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Evaluation of the concentration of minerals and heavy metals in some medicinal plants and the safety of their consumption

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Abstract
Besides their therapeutic value, medicinal plants can also contain varying levels of minerals and heavy metals, affecting the quality and safety for use. This could affect how safe and of what quality they are when taken by humans. In this case, we analyzed a series of elements (Na, K, Ca, Mg, Fe, Mn, Zn, Ni, Cr, and Pb) found in plants like *Trifolium pratense* L., *Urtica dioica* L., *Sideritis scardica* Griseb., *Origanum vulgare* L., *Allium ursinum* L., and *Althaea officinalis* L. The analytical method used in order to quantify these concentrations was Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), which is very precise for such an analysis. We noticed that the levels of these elements were not equal across the plants — they were significantly different from each other. Most of the mineral levels were within what is considered acceptable under the Recommended Dietary Allowance (RDA) and Estimated Daily Intake (EDI). In the case of heavy metals (i.e., Cr, Ni, and Pb), Target Hazard Quotient (THQ) and Hazard Index (HI) values came out lower than 1, which means that presumably there is no considerable health hazard in the event of consuming these plants in normal contents. These results indicate the necessity for the systematic evaluation of concentrations of such elements in plant-based drugs and general environmental assessment of their natural habitats to ensure consumer safety.

Keywords: *medicinal plants, minerals, heavy metals, ICP-OES, safety*

Introduction

Medicinal plants grow and mature under varied ecological environmnets, either by accepting different agricultural methods (artificial conditions) or by growing in other varied natural habitats. They are influenced by these varied conditions chemically, and thus most of the plant species and organs become suitable for medicinal uses (Chizzola, 2012). Their growth and development under fluctuating environmental conditions also expose them to various contaminants, e.g., pesticides, polycyclic aromatic hydrocarbons, heavy metals, and others (Tripathy et al., 2015). Plant growth processes require the presence of 19 essential elements, which fall into two very wide groups: macronutrients and micronutrients (Ernst, 2006). In addition to their role in plant physiological process development, these elements are a crucial source of human nutrition and health (Subramanian et al., 2012). However, plants inhabiting heavy metal-contaminated soils may possibly accumulate and concentrate such elements in their organisms (Gashi et al., 2020; Buqaj et al., 2023), whose action can be on various metabolic processes (Gashi et al., 2024). Medicinal plants are used worldwide in the treatment of various diseases; however, their native habitats are becoming more exposed to anthropogenic impacts such as environmental pollution. In this case, the presence of heavy metals in the environment poses a double threat: first, by causing toxicity to the plants, and second, by being stored in the plants and transmitted through the food chain, potentially ending up in humans (Karahan et al., 2020). Heavy metals have been defined as elements (metals and metalloids) with a high atomic number (>20) and high density (>5 g/cm³) that have been proven to be highly toxic in nature (Ali

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and Khan, 2018). Furthermore, as pointed out by Lajayer et al. (2017), the presence of heavy metals in plants affects, inter alia, the biosynthesis pathway of secondary metabolites and leads to qualitative and quantitative levels changes in them. Heavy metals pollution of various ecosystems is dangerous to human health because humans and other organisms come into contact with them through the food chain (Mitra et al., 2022). Therefore, the health of humans can also be harmed by the use of medicinal plants with high concentrations of heavy metals in their body (Asiminicesei et al., 2020). As a result of this, standardization and regulation of permissible levels of heavy metals in medicinal plants are required to prevent hazards and maintain public health (Sarma et al., 2011). Details of the source, uptake, and transport of metal ions and the plant's reaction towards them are explained in a review article by (Hlihor et al., 2022). Medicinal plants consist of several species in the entire world and are recognized for their therapeutic properties as a result of the active substances they have. In this context, they are applied for medication in order to cure and prevent different ailments (Rasool Hassan., 2012). It is also worth noting that, among other things, plants which have developed in soils rich in heavy metals are characterized by altered metabolism, precipitation of such heavy metals in their tissues, and decreased biomass production (Nagajyoti et al., 2010). Other research has been conducted on the determination of metal and heavy metal content in different medicinal plants (Bhat et al., 2010; Ozyigit et al., 2018; Karahan et al., 2020). In Kosovo, research has primarily focused on the determination of metal content in soil, particularly around industrial areas (Borgna et al., 2009; Nannoni et al., 2011; Šajn et al., 2013; Zogaj et al., 2014; Sahiti et al., 2023), with very few studies conducted on the content of heavy metals and minerals in medicinal plants (Faiku et al., 2022a; Faiku et al., 2022b). In addition, research on the therapeutic properties of plants that form part of our investigation exists. Especially the *Trifolium pratense* L. species and other species of this family have a variety of different active compounds (flavonoids, phenolic acids, saponins), which are known well enough for their antioxidant, anti-inflammatory, and anticancer activity (Kolodziejczyk-Czepas, 2012). Moreover, extracts of *Trifolium pratense* L. contain a high content of active compounds, particularly polyphenols, which exert therapeutic action in the relief of post-menopausal syndrome, reducing blood glucose and lipid levels, and anticancer effect (Akbaribazm et al., 2020). In this context, research on the therapeutic effects of *Trifolium pratense* L. has been reviewed in a review article by Akbaribazm et al. (2021). On the other hand, *Urtica dioica* L., being a perennial herb, has been well-known for its multifaceted use both in medicine and diet. As indicated by evidence presented by Asgarpanah & Mohajerani (2012), the plant has been discovered to possess many antibacterial, antiviral, antioxidant, and anticancer activities. The other authors have also presented pharmacological evidences on the use of *Urtica dioica* L. (Joshi et al., 2014; Dhouibi et al., 2020). Furthermore, *Sideritis scardica* Griseb., is among the species that are caracterized on the basis of variable pharmacological activities including antimicrobial, antioxidant, cytotoxic, and anti-inflammatory activities (Todorova et al.,2014). Moreover, the plant was reported to be differentiated on the basis of antioxidant activity because of having high ascorbic acid content, according to research conducted by Vassilevska-Ivanova et al. (2016). Besides, *Origanum vulgare* L., from the family Lamiaceae is a spice and medicinal plant and is important due to its bioactive compound composition with varied activities including antimicrobial, anticancer, and anti-inflammatory (Pezzani et al., 2017). The therapeutic activity of this plant has also been documented by Naquvi et al. (2019), where among others, the antifungal and anti-mutagenic activities *Origanum vulgare* L., extracts are highlighted. Moreover, according to Sobolewska et al. (2015), *Allium ursinum* L. is a medicinal and food plant containing numerous active constituents, displaying antimicrobial activity, antioxidant activity, and significant effect on the cardiovascular system. Also, research regarding antioxidant, antitumor, and antimicrobial activity of *Allium ursinum* L., has been demonstrated in a study by Oravetz et al. (2024). Leaves and roots of the *Althaea officinalis* L., carry secondary metabolites employed for their pharmacological effect as raported by Bonaterra et al. (2020), and characterized by the intense antioxidant and anti-inflammatory activities according to Shah et al. (2011). This research is relevant in many aspects but most importantly particularly relevant to food safety and the potential for plants to accumulate different minerals. This research also highlights the necessity to uncover where in the environment the levels of heavy metals at their highest concentrations and thus can be deposited in plant tissues. These plants are used for different pruposes, mainly in the gastronomic and health sectors, both locally in Kosovo as well as for exports to other countries in the European Union. The main hypothesis of the present

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research is that medicinal plants contain different levels of minerals and heavy metals, and the levels may affect their quality and safety for human use. In this specific case precisely, although as much as they contain therapeutic properties, many medicinal plants may contain heavy metals at levels potentially harmful to health, their quantities should be studied for safe consumption. The aim of the current research was to evaluate the content levels of various minerals and heavy metals in medicinal plants such as: *Trifolium pratense* L., *Urtica dioica* L., *Sideritis scardica* Griseb., *Origanum vulgare* L., *Allium ursinum* L., and *Althaea officinalis* L. The research goal was also to compare some nutritional parameters, i.e., RDA and EDI, and toxicological parameters, i.e., THQ and HI, and determine the impact of minerals and heavy metals on human health.

Material and methods

Sample collection

The plant material, consisting of herba (leaves, stems, and flowers), was collected from *Trifolium pratense* L., *Sideritis scardica* Griseb., *Origanum vulgare* L., and *Allium ursinum* L. Additionally, leaves from *Urtica dioica* L. and roots from *Althaea officinalis* L. were used. (Table 1). This material was sourced from the medicinal and aromatic plant collection center "Agroproduct" in Kosovo. The material collected (approximately 1 kg) analysis from these three parts (sections/layer) of the sacks was thoroughly mixed to create a homogeneous sample, which was then used for The plants collected at this location were supplied in by various harvesters operating in different regions of Kosovo. The sampling of the plant material was carried out randomly. The selection of the samples was done by taking material from different parts of the sacks to ensure an representative composition of the entire content: the upper part of the sacks, the middle part, and the bottom part.

Botanical name	Parts of plants that were used for analysis
Trifolium pratense L.	Herba
Sideritis scardica Griseb.	Herba
Origanum vulgare L.	Herba
Allium ursinum L.	Herba
Urticia dioica L.	Leaves
Althaea officinalis L.	Roots

Table 1. *Medicinal plants and their parts used for analysis*

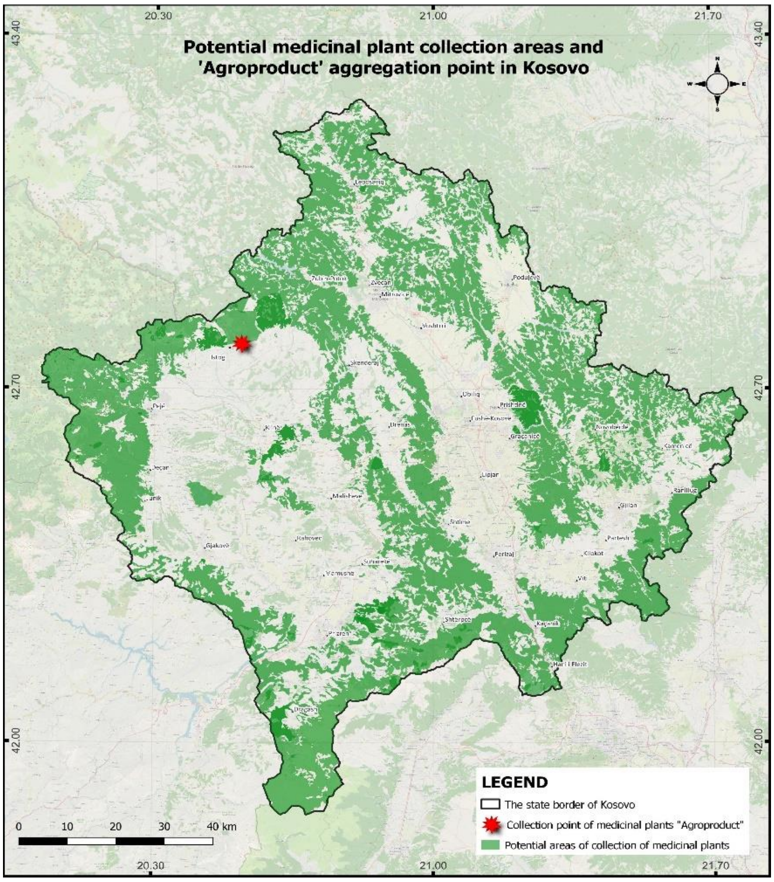

Figure 1
Map of potential medicinal plants areas and "Agroproduct" collection point in Kosovo (Source: Msc. Dardan Hoti)

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Sample preparation and chemical analysis

The concentration of the following elements was determined in the plant material: Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Ni, Cr, and Pb. The plant material was dried at 105°C for 24 hours to remove moisture content. After drying, the material was ground and sieved, and this processed material was used for chemical analysis. For digestion of the plant samples, a microwave digester (Berghof) was used. The plant material was dissolved by acid digestion in Teflon bombs by adding 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 to approximately 500 mg of powdered sample. To determine the mineral and heavy metal concentrations in the plant samples, an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was utilized. The analysis was carried out at the analytical laboratory "Agrovet" located in Fushë Kosovë, Kosovo according to the standardized protocol of the U.S. Environmental Protection Agency (EPA, 2007).

Evaluation of RDA, EDI, THQ and HI

To assess the nutritional contribution of the plants in this study, the percentage of the Recommended Daily Allowance (RDA%) for plants used as teas or herbs in everyday human consumption was calculated. RDA% calculation, based on WHO (2004) is expressed by:

$$RDA\% = \frac{\text{C for 100g sample}}{\text{RDA for mineral}} \times 100 \qquad [1]$$

where: C – the determined content of the mineral in 100g of dry plant matter in mg; RDA – adult individual's daily recommended allowance weighing 70 kg; RDA% – percentage of daily requirement covered by the intake of 100g plant product.

To evaluate the likely risk to human health due to consumption of these plants in the case of heavy metal contamination, we also calculated the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and overall Hazard Index (HI) per USEPA, 2024, which may represent risk the health of consumers. To assess the long-term human health risk from exposure to heavy metals such as Cr, Ni, and Pb through ingestion of the plants, the EDI for each metal was calculated separately using the formula below:

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \qquad [2]$$

where: C – the sample concentration of the metal (mg7 kg); IR – the rate of intake of the product per day (kg/day); ED - exposure duration (years); EF – frequency of exposure (days/year); BW – mean body weight (70 kg); AT – time of daily exposure (days).

EDI is the straightforward formula to calculate THQ, which is an index that estimates the non-carcinogenic health risk after the ingestion of a metal. Less than 1 indicates no significant health risk. THQ is calculated by the following formula:

$$THQ = \frac{EDI}{RfD} \qquad [3]$$

where: EDI – the estimated daily intake (mg/kg/day) for each metal; RfD – the reference dose (mg/kg/day), which refers to the maximum concentration of a contaminant that can be consumed without causing harmful health responses in an individual during their lifetime, according to US EPA (1998), EPA (1991), WHO (2022), and Khan et al. (2008).

The Hazard Index (HI) is then calculated by summing the individual THQ values for each metal using the following formula:

$$HI = THQ_{Cr} + THQ_{Ni} + THQ_{Pb} \qquad [4]$$

If the HI is below 1, this would mean no serious health hazard in long-term exposure. On the other hand, readings up to more than 1 (>1) suggest the potential for chronic health effects.

Data analysis

The study was conducted based on a random model, including three replicates. Statistical processing of the results was carried out using IBM SPSS Statistics 27. The results are presented as arithmetic means and standard error (±). Statistical analysis of variance among plants was performed using one-way ANOVA, while mean comparisons were conducted using the Duncan's Multiple Range Test at the 5% significance level. Additionally, the correlation coefficient (r) was analyzed using Pearson's method to examine relationships between the minerals and heavy metals.

Results and discussion

Concentration of minerals on medicinal plants

Mineral contents of Na, K, Ca, and Mg in medicinal herbs are presented in Table 2. The highest sodium concentration was found in *Althaea officinalis* L. with the value 1723.61 ±37.98 mg kg^{-1} and followed by *Origanum vulgare* L. with the value 1486.61 ±34.72 mg/

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kg^{-1}. The least content was for *Sideritis scardica* Griseb. with 540.15 ± 11.33 mg kg^{-1}. It is evident that there are plant species that can accumulate more of this element. This may be a significant factor while choosing plants for medicinal or culinary use where the content of this element comes into play. For potassium, the maximum concentration of this element was recorded in *Urtica dioica* L. leaves (15772.73 ± 152.97 mg kg^{-1}), as well as with high accumulating ability in nettle leaves. In contrast, lowest potassium was found in *Althaea officinalis* L. roots and *Sideritis scardica* Griseb. herb, 6875.49 ± 202.13 mg kg^{-1} and 7684.01 ± 324 mg kg^{-1}, respectively. The maximum calcium concentration was found in *Urtica dioica* L. (12984.28 ± 146.28 mg kg^{-1}), which higjlighting its exceptional ability to accumulate this mineral at nettle leaves. The outcome also shows that the contents of *Origanum vulgare* L. and *Trifolium pratense* L. are identical, as the species *Allium ursinum* L., *Sideritis scardica* Griseb., and *Althaea officinalis* L. Further, *Urtica dioica* L. leaves had the highest content of magnesium (4640.80 ± 26.40 mg kg-^{1}), whereas Allium ursinum L. had the minimum. Magnesium is a very important element involved in many metabolic processes, especially enzymatic activity. The statistical analysis revealed significant differences ($P < 0.05$) in Na, K, Ca, and Mg concentrations among the plant species analysed. The proportion of major macroelements, i.e., potassium (K), calcium (Ca), and magnesium (Mg), in medicinal plants, i.e., *Urtica dioica* L., *Sideritis sp.*, and *Origanum vulgare* L., follows a definite pattern of distribution: K > Ca > Mg, as cited by Chizzola (2012). These findings are fully consistent with our research work, which also identifies the same general trends in the abundance of these elements and therefore confirms previously established scientific research results in this regard. Their mineral content, i.e., Na, K, Ca, and Mg plays a crucial role in deter-mining their medically beneficial and traditional medi-cinal uses. Specifically, studies by Radha et al. (2021), on some medicinal plants indicate the concentration of such elements in plants and also indicate the impor-tance of elements such as potassium and sodium in the cardiovascular system, calcium in skeletal and muscular systems, and magnesium in the activity of many enzymes. Therefore, it can be argued that plants with high-content of such minerals have greater potential health benefits. On the other hand, different plant species showed varying levels of accumulation of these elements. Considering the obtained data, it is clear that there are differences in the mineral concentrations among medicinal plants. Various environmental factors, agricultural practices, environmental pollution, the presence of these elements in the soil, and the plant species itself, all influence the mineral content within plant tissues. In this context, according to a study by Ceccanati et al. (2021), the concentration of various minerals was analyzed in several medicinal plants. The data from this study suggest that different environmental conditions in various geographical regions, as well as agricultural practices, influence the absorption of minerals from the substrate. In another study by Mohammadi & Asadi-Gharneh (2018), the species of *Mentha longifolia* was found to accumulate minerals based on the ecotype of the plant and the physico-chemical properties of the soil. Therefore, in our study, factors of different nature as mentioned above may have influenced the mineral concentrations in the medicinal plants.

Plants	Na	K	Ca	Mg
Trifolium pratense L.	1147.44^{C} ±12.89	13101.05^{A} ±135.05	4443.38^{C} ±80.99	2252.06^{C} ±36.36
Urticia dioica L.	1229.38^{C} ±133.42	15772.73^{A} ±152.97	12984.28^{A} ±146.28	4640.80^{A} ±26.40
Sideritis scardica Griseb.	540.15^{D} ±11.33	7684.01^{B} ±328.15	2690.03^{E} ±51.39	1808.32^{D} ±44.98
Origanum vulgare L.	1486.61^{B} ±34.72	14958.28^{A} ±500.85	5320.74^{B} ±41.99	2224.40^{C} ±15.32
Allium ursinum L.	1268.35^{C} ±21.85	15246.38^{A} ±128.03	2991.01^{D} ±86.80	1437.52^{E} ±11.39
Althaea officinalis L.	1723.61^{A} ±37.98	6875.49^{B} ±202.13	2168.06^{F} ±87.90	3229.18^{B} ±97.91
F	44.87	8.53	2053.27	583.14
P <0.05	0.000	0.001	0.000	0.000

Table 2.
Concentrations (mg kg^{-1}) of sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) on medicinal plants .

Note:
Means in each column followed by same letters are not significantly different at *P* 0.05 by one-way ANOVA with Duncan's multiple range tests.

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Concentration of heavy metals on medicinal plants

Heavy metal contents of Fe, Mn, Cu, Zn, Ni, Cr, and Pb in medicinal plants are presented in Table 3. The highest Fe (iron) content was found in *Origanum vulgare* L. (561.42±10.16 mg kg⁻¹), followed by *Urtica dioica* L. (377.98 ± 12.40 mg kg⁻¹), and *Allium ursinum* L. (340.76 ± 12.11 mg kg⁻¹), was found. In contrast to this, the lowest content of this element was found in *Althaea officinalis* L. roots (23.93 ± 2.33 mg kg⁻¹). This could be due in the distribution of this essential microelement to the aerial parts of the plant. Mn (Manganese) exhibited a broad spectrum of values in the investigated species. It was greatest in *Origanum vulgare* L. (100.46 ± 1.28 mg kg⁻¹) and lowest in the roots of *Althaea officinalis* L. (8.21 ± 0.19 mg kg⁻¹). Manganese is a vital microelement required to activate enzymes. Copper (Cu) content varied not broadly and was highest in roots of *Althaea officinalis* L. (9.02 ± 0.33 mg kg⁻¹) and lowest in *Allium ursinum* L. (3.66 ± 1.42 mg kg⁻¹). P-value 0.069 indicates the absence of statistically significant differences among species and that the content of the specified element is very homogeneous in the analyzed plants. The greatest content of zinc (Zn) was found in *Allium ursinum* L. (31.31 ± 0.71 mg kg⁻¹) with high microelements accumulation capacity. The lowest content of zinc was established in *Sideritis scardica* Griseb. (19.80 ± 0.47 mg kg⁻¹). Regarding nickel (Ni), its maximum contents were recorded in *Allium ursinum* L. (5.28 ± 0.14 mg kg⁻¹). Although nickel is a useful microelement, excessive nickel concentrations can be phytotoxic and even dangerous to humans. Its content in plants should therefore be strictly analyzed prior to being used for medicinal or food purposes. The maximum levels of chromium (Cr) were found in *Allium ursinum* L. (3.66 ± 0.41 mg kg⁻¹) and *Origanum vulgare* L. (3.51 ± 0.24 mg kg⁻¹), while the minimum level was found in roots of Althaea officinalis L. (0.74 ± 0.05 mg kg⁻¹). For lead concentration, the highest were in *Origanum vulgare* L. (4.11 ± 0.24 mg kg⁻¹) and *Allium ursinum* L. (3.45 ± 1.32 mg kg⁻¹), showing that the two plant species are prone to lead accumulation. The lowest level of lead was found in *Althaea officinalis* L. (0.17 ± 0.17 mg kg⁻¹). Statistical analyses indicate significant differences in concentrations of the majority of the heavy metals ($P < 0.05$) except for copper. Generally, the concentrations of these elements are not homogeneous, suggesting differences in the ability of the plants to absorb or filter out these elements. One can also infer that various factors regulate the concentration of these elements in the plants. The findings published by Chizzola (2012), indicate that the levels of the major heavy metals in medicinal plants *Urtica dioica* L., *Sideritis sp*., and *Origanum vulgare* L. follow the order: Fe > Mn > Zn > Cu. The same distribution was observed in our study, validating previous reports and highlighting the accumulation habits of these elements by these plants. There have been various studies on *Trifolium pratense* L. depending on the heavy metal level. In one study conducted by Cakaj et al. (2023), there was a assessment of metal concentrations such as Cr, Ni, Cu, and Pb. The study revealed higher values of the bioaccumulation factor (BCF) and the translocation factor (TF) for metals such as Ni and Cr. In Romania, another study was conducted by Oprea et al. (2024), and the authors were interested in metal contents of Cd, Zn, Cu, and Pb. According to the results of the study, high levels of metals in the soil caused their accumulation in *Trifolium pratense* L. These studies illustrate that when *Trifolium pratense* L. is grown on polluted areas or farmland, it may absorb heavy metals. They also point to the effect of the concentration of metals in the soil, agricultural and industrial operations, and the effect of physico-chemical factors of the soil on the uptake process of such elements. Therefore, there should be caution when collecting this plant from regions with high anthropogenic pressure, where contamination levels may be higher. Similarly, such studies have also been conducted on *Urtica dioica* L. A study by Sahiti et al. (2023), in Kosovo determined the concentration of Pb, Ni, Zn, and Fe . Higher concentrations of these elements were found in plants near industrial areas, suggesting a clear relationship between metal concentrations in the plant and those in the soil. Other studies of this type in Kosovo were conducted near traffic areas, such as by Daci-Ajvazi et al. (2018), and near industrial zones, as seen in the work of Bislimi et al. (2021). *Sideritis scardica* Griseb. is an endemic plant of the Balkan Peninsula and is traditionally used for the treatment of various respiratory and gastrointe-stinal ailments. According to Qazimi et al. (2022), an analysis of 25 elements in samples of *S. scardica* Griseb. grown under natural conditions and those cultivated under controlled conditions revealed slight variations in element concentrations, depending on the growing environment. Another study Karapandzova et al. (2013), in North Macedonia and Albania investigated the concentration of heavy metals in *S. scardica* Griseb.,

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Table 3. *Heavy metals concentration on medicinal plants (mg kg-1 dry weight)*

Plants	Fe	Mn	Cu	Zn	Ni	Cr	Pb
Trifolium pratense L.	69.06^{E} ±7.55	27.40^{D} ±0.37	6.17^{AB} ±0.25	28.11^{AB} ±0.71	4.59^{B} ±0.14	1.28^{C} ±0.20	0.57^{B} ±0.03
Urtica dioica L.	377.98^{B} ±12.40	85.34^{B} ±3.67	5.26^{B} ±0.06	24.22^{CD} ±1.51	2.30^{E} ±0.12	2.19^{B} ±0.41	0.89^{B} ±0.35
Sideritis scardica Griseb.	305.09^{D} ±4.37	99.43^{A} ±9.48	5.75^{AB} ±2.14	19.80^{E} ±0.47	3.78^{C} ±0.09	3.40^{A} ±0.15	0.93^{B} ±0.17
Origanum vulgare L.	561.42^{A} ±10.16	100.46^{A} ±1.28	6.71^{AB} ±0.12	21.56^{DE} ±0.52	3.76^{C} ±0.11	3.51^{A} ±0.24	4.11^{A} ±0.24
Allium ursinum L.	340.76^{C} ±12.11	54.69^{C} ±4.05	3.66^{B} ±1.42	31.31^{A} ±0.71	5.28^{A} ±0.14	3.66^{A} ±0.41	3.45^{A} ±1.32
Althaea officinalis L.	23.93^{F} ±2.33	8.21^{E} ±0.19	9.02^{A} ±0.33	25.64^{BC} ±2.18	3.14^{D} ±0.04	0.74^{C} ±0.05	0.17^{B} ±0.17
F	501.92	74.61	2.76	12.43	78.49	19.60	8.23
P <0.05	0.000	0.000	0.069	0.000	0.000	0.000	0.001

Note: Means in each column followed by same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

reporting the following order of metal concentrations: Zn > Mn > Cu > Ni > Cr > Pb. In contrast, our study found a somewhat different trend: Mn > Zn > Ni > Cu > Cr > Pb. *Origanum vulgare* L. is considered an important medicinal plant and is also used in culinary applications. Several studies have been conducted to determine the mineral and heavy metal concentrations in this species (Antal et al., 2015; Marinescu et al., 2020). These studies emphasize the influence of various factors on the accumulation of minerals and heavy metals in *O. vulgare* L.. Additionally, results presented by Behmmen et al. (2020), regarding the concentration of minerals and heavy metals in *O. vulgare* L., based on a study conducted in Bosnia and Herzegovina, are consistent with the findings of our study. On the other hand, Gordanić et al. (2023), examined wild garlic (*Allium ursinum* L.) and showed that this plant is very effective in accumulating essential and non-essential elements. It can thus be concluded that *A. ursinum* L. is a source of major minerals and also effective in accumulating toxic heavy metals such as Cd, Pb, and Cr. These findings are consistent with our findings on levels of Cr and Pb. Furthermore, other data regarding the levels of biogenic elements and their biochemical interactions of them in *A. ursinum* L. were presented in a publication by Zaimenko et al. (2024). At last, some determination studies of heavy metal concentrations in *Althaea officinalis* L. has been conducted, such as by Jurowski & Krośniak (2022), and Memushaj et al. (2024). Based on the findings of various research studies, there is sufficient evidence to address the in-volvement of several factors in the accumulation of heavy metals in plants. Rashid et al. (2023), in a review study, provide an overview of the factors that affect metal uptake. All of these are plant species and genotype, metal characteristics such as type, concentration, mobility and bioavailability forms, solubility, and oxidation state, and soil pH, cation exchange capacity, organic matter content, soil texture, microbial activity, and soil water level. Since the plants under study were harvested from different places with different environmental conditions, future studies should emphasize conducting site-specific analysis to understand the heavy metal accumulation in medicinal plants more effectively. The toxic and normal concentration values of heavy metals in plants are presented in Table 4, as per a study conducted by Ozigyt et al. (2023). Specifically, the table provides data on normal and toxic concentrations of Fe, Mn, Cu, Zn, Ni, Cr, and Pb. Plant heavy metals at concen-trations higher than tolerable values induce phytotoxicity, which impairs normal growth and development. It is also of atmost priority to provide maximum permissible concentrations of the metals in plants that are utilized for various human activities. The highest permissible concentrations of certain heavy metals in medicinal plants are presented below, based on information reported by Kohzadi et al. (2018) : Zn 27.4 mg kg^{-1}, Cr 0.2–2 mg kg^{-1}, Ni 1.63 mg kg^{-1}, Pb 10 mg kg^{-1}. The findings of this research in-dicate that Zn content is above acceptable limits in *Trifolium pretense* L., and Allium ursinum L. Moreover, Cr content exceeds the allowed levels in *Urtica dioica* L,.

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Table 4. *Reference values for normal and toxic heavy metal concentrations (mg kg⁻¹) in plants based on data by Ozyigit et al. (2023).*

Heavy metal	Normal concentration	Toxic concentration
Fe	2–250	400–1000
Mn	30–300	300–500
Cu	5–30	20–100
Zn	25–150	100–400
Ni	0.1–5	30
Cr	0.006–18	>100
Pb	0.1–10	30–300

Sideritis scardica Griseb., *Origanum vulgare* L., and *Allium ursinum* L. As for Ni, its content exceeds acceptable levels in all plant species under study in this study. However, Pb contents are within acceptable levels based on prior research findings. These results emphasize the ability of some medicinal plants to uptake heavy metals to levels that may be toxic to plant growth as well as human ingestion. Regular monitoring and research are therefore critical to ascertain the safety and quality of these plants for medicinal and food purposes.

Correlation matrix between minerals and heavy metals in medicinal plants

The correlation between minerals and heavy metals are presented in Table 5. Significant positive correlations were observed between potassium and calcium (r = 0.528, $P < 0.05$), potassium and iron (r = 0.518, $P < 0.05$), and zinc and nickel (r = 0.534, $P < 0.05$). Additionally, strong positive correlations were found between calcium and magnesium (r = 0.796, $P < 0.01$), iron and manganese (r = 0.866, $P < 0.01$), iron and chromium (r = 0.800, $P < 0.01$), iron and lead (r = 0.711, $P < 0.01$), manganese and chromium (r = 0.747, $P < 0.01$), and between chromium and lead (r = 0.725, $P < 0.01$). In contrast, significant negative correlations were identified between sodium and manganese (r = -0.510, $P < 0.05$), magnesium and chromium (r = -0.489, $P < 0.05$), and manganese and zinc (r = -0.587, $P < 0.05$). Furthermore, strong negative correlations were identified between potassium and copper (r = -0.666, $P < 0.01$), calcium and nickel (r = -0.630, $P < 0.01$), and magnesium and nickel (r = -0.878, $P < 0.01$).According to various studies, synergistic and antagonistic interactions exist between metals in terms of their accumulation in plants. Furthermore, according to Kaur et al. (2009), the synergistic or antagonistic influence on the accumulation of different metals such as Cr, Mn, Zn, and Cu in *Brassica juncea* depended on their concentration in the substrate and the type of metal present. Similarly, Khan et al. (2019), reported comparable findings in tomato and potato plants. Overall, the issue of metal accumulation in plants, particularly the synergistic or antagonistic effects, is highly complex in nature. This is because the issue of heavy metal accumulation in plants requires a multidisciplinary approach, involving pedology, aspects of plant physiology, and genetic-level research related to gene expression in the biosynthesis of heavy metal transporters in plants (Khan et al., 2021).

Table 5. *Correlation between minerals and heavy metals across all plants*

	Na	K	Ca	Mg	Fe	Mn	Cu	Zn	Ni	Cr	Pb
Na	1										
K	0.141	1									
Ca	0.028	0.528*	1								
Mg	0.311	0.091	0.796**	1							
Fe	-0.119	0.518*	0.375	-0.073	1						
Mn	-0.510*	0.216	0.375	-0.038	0.866**	1					
Cu	0.342	-0.666**	-0.182	0.232	-0.343	-0.207	1				
Zn	0.350	0.258	-0.129	-0.171	-0.336	-0.587*	-0.232	1			
Ni	-0.137	0.161	-0.630**	-0.878**	-0.059	-0.202	-0.370	0.534*	1		
Cr	-0.368	0.297	-0.040	-0.489*	0.800**	0.747**	-0.375	-0.133	0.339	1	
Pb	0.150	0.447	-0.071	-0.415	0.711**	0.416	-0.207	0.068	0.346	0.725**	1

Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

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Evaluation of RDA, EDI, THQ and HI

Evaluation of recommended dietary allowance (RDA)

Na, K, Ca, Mg, Fe, Mn, Cu, and Zn RDA values and daily requirement in milligrams are presented in Table 6. The minimum RDA value of 3.6 for sodium was that of *Sideritis scardica* Griseb., and maximum value of 74.3 in *Trifolium pratense* L. The minimum RDA value for potassium was 14.6 in *Althaea officinalis* L., and maximum value of 33.6 in *Urtica dioica* L. The minimum 21.7 content of calcium was present in *Althaea officinalis* L., while the maximum 129.8 was present in *Urtica dioica* L. The contents of magnesium was 34.2 in *Allium ursinum* L., and 110.5 in *Urtica dioica* L. For iron, however, the minimum value of 29.9 was in *Althaea officinalis* L., but the maximum value of 701.8 was present in *Origanum vulgare* L. The lowest concentration of manganese (35.7) was found in *Althaea officinalis* L., and the highest (436.8) in *Origanum vulgare* L. For copper, the lowest RDA value of 40.7 was in *Allium ursinum* L., and the highest in *Althaea officinalis* L. For zinc, the lowest concentration of 18.0 was in *Sideritis scardica* Griseb., and the highest of 28.5 *A*was in *llium ursinum* L. Overall, in the present study, *Urtica dioica* L. showed the highest RDA values for K, Ca, and Mg, whereas, *Origanum vulgare* L., has the highest for Fe and Mn. *Althaea officinalis* L., has the lowest for nearly all of the elements, particularly for K, Fe, and Mn. The evidence clearly indicates that the mineral requirements for minerals such as Na, K, Ca, and Mg are not met by ingestion of 100 g dw^{-1} of medicinal plants under investigation. For example, the required dose per day (RDA) of Na is 1500 mg and the maximum RDA value from plants studied for sodium is *Trifolium pratense* L. (74.43 mg) and the plant provides only approximately 5% of the dose. In contrast, RDA values of Fe, Mn, Cu, and Zn are exceeded in all cases when 100 g dw^{-1} of respective medicinal plants is consumed. The RDA for Fe is 8 mg in comparsion, whereas the maximum in RDA among the plants investigated for iron, *Origanum vulgare* L. (701.8 mg), is well above the requirement. RDA is a recommendation that allows us to determine if the quantity of the vital minerals in medicinal plants is sufficient or deficient to support health functions in human beings (Karahan et al., 2023).

Table 6. *Recommended dietary allowance (RDA) values for nutrients in parts of plants consumption.*

Plants	Na (1500 mg)	K (4700 mg)	Ca (1000mg)	Mg (420 mg)	Fe (8 mg)	Mn (2.3 mg)	Cu (0.9 mg)	Zn (11 mg)
Trifolium pratense L.	74.3	27.9	44.4	53.6	86.3	119.1	68.6	25.6
Urtica dioica L.	8.2	33.6	129.8	110.5	472.5	371.0	58.5	22.0
Sideritis scardica Griseb.	3.6	16.3	26.9	43.1	381.4	432.3	63.9	18.0
Origanum vulgare L.	9.9	31.8	53.2	53.0	701.8	436.8	74.6	19.6
Allium ursinum L.	8.5	32.4	29.9	34.2	426.0	237.8	40.7	28.5
Althaea officinals L.	11.5	14.6	21.7	76.9	29.9	35.7	100.2	23.3

Note: 70 kg adults-based on 100 g dw^{-1}

Evaluation of Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI)

Our Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) levels of heavy metals such as Cr, Ni, and Pb in plant samples are presented in Table 7. The highest EDI value of chromium was calculated in *Allium ursinum* L. (2.51E-02), nickel (2.58E-02) was also found un the same plant, and the maximum EDI value of lead was found in the plant *Sideritis scardica* Griseb. (1.51E-02). The lowest values of EDI for chromium (5.08E-03), for nickel (1.13E-02), and for lead (5.70E-04) were found in *Althaea officinalis* L. The maximum THQ values of chromium (1.25E-02) and nickel (1.72E-02) were found in *Allium ursinum* L., while the maximum THQ value of lead (1.51E-03) was recorded in *Sideritis scardica* Griseb. The minimum THQ values of chromium (2.54E-03) and lead (5.70E-05) were recorded in *Althaea officinalis* L., while the minimum THQ value of nickel (7.50E-03) was recorded in *Urtica dioica* L. The maximum HI value (3.08E-02) was noted in *Allium ur-*

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sinum L., and the minimum value of HI (1.28E-02) was noted in *Althaea officinalis* L. THQ and HI values are below 1 (THQ <1; HI <1) and do not pose any health risk on oral ingestion of the plants under study. Inclusion of EDI, THQ, and HI provide an overall picture for safety and efficacy assessment of medicinal and aromatic plants intended for therapeutic use. The guidelines provide a nutritional as well as toxicological fingerprint which helps in the selection of various medicinal plants for use. Such studies involving the analysis of these guidelines have been documented by various scientists like (Woldetsadik et al., 2020; Gjergjizi -Nallbani et al., 2024).

Moreover, according to Naz et al. (2024), they emphasized the importance of studying such parameters to safeguard human health from different concentrations of heavy metals. Therefore, constant monitoring of the concentration of heavy metals in medicinal plants is essential to reduce their negative effect on human health.

Table 7. *Estimated daily intake (EDI), target hazard quotient (THQ) of heavy metals and hazard index (HI) values for adults associated with the consumption of plants samples*

Plants	Cr		Ni		Pb		HI
	EDI	THQ	EDI	THQ	EDI	THQ	
Trifolium pratense L.	8.78E-03	4.39E-03	2.24E-02	1.50E-02	1.85E-03	1.85E-04	1.95E-02
Urtica dioica L.	1.50E-02	7.50E-03	1.13E-02	7.50E-03	2.88E-03	2.88E-04	1.53E-02
Sideritis scardica Griseb.	2.33E-02	1.17E-02	1.85E-02	1.23E-02	1.51E-02	1.51E-03	2.55E-02
Origanum vulgare L.	2.40E-02	1.20E-02	1.84E-02	1.23E-02	1.33E-02	1.33E-03	2.56E-02
Allium ursinum L.	2.51E-02	1.25E-02	2.58E-02	1.72E-02	1.11E-02	1.11E-03	3.08E-02
Althaea officinalis L.	5.08E-03	2.54E-03	1.54E-02	1.02E-02	5.70E-04	5.70E-05	1.28E-02

Research limitations

This study has certain limitations because that the plant samples were collected from a central aggregation point without knowing the precise area or exact location of their origin (the plants brought to this point originate from various localities across Kosovo). The study aimed to analyze only the current status of selected medicinal plants in terms of their concentrations of minerals and heavy metals.

Conclusions

This article provides a quantitative analysis of certain minerals and heavy metals in medicinal plants, with an emphasis on the impact of these contents on the quality and safety of their use. The research revealed that there was a great variation in mineral and heavy metal content in the studied plants, which resulted from the influence of environmental factors and soil properties on which the respective plants grew and developed. Correlation analysis revealed that there were significant correlations between minerals and heavy metals, suggested synergistic and antagonistic interacttions. Moreover, exposure assessment based on medicinal plants' ingestion, as estimated by EDI and RDA indices, indicated that mineral levels, in most cases, are safe to ingest. Health risk assessment, through THQ and HI indices, indicated that for heavy metals such as Cr, Ni, and Pb, non-carcinogenic remains within the tolerable range. This study stresses the importance of examining the mineral content and heavy metals in various medicinal plants regading their quality and safe use. There is also a need to emphasize the need to inform collectors of medicinal and aromatic plants so that they are not gathered in the surroundings of intensive agricultural production areas, in the proxymity of metal industries, or along roads with heavy traffic. More studies should be conducted to evaluate the factors influencing metal accumulation and to determine their long-term impacts on human health.

Conflict of Interests

The authors state that there are no conflicts of interest related to the publication of this paper.

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