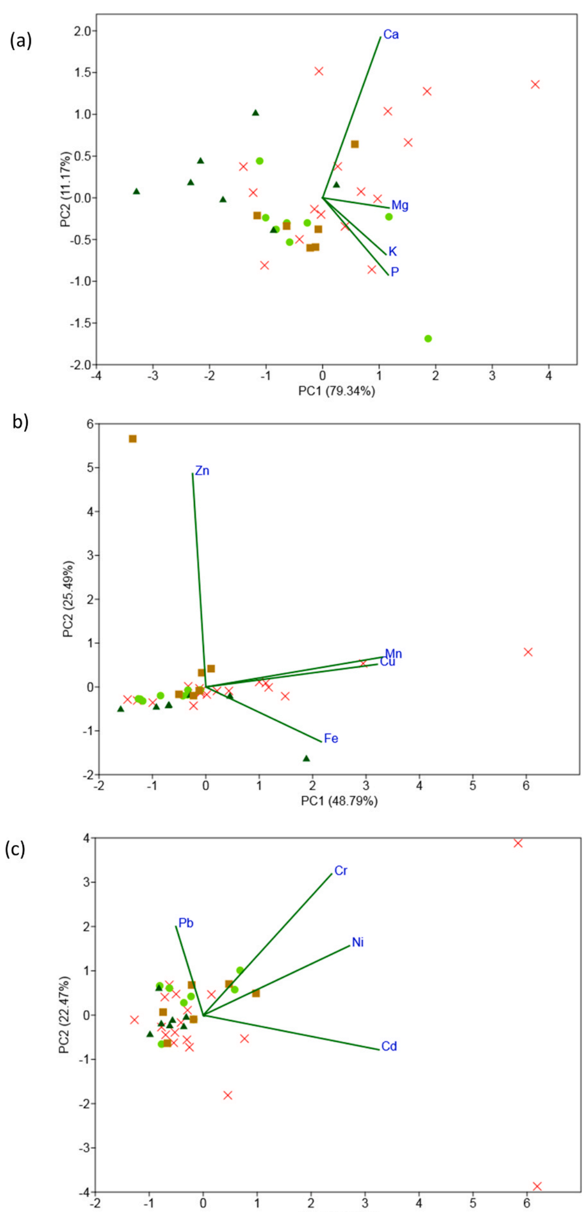

Fig. 1. PCA correlation bi-plot visualizing distributions of (a) macroelements, (b) microelements and (c) trace elements between studied legume species: *Phaseolus* spp. (red crosses), *Vicia* spp. (light-green dots), *Pisum* spp. (dark-green triangles), and *Lathyrus* spp. (brown squares).

4. Discussion

4.1. Macroelements

The macroelement concentration in the analyzed samples of legumes was quite variable, both between and within the groups (Tables 2 and 3). The studied legume varieties were characterized by highest average content of K (14200 mg kg^{-1}), followed by P (4320 mg kg^{-1}).

According to Maeaba and Prasad (2023), insufficient Ca consumption and consequently fragile bones, heart palpitation, muscle cramps and irritability are the great challenge for human health nowadays; for this reason, new natural sources of Ca are requested for inclusion in a diet. The Mg and Ca mean contents in the analyzed legumes were within the range of 961.–1400 mg kg^{-1}. *Phaseolus* spp. contained the highest levels of K, Ca and Mg. The elemental screening performed by Oliviera et al. (2018) using ICP-OES showed lower levels of Ca in seven different varieties of *Phaseolus* purchased at a local market in Sao Paulo (Brazil). Sinkovič et al. (2022) studied by ICP-MS six *P. vulgaris* L. and one *P. coccineus* L. samples grown in experimental fields in Slovenia and found contents of macroelements comparable to the present study. However, the range of K content (1350–1900 mg kg^{-1}) among varieties was broader than in the present study. Grembecka and Szefer (2022) reported much lower K levels (8910 mg kg^{-1} (7600–10100)) in six *P. vulgaris* L. obtained from the Poland market determined by flame atomic absorption spectrometry (F-AAS), but higher levels of Mg (1660 mg kg^{-1} (1340–2020)). Higher Mg levels (1680–1720 mg kg^{-1}) were also reported in the study by Blair et al. (2016), who evaluated the accumulation of minerals and elements in *P. vulgaris* L. grown across Colombia fields.

The determined content of K (13,680 mg kg^{-1}) in *Vicia* spp. was higher than that reported by Sinkovič et al. (2022) (1270 mg kg^{-1}). In the same study, *Vicia* spp. had comparable average levels of Mg and Ca.

In contrast, *Pisum* spp. contained a rather lower content of macroelements than the other studied species. Zhang et al. (2018) reported that the amounts of Ca and Mg in commercially available cultivars of Australian raw *P. sativum* analyzed by the ICP-OES were 304. and 817. mg kg^{-1}, respectively, being lower than the values reported here. In the same study, comparable contents were found for K (11,100 mg kg^{-1}) and P (3870 mg kg^{-1}). Comparable values were also reported in the study of Sinkovič et al. (2022), while Grembecka and Szefer (2022) reported higher levels of P in different pea species collected from the market in Poland (7340–7660 mg kg^{-1}).

Phosphorus main role is in bone formation, it is a component of high-energy compounds and is necessary for the helical structure of nucleic acids (Hernando et al., 2021; Reddi, 2023). Phosphorus was determined in the highest average concentration in *Lathyrus* spp., followed by *Phaseolus* spp. Among the macroelements present in *Lathyrus* spp., the highest concentration was exhibited for K, while registering the lowest measured levels of Ca and Mg. According to Islam et al. (2013), *Lathyrus sativus* L. samples from the Dhaka city market (Bangladesh) were characterized with slightly lower K (8790–12400 mg kg^{-1}), Ca (921–1160 mg kg^{-1}) and Mg (1190–1630 mg kg^{-1}) contents than in this study.

4.2. Microelements

Great variability was observed in microelements' concentration in the analyzed legumes (Table 2). The data revealed that legumes generally contained the highest amounts of Zn and Fe. Particularly, *Lathyrus* spp. were the richest in Zn content. On the other side, they were characterized by low amounts of Mn and Cu.

Iron deficiency anemia, considered the most common anemia in all age groups, mostly coexists with zinc deficiency (Jeng and Chen, 2022). Zinc and iron deficiencies are common in developing countries but also in low-income populations in developed countries (Cole et al., 2010). The WHO published recommendations for prevention and treatment of malnutrition and diarrhea using zinc supplementation (Kopru et al., 2022).For this element, the RDI value was 11 mg day^{-1} (Orecchio et al., 2014). According to Islam et al. (2013), *Lathyrus* spp. samples contained Zn concentration in the range of 46.7–49.3 mg kg^{-1}, comparable with the results from this study. However, the results from the same investigation revealed that *Lathyrus* spp.contained a higher amount of Mn (34.2–39.8 mg kg^{-1}), Fe (29.9–62.5 mg kg^{-1}), and Cu (6.74–7.28 mg kg^{-1}).

Copper is involved in many aspects of energy metabolism as it acts as enzyme biocatalysts; Cu is also important component in the synthesis of hemoglobin, myoglobin and cytochromes and is interrelated with the function of Zn and Fe in the body (Kumari and Platel, 2020).The suggestions made by WHO for a dietary reference adequate intake and a tolerable upper intake for males and females (aged 19–70 years), were 0.90 mg day^{-1} and 10 mg day^{-1}, respectively (Orecchio et al., 2014). On the other hand, manganese is essential for nerve and brain development and cognitive functioning (Kumari and Platel, 2020). The recommended daily allowance for manganese is 2.3 mg day^{-1} for adult males and 1.8 mg day^{-1} for adult females (Kopru et al., 2022).

Compared with the literature data, *Phaseolus* spp. was found to be a distinct source of Zn and Fe, making both elements necessary for the performance of various bioprocesses required for the growth of this plant (El-Sweify et al., 2007). *Phaseolus* spp. Samples of this study contained Zn in the range 25.4–67.0 mg kg^{-1}, which is higher than the data presented by Sinkovič et al. (2022) and Grembecka and Szefer (2022). Another study investigating mineral content in whole seeds of *P. vulgaris* from Colombia showed a wide range of Fe content (40.0–84.6 mg kg^{-1}) (Blair et al., 2009). On the other hand, the determined content of Cu and Mn were lower than the ones reported by Blair et al. (2016) (7.8 and 15.5 mg kg^{-1}, respectively).

The highest Zn concentration found in *Vicia* spp. was similar to those reported for *Vicia faba* L. in the study by Sinkovič et al. (2022). According to the same authors, faba beans were characterized with more than two-fold higher Fe (42.3–47.0 mg kg^{-1}), Mn (16.4–16.8 mg kg^{-1}) and Cu (16.3 mg kg^{-1}) contents than in the present study. *Pisum* spp. was characterized by the highest average content of Fe in comparison to other legumes. Results found for Zn, Mn and Cu levels through this study were of the similar or slightly lower levels as those reported in the literature (Sinkovič et al., 2022; Grembecka and Szefer,2022). However, Zhang et al. (2018) found significantly higher levels of all analyzed microelements in *P. sativum* L. than in this study.

Different elements' concentrations in legume species and those published by other researchers can be partially attributed to the differences in plant genetic material, soil temperature, pH, reductive-oxidative conditions, fertilization and seeding density, but also to the usage of different analytical instruments (AAS, ICP-OES and ICP-MS) (Shahid et al., 2015). According to Anjum et al. (2015), soil pH, high organic matter, low temperature, and high salt concentration strongly determines the availability of some elements such as Fe, Mn, and Zn in leguminous plants. Additionally, the differences in the content of Ca, Cu, Fe and Zn may originate from the presence of a higher P level, as confirmed by Murtaza et al. (2015).

4.3. Trace elements

The mean concentration of trace elements in the analyzed legume samples was in the following order: Ni > Cr > Pb > Cd for all varieties (Table 2). Arsenic concentration was below the detection limit of the applied ICP-OES method for all the samples. *Vicia* spp. samples showed the highest average content in trace elements, while *Lathyrus* spp. varieties were characterized by a quite varied Ni content.

Nickel is classified as an immunotoxic agent and carcinogen to humans (group 1) by the International Agency for Research on Cancer (Begum et al., 2022; IARC, 1990). The EU has not yet defined a maximum Ni concentration in legumes. However, according to the Institute of Medicine (US) Panel on Micronutrients (Institute of Medicine US, 2001), the tolerable upper intake level (UL) for nickel is set at 1 mg day^{-1}. The levels of Ni in analyzed legume samples ranged from 24.2 in the *Pisum* spp. samples to 57.0 mg kg^{-1} in the *Lathyrus* spp. Previous research revealed a Ni content of 1.7 mg kg^{-1} in *Phaseolus* spp. and 0.8–1.1 mg kg^{-1} in *Pisum* spp. (Grembecka and Szefer, 2022) and 0.9 mg kg^{-1} in *Phaseolus* spp. (Blair et al., 2016).

Chromium has been related to the enhancement of the insulin action and regulation of the glucose level (Kumari and Platel, 2020). The Cr content in legume samples ranged from 0.8 in *Phaseolus* spp. to 4.1 mg kg^{-1} in *Vicia* spp. Other researchers have reported Cr concentrations of 0.720 ± 0.034 mg kg^{-1} in *Phaseolus* spp. 1.16 ± 0.074 mg kg^{-1} in *Vicia faba* L. determined using instrumental neutron activation analysis (El-Sweify et al., 2007). A limit for Cr in legumes has also not been established. However, Orecchio et al. (2014) reported a Recommended Daily Intake of 25 mg day^{-1} for most adult females and 35 mg day^{-1} for most adult males. The National Research Council (National Research Council, Recommended dietary allowances, 1989) has determined an estimated safe and adequate daily dietary intake for Cr of 50–200 μg day^{-1} for children from 7 years to adulthood, which is in line with the Recommended Daily Allowance for Cr for adult men and women established by the US National Academy of Sciences (NAS National Academy of Sciences, 1974).

Lead is classified by the IARC (Begum et al., 2022) in group 2a (probably carcinogenic to humans) and as the most toxic compound or human carcinogen by the US Environmental Protection Agency (US EPA US Environmental Protection Agency, 2004). In Regulation (EC) (No 915/2023), the EU Commission has set the maximum admitted levels for Pb in legumes as 0.10 mg kg^{-1} wet weight and a provisional tolerable weekly intake (PTWI) of 0.025 mg kg^{-1} body weight. The same limit was established by Codex Alimentarius CXS (193–1995), 2023 of the Food and Agriculture Organization and the World Health Organization. According to the Serbian Regulation (2019, amended in 2020 and 2021) on the "maximum levels of certain contaminants in food", the regulatory limit for Pb establishes maximum tolerable values of 0.20 mg kg^{-1} of fresh weight within legumes. In the present study, the concentration of Pb ranged from 0.07 in *Phaseolus* spp. to 1 mg kg^{-1} in *Vicia* spp. In all investigated samples (except in some *Phaseolus* spp. varieties), the Pb levels were found to be higher than the maximum levels set by EC, WHO/FAO and Serbian standards, thus surpassing legislation safety limitations for human consumption.

Cadmium is another trace metal that is classified as carcinogenic to humans (group 1) by the IARC (Begum et al., 2022), while its PTWI is 2.5 μg kg^{-1} as established by EFSA European Food Safety Authority, 2023.The Cd content of the analyzed legume samples did not significantly differ and ranged from 0 to 0.07 mg kg^{-1}, being below the limit (0.1 mg kg^{-1}) allowed by the Codex Alimentarius CXS (193–1995), 2023, but higher than the limit 0.02 mg kg^{-1} wet weight set by EU (Regulation (EC) (915/2023). Comparing the results with the Serbian regulation on maximum concentrations of certain contaminants in food (2019, 2020, 2021, and 2021), which defines the maximal values for Cd (<0.02 mg kg^{-1}) in legume samples, it can be concluded that samples in many cases exceed the permitted levels. These higher Pb and Cd levels might be caused by various factors, including the growth environment of legumes (soil and water used for irrigation, and usage of artificial fertilizers or pesticides), aging of the machine during the production, or atmospheric deposition and the proximity of the cultivation area to urban areas where pollution may be a contributing factor. According to the European Environment Agency (EEA European Environment Agency, 2023), toxic heavy metals like Cd, and Pb pose a threat to living organisms. While Europe generally maintains acceptable ambient air concentrations, often tied to particular industrial sites, the deposition of these hazardous metals into the atmosphere ultimately exposes ecosystems and organisms to them, resulting in their bioaccumulation within the food chain, thereby endangering human health. Consequently, the reduction of heavy metal emissions remains a key priority in both international and EU initiatives (EEA European Environment Agency, 2023). Todorović et al. (2019) determined that the mean annual concentrations of PM_{10}, which contain heavy metals, exceeded the guideline value of 20 μg m^{-3} in all three major cities in Serbia: Belgrade, Novi Sad and Niš. The utilization of standard water quality for cultivation on previously unused land, combined with conventional agricultural practices, suggests that air pollution in Serbia could be a significant contributing factor to the elevated concentrations of these heavy metals observed in the analyzed samples. It is worth to mention that no maximum levels have been established for other metals in analyzed legume species in Serbia, and the data provided herein should be helpful in future efforts to establish regulatory limits. According to Blair et al. (2016), the Cd content analyzed by ICP-OES in whole seeds of *P. vulgaris* L. was 0.3 mg kg^{-1}. Cd in *P. vulgaris* L. and *Pisum* spp. was not detected with AAS in the study by Grembecka and Szefer (2022). No prior reports by other researchers of Cd, as well as Pb, Ni, and As contents in *Vicia* spp. were found in the literature. The difference with the literature must be carefully evaluated since part of the difference can be attributed to the sensitivity of the detection method and/or the instrument used. In general, ICP has a higher sensitivity than the flame AAS and certain elements are only detectable by more sensitive instruments.

4.4. Principal components analysis

The bi-plot resulting from the PCA analysis of macroelements does not exhibit clear groupings among the studied legume species. However, there are notable differences regarding the positioning of specific samples. The majority of the *Pisum* spp. samples are grouped on the left side of the bi-plot, while the majority of the *Vicia* spp. and *Lathyrus* spp. samples are located in the middle of the diagram. The *Phaseolus* spp. samples are spread widely across the PCA bi-plot, exhibiting the highest concentrations of Ca. The loadings of Mg, K, and P tend towards the right side of the bi-plot. A few samples of *Phaseolus* spp. and *Vicia* spp. are positioned in this region. Consequently, it can be inferred that these legume species have a higher content of these macroelements.

The presence of narrow groupings of legume samples on the PCA bi-plot of microelements, displayed in Fig. 1b can also be explained in terms of similar profiles of their microelement content. This means that microelements have comparable concentrations, which results in their clustering together on the diagram. The two *Phaseolus* spp., one *Lathyrus* spp. and one *Pisum* spp. samples that are distant from the grouping have maximal levels of Mn and Cu, Zn, and Fe, respectively, observed among all the samples studied.

Based on the PCA analysis for trace elements (bi-plot Fig. 1c), can be observed that data do not exhibit clear groupings, which in turn suggests a lack of distinct clusters or patterns in the distribution of trace elements across the analyzed legume samples. This observation indicates a lack of significant differences in the trace element composition among the legume species under investigation. The absence of distinct groupings in the PCA bi-plots further points towards homogeneity in the Cd, Pb, Ni, and Cr profiles of legume samples, implying that they may have similar compositions or share common characteristics. Apart from the majority of legume samples, two common bean samples show an increased accumulation of Cr, Ni, and Cd.

In summary, the narrow groupings of legume samples observed on the PCA bi-plots of macro-, micro- and trace elements can be attributed to their similar elemental profiles. In their study, dos Santos et al. (2013) identified and verified the grouping tendency of bean samples in relation to their species and their geographical origin. The similar profile observed in the present study is possibly a result of interactions among genetic and environmental factors. Firstly, legume samples that exhibit narrow groupings on the PCA bi-plots likely share similar profiles in terms of their macro-, micro- and trace element contents. Secondly, these elements can interact with each other within the legume samples, which can influence their overall distribution and clustering patterns in the PCA bi-plots. If certain legume species have a consistent balance or ratio of these elements, it would lead to the formation of tight groupings on the diagram. Furthermore, the narrow groupings on the PCA bi-plots can also be influenced by genetic and environmental factors. Legume species that are genetically similar or have been exposed to similar environmental conditions may exhibit similar macro-, micro- and trace element profiles, leading to their proximity in the bi-plot. In the present study, it is important to note that all the samples were cultivated under identical environmental conditions. This uniformity in environmental factors serves to reduce disparities among the samples and encourages their clustering. However, it's noteworthy that significant variations in the elemental composition were observed among varieties within the same species. As a result, the possibility of grouping based solely on species is limited (Bookstein, 2019).

While this study offers valuable insights into the elemental composition of legume species cultivated in Serbia, it is essential to acknowledge certain limitations. Firstly, the investigation focused on a specific set of macro-, micro-, and trace elements, and did not encompass a comprehensive analysis of all potentially relevant elements. Additionally, the study did not delve into the specific sources of contamination, such as detailed assessments of soil properties, water quality, or atmospheric conditions in the cultivation areas, which could provide a better understanding of the observed elemental variations. Furthermore, the sampling was limited to a specific timeframe and geographic region, potentially overlooking seasonal or regional variations that might influence elemental concentrations. Lastly, the study primarily relied on the Metal Pollution Index (MPI) as an indicator of contamination, yet another risk assessment, considering factors like bioavailability and potential health impacts, could enhance the study's depth. Future research endeavors should address these limitations to provide a more holistic understanding of the factors influencing the elemental composition of legumes.

5. Conclusions

In the present work the levels of macro-, micro- and trace elements in 38 varieties of legumes cultivated in Serbia using the ICP-OES technique after microwave digestion were determined. The data indicated a considerable variability across and within species with respect to elemental concentrations. However, the levels of macro- and microelements in all the samples analyzed were within the range reported for similar legumes from various parts of the world. Among the macroelements, the most abundant was K (8980.7–14177.4 mg kg^{-1}, *Phaseolus* spp.), while Zn and Fe were the microelements present in the highest concentration. The mean concentration of trace elements in the analyzed legume samples was in the following order: Ni > Cr > Pb > Cd for all varieties. The detected Pb and Cd contents in every cultivar were above the limits set by national and international legislation, indicating air pollution, soil, or irrigation water as sources of pollution in the growing area. Moreover, the obtained results reveal notable distinctions among the legume species. *Pisum* spp. exhibits the lowest MPI levels, suggesting minimal contamination with toxic elements in comparison to the other legume species. On the nutritional front, *Phaseolus* spp. stands out with the highest values for K, Ca, and Mg. In contrast, *Lathyrus* spp. emerges as the richest in P. Overall, the narrow groupings of legume samples observed on the PCA bi-plots of macro-, micro- and trace elements, can be attributed to their similar profiles derived from genetic and environmental factors.

CRediT authorship contribution statement

Marijana Ačanski: Software, Data curation. **Maria Papageorgiou:** Writing – review & editing, Supervision, Funding acquisition. **João Miguel Rocha:** Writing – review & editing, Supervision, Funding acquisition. **Nataša Nastić:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Marko Ilić:** Software, Data curation. **Kristian Pastor:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Adriana Skendi:**

Writing – review & editing, Formal analysis. **Stefanos Stefanou:** Writing – review & editing, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106127.

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