

# TOXIC METAL ACCUMULATION IN MEDICINAL PLANTS COLLECTED FROM ROADSIDE ENVIRONMENTS: IMPLICATIONS FOR HUMAN CONSUMPTION

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## ABSTRACT

Medicinal plants can absorb heavy metals from the environment, which may pose risks to human health when consumed. These elements can negatively affect plant growth and metabolism by disrupting key physiological and biochemical processes. This study aimed to evaluate environmental conditions and determine the concentrations of selected minerals (Ca, K, Na and Mg) and heavy metals (Cr, Ni, Pb, Zn, Cu, Mn and Fe) in three medicinal plant species [*Silybum marianum* (L.) Gaertn., *Achillea millefolium* L. and *Convolvulus arvensis* L.], as well as to assess potential human health risks associated with their consumption. Plant samples were collected from roadside areas in the municipality of Prizren, Kosovo, and elemental concentrations were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The results revealed species-dependent variability in both mineral and heavy metal concentrations. *S. marianum* exhibited elevated levels of several essential minerals, whereas *C. arvensis* showed a higher accumulation potential for chromium and nickel, exceeding the permissible WHO/FAO limits. Correlation analysis indicated both synergistic and antagonistic relationships between minerals and heavy metals, suggesting shared sources of contamination and element-specific uptake mechanisms. Health risk assessment based on estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI) values indicated no significant non-carcinogenic risk for adult consumers under the assumed consumption scenario. Nevertheless, the accumulation of certain toxic elements highlights the importance of regular environmental monitoring and preliminary assessment of medicinal plants harvested from roadside environments. Future studies should also include heavy metal content and physicochemical properties of soils from harvesting sites to better understand plant–soil interactions and contamination pathways.

**Keywords:** environmental monitoring; human health risk; ICP-OES; roadside contamination; trace elements

## Introduction

Medicinal plants have been used by humans for centuries in traditional medicine and culinary practices, serving as valuable sources of bioactive compounds for the prevention and treatment of various diseases (Hossein-zadeh et al. 2015). A growing body of scientific research has confirmed their therapeutic potential in both traditional and contemporary medical systems, particularly for managing a wide spectrum of health disorders (Yuan et al. 2016). In addition to bioactive constituents, medicinal plants may also provide essential minerals such as calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg). These elements are involved in key metabolic processes, including protein synthesis and the regulation of water balance in the human body, thereby contributing to the medicinal and nutritional value of these plants (Shakya 2016).

Although herbal teas have historically been used for health-related purposes, their consumption increased markedly during the COVID-19 pandemic, when they were widely used as supportive approaches for managing and preventing infections (Luo et al. 2021). Several medicinal species have been investigated for their therapeutic potential. For example, *Silybum marianum* (milk thistle) has demonstrated anticancer properties and is widely

used in the management of liver-related conditions (Porwal and Muath 2019; Emadi et al. 2022). *Achillea millefolium* (yarrow) is traditionally used to treat ulcers and inflammatory disorders of the gastrointestinal tract (Far et al. 2023). *Convolvulus arvensis* exhibits antibacterial and antioxidant activities (Salamatullah 2022), while other species within the genus *Convolvulus* have shown potential in improving cognitive functions and alleviating symptoms of neurodegenerative diseases, including Alzheimer’s disease (Agarwal et al. 2014; Bihaqi et al. 2016).

In this context, *S. marianum* contains bioactive compounds such as silybin and silymarin, which exert hepatoprotective, anti-inflammatory, antioxidant, and anticancer effects, and have been implicated in the treatment of diseases affecting the liver, pancreas, prostate, and nervous system (Křen and Walterova 2005). In addition, *S. marianum* has been reported to act as an anti-fibrotic agent by neutralizing hepatic toxins (Abenavoli et al. 2010). *A. millefolium* contains constituents such as camphor, borneol, and eucalyptol, which contribute to its anti-inflammatory and antimicrobial properties (Candan et al. 2003). These effects have been associated with inhibitory activity against elastase and matrix metalloproteinases, enzymes involved in inflammatory processes (Benedec et al. 2007).

Meanwhile, *C. arvensis* has been traditionally used for its laxative, diuretic, and anti-inflammatory properties (Salehi et al. 2020). Experimental studies have confirmed that *C. arvensis* exhibits anti-inflammatory, antimicrobial, and antifungal activities due to the presence of active compounds such as cuprenene, thymol, and chamazulene, which contribute to antioxidant activity and the inhibition of pathogenic microorganisms (Salamatullah 2022).

The documented therapeutic potential of medicinal plants may be hindered or compromised by environmental pollutants, particularly heavy metals. These toxic elements can interfere with metabolic pathways and disrupt the biosynthesis of active compounds, thereby diminishing the medicinal value of plants. Moreover, many medicinal species can accumulate substantial concentrations of heavy metals, occasionally exceeding thresholds considered safe for human health. For example, *S. marianum* has been reported to accumulate significant levels of heavy metals under natural field conditions, suggesting its potential use in environmental monitoring and phytoremediation strategies (Angelova et al. 2018), including possible applications within crop rotation systems (Angelova and Inhtyarova 2023). Similarly, *A. millefolium* has demonstrated the ability to accumulate heavy metals, which may alter the composition of essential oils and highlight its relevance in phytotoxicological assessments (Angelova and Inhtyarova 2023). In the case of *C. arvensis*, a high capacity to accumulate chromium (Cr), cadmium (Cd) and copper (Cu) has been reported, suggesting potential for phytoremediation of contaminated soils (Gardea-Torresdey et al. 2004). In addition, *C. arvensis* has been identified as a potential hyperaccumulator of chromium (Angelova and Inhtyarova 2023).

Certain metals such as copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni) and manganese (Mn) are essential micronutrients for both plants and animals, contributing to numerous metabolic and enzymatic processes. However, at excessive concentrations these elements may become toxic. In contrast, metals such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) do not serve known biological functions and are considered highly toxic even at trace levels (Luo et al. 2021). Due to their density, toxicity and reactivity, heavy metals can accumulate in plant tissues and may pose health risks when transferred through the food chain (Fahimirad and Hatami 2017).

Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni) and chromium (Cr), even at low concentrations, as well as zinc (Zn) and copper (Cu) at elevated concentrations, have been shown to exert detrimental effects on both soft tissues (e.g. liver, kidneys and testes) and hard tissues (e.g. femur and tibia) in animals exposed to industrial pollution (Plakiqi Milaimi et al. 2016). In addition, exposure to moderate levels of these metals has been associated with anemia, erythropenia, leukocytosis and carcinogenic effects in human popula-

tions (Briffa et al. 2020), as well as with the inhibition of key enzymatic activities in plants (Gashi et al. 2024).

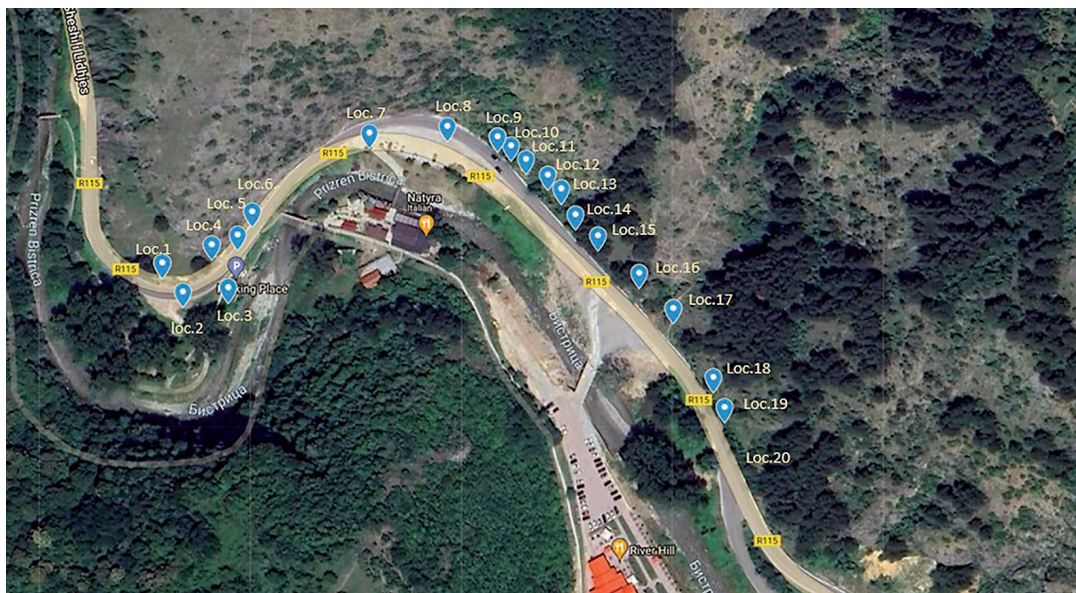
Although medicinal plants are often perceived as inherently safe due to their natural origin, several studies have shown that herbal products may contain toxic substances (WHO 2007). Given the increasing global consumption of medicinal herbs, ensuring their safety is essential, particularly with respect to contamination by heavy metals and, in cultivated species, pesticide residues. Such contaminants pose direct health risks and may contribute to their accumulation and potential bio-magnification along the food chain.

Raising awareness and educating medicinal plant collectors regarding acceptable levels of heavy metals is therefore important for protecting public health. In Kosovo, previous studies have investigated heavy metal contamination in medicinal plants (Dreshaj et al. 2018); however, no specific research has been conducted on the three plant species examined in the present study. Many medicinal herbs available in local markets originate from both domestic and international sources. Nevertheless, species such as *S. marianum* and *C. arvensis*, which are commonly sold in informal herbal markets, may pose additional risks due to limited quality control, potential misidentification, uncertain dosing and regulatory gaps. The implementation of preventive measures, the establishment of safety standards, and rigorous contamination monitoring are critical steps in minimizing human exposure to heavy metals through medicinal plants.

Heavy metals can enter plants primarily through root uptake from contaminated soils, but also via foliar absorption following atmospheric deposition, making medicinal plants effective accumulators of environmental pollutants (Wild et al. 2005). The extent of accumulation depends on the type and chemical characteristics of each metal, as well as on the anatomical and physiological traits of the plant species involved (Sarma et al. 2011; Asimnicesei et al. 2024). Consequently, different plant species may accumulate varying concentrations of heavy metals even when growing under similar environmental conditions.

The main hypothesis of this study is that medicinal plants growing and being collected along roadsides accumulate higher levels of heavy metals compared to plants from uncontaminated areas. This is attributed to pollution at collection sites, from which these plants may subsequently enter formal and informal markets. Accumulation potential is expected to vary depending on environmental factors, such as contaminant levels in air, water and soil near high-traffic roads, as well as on intrinsic characteristics of each plant species.

This study focuses on the analysis of heavy metals and minerals, including chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), calcium (Ca), potassium (K), sodium (Na) and magnesium (Mg), in medicinal plants collected along the Prizren-Prevallë roadside, a high-traffic area influenced by agricultural activity and tourism development. Specifi-



**Fig. 1** Plant sampling locations in the study area (source: authors' GPS data and Google Maps, June 2025).

cally, the study aims to (1) identify interspecific differences in the capacity of medicinal plants to accumulate heavy metals and minerals under shared environmental conditions, (2) raise public health awareness in Kosovo and beyond regarding the risks associated with the identification, collection and sale of medicinal plants in unregulated markets, and (3) assess potential health risks arising from human consumption based on variations in mineral and heavy metal content among the investigated species.

## Material and Methods

### Study area

The study area is located along the Prizren-Prevallë road (R115) in southern Kosovo. This mountainous region is characterized by alpine forests, pastures, fertile lowland soils and rocky terrain at higher elevations. The area is geologically rich and contains mineral deposits including nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), lead (Pb), iron (Fe) and manganese (Mn), as documented in the Kosovo Geological Map (Kosovo Mining Agency 2024).

The predominant minerals in the region include quartz, olivine and serpentine, together with iron-bearing minerals such as hematite and magnetite. These geological characteristics influence soil chemistry and may contribute to the accumulation of heavy metals in plant tissues. In addition to natural geological factors, the region is influenced by anthropogenic activities such as intensive road traffic, agricultural practices and increasing tourism. The Prizren-Prevallë area is among the most frequently visited tourist destinations in Kosovo, which may increase environmental exposure to pollutants in

both air and soil. Watercourses and natural springs may further contribute to pollutant transport and potential uptake by plants.

Local inhabitants are engaged in rural tourism and operate livestock and crop farms. Collection and sale of medicinal and aromatic plants is a longstanding tradition in the area, often conducted through informal roadside markets. However, this practice raises concerns regarding accurate plant identification and the absence of standardized quality control. Despite these limitations, local consumers continue to purchase and consume such products under the assumption that natural origin guarantees safety.

### Plant sampling and sample handling

Samples of three medicinal plant species [*Silybum marianum* (L.) Gaertn., *Achillea millefolium* L. and *Convolvulus arvensis* L.] were collected from roadside areas along the Prizren-Prevallë road (R115) in June 2025, within an approximate stretch of 1 km. The sampling sites were located at a distance of 1.5–2.0 m from the main road to ensure comparable exposure conditions. The geographic coordinates of the sampling sites ranged from 42.20610° N to 42.207516° N and from 20.752597° E to 20.756254° E, with elevations between 439.6 m and 470.8 m a. s. l. Sampling locations are shown in Fig. 1.

For each plant species, 20 individual plants were collected. Aboveground parts (stems, leaves and flowers) were sampled, placed in sterile nylon bags, labelled and transported to the laboratory for further processing.

### Sample preparation

Plant material was dried for 48 h at 50 °C in a drying oven. Dried samples were ground separately using a blender. The ground material obtained from the 20 in-

**Table 1** Microwave digestion program used for plant samples.

Step	I	II	III	IV
Temperature (°C)	170	190	210	100
Pressure (bar)	30	30	30	0
Time (minutes)	15	10	15	10

dividuals per species was homogenized and subsampled. Approximately 10 g of each homogenized plant sample was placed into three sterile plastic cups to produce three analytical replicates per species.

### Acid digestion

Sample digestion and elemental determination were conducted at the Agrovet Laboratory (Fushë Kosovë, Kosovo) using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The analytical procedure followed the standardized U.S. EPA Method 6010C (EPA 2007).

Approximately 0.5 g of ground plant material was weighed using an analytical balance and transferred into Teflon digestion vessels. A mixture of 6 mL nitric acid (HNO<sub>3</sub>, 65%) and 2 mL hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) was added. Samples were left at room temperature for 2 h before microwave digestion. Digestion was performed in a Berghof Speedwave MWS 3+ microwave system using a multi-step temperature and pressure program (Table 1).

After digestion, vessels were cooled for approximately 30 min at room temperature. Digests were then filtered and transferred to 50 mL containers.

### ICP-OES measurement and wavelengths

Digested samples were diluted to the required volume using distilled water. Element concentrations were determined by ICP-OES by selecting appropriate analytical wavelengths according to EPA Method 6010C (EPA 2007). The wavelengths used for each element are shown in Table 2.

For each sample, three measurements were performed. Concentrations were determined for heavy metals (Cr, Ni, Pb, Zn, Cu, Mn and Fe) and minerals (Ca, K, Na and Mg).

Certified reference material (CRM) was applied during ICP-OES quality control procedures. However, the

**Table 2** ICP-OES analytical wavelengths used for element determination.

Element	Wavelength (nm)	Element	Wavelength (nm)
Ca	317.933	Mn	257.610
Cr	267.716	Na	589.592
Cu	327.393	Ni	231.604
Fe	238.204	Pb	220.353
K	766.490	Zn	213.857
Mg	285.213		

corresponding recovery (%) values were not retained in the archived analytical records from the period when the measurements were conducted.

### Statistical analyses and human health risk assessment

Element concentrations were statistically processed using Sigma Stat 4.0 software. Arithmetic mean, standard error and Pearson correlation coefficients were calculated. Correlations were considered statistically significant at  $p \leq 0.01$ . Interspecific variation in metal and mineral concentrations among plant species was also evaluated.

To assess nutritional contribution, the percentage of the Recommended Daily Allowance (RDA%) was calculated for mineral elements, assuming plants were consumed as teas or herbs. Calculations were based on WHO recommendations (WHO 2004) using the following equation:

$$RDA\% = (C_{100g} / RDA) \times 100$$

where  $C_{100g}$  is the mineral concentration in 100 g dry plant material (mg), and RDA is the recommended daily allowance for an adult (70 kg body weight).

Potential non-carcinogenic health risks associated with heavy metal intake through plant consumption were evaluated by calculating the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and the overall Hazard Index (HI), following U.S. EPA methodology (US EPA 2024). EDI for each metal was calculated as:

$$EDI = (C \times IR \times EF \times ED) / (BW \times AT)$$

where C is the metal concentration in the sample (mg kg<sup>-1</sup>), IR is the ingestion rate (kg day<sup>-1</sup>), EF is exposure frequency (days year<sup>-1</sup>), ED is exposure duration (years), BW is body weight (70 kg), and AT is averaging time (days).

THQ was calculated as:

$$THQ = EDI / RfD$$

where RfD is the reference dose (mg kg<sup>-1</sup> day<sup>-1</sup>) reported by regulatory agencies and literature sources (US EPA 1991; US EPA 1998; Khan et al. 2008; WHO 2022).

The Hazard Index (HI) was calculated as the sum of THQs for selected metals:

$$HI = THQ_{Cr} + THQ_{Ni} + THQ_{Pb}$$

Values of THQ or HI < 1 indicate no significant non-carcinogenic risk, whereas values > 1 suggest potential long-term health effects.

## Results and Discussion

### Concentration of minerals and heavy metals in medicinal plants

The concentrations of heavy metals and essential minerals determined in the investigated medicinal plant species are presented in Table 3, while the relative percentage

**Table 3** Concentration of heavy metals and minerals (mg kg<sup>-1</sup>) in selected medicinal plants (*Silybum marianum*, *Achillea millefolium*, *Convolvulus arvensis*).

Parameter	<i>Silybum marianum</i> (L.) Gaertn.	<i>Achillea millefolium</i> L.	<i>Convolvulus arvensis</i> L.	WHO/FAO (2004) permissible limits (mg kg <sup>-1</sup> )	WHO/FAO toxic concentration (mg kg <sup>-1</sup> )
Ca	14215.53 ± 264.20	5946.23 ± 91.49	5641.99 ± 129.81	/	/
K	13884.84 ± 113.48	12630.56 ± 109.77	11219.38 ± 109.94	/	/
Mg	3008.99 ± 58.66	1259.42 ± 10.53	1741.99 ± 27.32	/	/
Na	1491.95 ± 61.98	1302.48 ± 38.31	1285.00 ± 17.77	/	/
Cr	2.88 ± 0.09	3.04 ± 0.12	7.23 ± 0.23	<0.1–1.0	2
Cu	6.88 ± 0.71	5.01 ± 0.09	4.67 ± 0.06	3–15	20
Fe	713.96 ± 23.73	281.83 ± 12.06	790.67 ± 50.37	50–250	>500
Mn	43.11 ± 1.49	63.46 ± 0.50	64.07 ± 2.41	15–100	400
Ni	3.99 ± 0.44	3.16 ± 0.27	5.17 ± 0.28	0.1–5.0	30
Pb	1.84 ± 0.02	1.50 ± 0.39	1.29 ± 0.14	1–5	20
Zn	41.78 ± 0.59	30.49 ± 0.67	20.97 ± 0.63	15–150	200

Note: Values are presented as mean ± SE; n = 20 individuals per species (composited sample), measured in three analytical replicates.

differences in elemental composition between the plant species are summarized in Table 5.

As shown in Table 3, *Silybum marianum* exhibited the highest concentrations of macrominerals among the analyzed species. Calcium (Ca) and potassium (K) were particularly abundant, reaching 14,215.53 mg/kg and 13,884.84 mg/kg dry weight, respectively. Magnesium (Mg; 3008.99 mg/kg) and sodium (Na; 1491.95 mg/kg) were also present at higher levels compared to the other studied plants. In addition, relatively elevated concentrations of copper (Cu; 6.88 mg/kg), chromium (Cr; 2.88 mg/kg), and zinc (Zn; 41.78 mg/kg) were detected in *S. marianum*.

Iron (Fe) concentration in *S. marianum* was high (713.96 mg/kg), exceeding the toxic threshold proposed by WHO/FAO (>500 mg/kg). However, both iron and manganese (Mn; 43.11 mg/kg) concentrations in *S. marianum* were lower than those recorded in *Convolvulus arvensis* (Fe: 790.67 mg/kg; Mn: 64.07 mg/kg).

Most of the analyzed heavy metals in *S. marianum*, including manganese (Mn; WHO/FAO toxic level: 400 mg/kg), nickel (Ni; 3.99 mg/kg; WHO/FAO toxic level: 30 mg/kg), and lead (Pb; 1.84 mg/kg; WHO/FAO toxic level: 20 mg/kg), were within permissible limits according to WHO/FAO guidelines and previously published studies (Stanojković-Sebić et al. 2017; Atta et al. 2023). Chromium was the only element exceeding the recommended toxic threshold (Cr >2 mg/kg), indicating potential environmental contamination.

In *Achillea millefolium*, potassium (12,630.56 mg/kg) and calcium (5946.23 mg/kg) were present at high concentrations, although lower than those observed in *S. marianum*. Magnesium concentration (1259.42 mg/kg) was the lowest among all investigated species, whereas sodium (1302.48 mg/kg) was slightly lower than in *S. marianum* but higher than in *C. arvensis*. Iron concentration

(281.83 mg/kg) was markedly lower than in *S. marianum*, while manganese (63.46 mg/kg) was elevated but still lower than in *C. arvensis*. Zinc (30.49 mg/kg) and copper (5.01 mg/kg) were within normal plant concentration ranges and followed the decreasing trend *S. marianum* > *A. millefolium* > *C. arvensis*.

Nickel (3.16 mg/kg) and chromium (3.04 mg/kg) concentrations in *A. millefolium* were comparable to those found in *S. marianum*, whereas lead concentration (1.50 mg/kg) was lower. Similar to *S. marianum*, chromium was the only element exceeding the WHO/FAO toxic threshold (2 mg/kg), with values slightly higher than those observed in *S. marianum*.

In *Convolvulus arvensis*, potassium (11,219.38 mg/kg) and calcium (5641.99 mg/kg) concentrations were comparable to *A. millefolium* but remained lower than in *S. marianum*. Magnesium (1741.99 mg/kg) and sodium (1285 mg/kg) were detected at moderate levels.

Notably, *C. arvensis* showed the highest iron concentration among all studied species (790.67 mg/kg), exceeding the WHO/FAO toxic threshold (>500 mg/kg). In addition, this species exhibited the highest chromium (7.23 mg/kg) and nickel (5.17 mg/kg) concentrations, surpassing both the chromium toxic limit (>2 mg/kg) and the upper normal range for nickel (1–5 mg/kg). In contrast, lead (1.29 mg/kg), zinc (20.97 mg/kg), and copper (4.67 mg/kg) concentrations were lower than in the other two species and remained within acceptable ranges.

Overall, *S. marianum* was characterized by the highest concentrations of macrominerals (Ca, K, Mg, Na) and essential trace elements such as Cu and Zn, whereas *C. arvensis* accumulated the highest levels of potentially toxic metals, including Cr, Fe, Mn, and Ni. *Achillea millefolium* generally exhibited intermediate concentrations for most elements, with manganese levels comparable to those observed in *C. arvensis*.

**Table 4** Comparison of toxicologically relevant heavy metal concentrations ( $\text{mg kg}^{-1}$  DW) measured in this study with literature data from the region and beyond, including WHO/FAO guideline limits.

Species	Metal	Results in our study ( $\text{mg kg}^{-1}$ DW)	Typical / average concentration in literature ( $\text{mg kg}^{-1}$ DW)	WHO/FAO guideline limit ( $\text{mg kg}^{-1}$ DW)	Plant toxicity range / reported toxic threshold ( $\text{mg kg}^{-1}$ DW)	Literature sources
<i>Silybum marianum</i>	Cr	2.88	0.5–2.0	<0.1–1	>2	Kulhari et al. 2013; Stanojković-Sebić et al. 2017
	Cu	6.88	5–12 (seeds)	3–15	>20	Özcan 2021; Stanojković-Sebić et al. 2017
	Fe	713.96	50–250	50–250	>500	Stanojković-Sebić et al. 2017; Özcan 2021
	Mn	43.11	15–100	15–100	>400	Stanojković-Sebić et al. 2017
	Ni	3.99	1–3 (seeds); <5	0.1–5	>30	Stanojković-Sebić et al. 2017; Papadimou et al. 2024
	Pb	1.84	0.5–3	1–5	>20 (WHO/FAO)	Kulhari et al. 2013
	Zn	41.78	30–80 (seeds)	15–150	>200	Dghaim et al. 2015; Stanojković-Sebić et al. 2017
<i>Achillea millefolium</i>	Cr	3.04	0.1–1.0	<0.1–1	>2	Stanojković-Sebić et al. 2017
	Cu	5.01	4–10 (clean); 16–23 (polluted)	3–15	>20	Hlihor et al. 2022
	Fe	281.83	up to 100 (clean); up to 300 (polluted)	50–250	>500	Stanojković-Sebić et al. 2017
	Mn	63.46	16–163 (clean); up to 300 (polluted)	15–100	>400	Stanojković-Sebić et al. 2017; Abebe et al. 2024
	Ni	3.16	0–6 (rural); up to 15 (Ni-polluted)	0.1–5	>30	Hlihor et al. 2022
	Pb	1.50	~1 (clean); up to 15.9 (near traffic)	1–5	>20 (WHO/FAO)	Hlihor et al. 2022
	Zn	30.49	23–36 (rural); up to 211 (polluted)	15–150	>200	Hlihor et al. 2022
<i>Convolvulus arvensis</i>	Cr	7.23	Lack of data	<0.1–1	<b>30–50</b> (plant toxicity range reported in literature)	Kulhari et al. 2013
	Cu	4.67	5–15	3–15	>20	Stanojković-Sebić et al. 2017
	Fe	790.67	50–200	50–250	>500	Stanojković-Sebić et al. 2017
	Mn	64.07	20–150	15–100	>400	Stanojković-Sebić et al. 2017
	Ni	5.17	<5	0.1–5	>30	Stanojković-Sebić et al. 2017
	Pb	1.29	<5	1–5	>20 (WHO/FAO)	Kulhari et al. 2013
	Zn	20.97	20–70	15–150	>200	Dghaim et al. 2015; Stanojković-Sebić et al. 2017

Note: WHO/FAO guideline limits are provided for comparison based on reported permissible ranges (Table 3). “Plant toxicity range / reported toxic threshold” refers to concentrations associated with toxicity effects reported in plants or in environmental studies, and should not be interpreted as direct human safety limits. DW = dry weight.

The relative ranking of heavy metal concentrations (Ni, Cr, Pb) within each plant species followed distinct patterns:

- S. marianum*: Ni > Cr > Pb;  
*A. millefolium*: Ni ≈ Cr > Pb;  
*C. arvensis*: Cr > Ni > Pb.

The results presented in Table 3 were further evaluated through comparison with regional and international literature data, summarized in Table 4, which provides reference concentration ranges and threshold values for heavy metals commonly reported in medicinal plants. For instance, Kulhari et al. (2013) documented lead concentrations in *Achillea millefolium* reaching up to 15.9 mg/kg in samples collected from industrial areas, whereas Pb was absent or present at trace levels in plants harvested from uncontaminated environments. The same authors reported nickel concentrations as high as 42.9 mg/kg in *A. millefolium* growing on nickel-rich soils, highlighting the strong influence of soil geochemistry on elemental uptake by medicinal plants.

#### Comparative evaluation with literature data and interspecific differences

The concentrations of toxicologically relevant heavy metals determined in the medicinal plants investigated were further evaluated through comparison with data reported in regional and international literature, as summarized in Table 4. This table presents typical or average concentration ranges, WHO/FAO guideline limits, and reported plant toxicity thresholds for each metal, allowing a broader interpretation of the measured values.

For *Silybum marianum*, the concentrations of most trace elements were generally consistent with literature-reported values for medicinal plants. Chromium concentration (2.88 mg/kg) exceeded the WHO/FAO guideline limit (<0.1–1 mg/kg) and slightly surpassed the plant toxicity threshold (>2 mg/kg), suggesting environmental enrichment rather than species-specific hyperaccumulation.

Copper (6.88 mg/kg), manganese (43.11 mg/kg), nickel (3.99 mg/kg), lead (1.84 mg/kg), and zinc (41.78 mg/kg) remained within typical concentration ranges reported for *S. marianum*, particularly for seed material, and well below plant toxicity thresholds. Iron concentration (713.96 mg/kg), however, exceeded both the typical literature range (50–250 mg/kg) and the WHO/FAO toxic

reference value (>500 mg/kg), indicating substantial iron uptake from the surrounding environment.

In *Achillea millefolium*, chromium concentration (3.04 mg/kg) also exceeded the WHO/FAO guideline limit and reported plant toxicity threshold (>2 mg/kg), consistent with findings from polluted or traffic-affected areas. Iron concentration (281.83 mg/kg) slightly exceeded the upper limit of the typical range for unpolluted sites but remained within values reported for plants growing under moderate environmental stress. Copper, manganese, nickel, lead, and zinc concentrations were comparable to literature values for *A. millefolium* collected from rural or lightly polluted environments and did not approach plant toxicity thresholds.

For *Convolvulus arvensis*, available literature data on heavy metal accumulation are limited, particularly for chromium. Nevertheless, the chromium concentration measured in this study (7.23 mg/kg) substantially exceeded WHO/FAO guideline limits and the reported plant toxicity threshold for Cr, indicating pronounced environmental contamination. Similarly, iron concentration (790.67 mg/kg) was markedly higher than typical literature values and exceeded the toxic reference level (>500 mg/kg). Nickel concentration (5.17 mg/kg) slightly exceeded the upper limit of the normal range (0.1–5 mg/kg) but remained well below the reported toxic threshold (>30 mg/kg). Other elements, including copper, manganese, lead, and zinc, were within ranges commonly reported for medicinal plants.

The interspecific differences in elemental composition are further illustrated by the percentage comparisons presented in Table 5. These data highlight pronounced variability among species, particularly for macrominerals and selected trace elements. *Silybum marianum* exhibited substantially higher concentrations of calcium, magnesium, potassium, sodium, copper, and zinc compared to both *A. millefolium* and *C. arvensis*. In contrast, *C. arvensis* showed markedly higher concentrations of chromium, iron, manganese, and nickel, emphasizing its greater tendency to accumulate potentially toxic metals.

Based on these findings, the investigated plant species can be considered valuable sources of essential minerals, although considerable interspecific variation was observed. The particularly high calcium concentration in *S. marianum* may be related to its more robust anatomical structure and well-developed root system, which could enhance mineral uptake efficiency. According to

**Table 5** Percentage differences (%) in element concentrations among medicinal plant species.

Comparison (Plant 1 / Plant 2)	Ca	K	Mg	Na	Cr	Cu	Fe	Mn	Ni	Pb	Zn
<i>Silybum marianum</i> / <i>Achillea millefolium</i>	139.07	9.93	138.92	14.55	-5.26	37.33	153.33	-32.07	26.27	22.67	37.03
<i>Silybum marianum</i> / <i>Convolvulus arvensis</i>	151.96	23.76	72.73	16.11	-60.17	47.32	-9.70	-32.71	-22.82	42.64	99.24
<i>Achillea millefolium</i> / <i>Convolvulus arvensis</i>	5.39	12.58	-27.70	1.36	-57.95	7.28	-64.36	-0.95	-38.88	16.28	45.40

Note: Values represent the percentage difference in element concentration between Plant 1 and Plant 2, calculated as: [(Plant1–Plant2)/Plant2]×100. Positive values indicate higher concentrations in Plant 1, whereas negative values indicate lower concentrations in Plant 1 relative to Plant 2.

Stanojković-Sebić et al. (2017), such differences in mineral composition are often driven by a combination of soil characteristics and species-specific physiological adaptations.

Importantly, the observed variation in mineral content among the studied plants does not imply toxicological concern with respect to essential elements. Instead, these differences are relevant for assessing the phytotherapeutic and nutritional potential of the species, especially in terms of their contribution to dietary mineral intake. The elevated concentrations of certain heavy metals, particularly chromium and iron, appear to reflect environmental conditions rather than intrinsic plant toxicity, underscoring the importance of site selection when harvesting medicinal plants.

### Heavy metals

**Chromium** concentrations detected in the analyzed plant samples ranged from 2.88 mg/kg in *Silybum marianum* to 7.23 mg/kg in *Convolvulus arvensis* (Table 3). Although these values may appear relatively low in absolute terms, they exceed the toxic threshold defined by the World Health Organization and several authors, who consider concentrations above 2 mg/kg (dry weight) as potentially hazardous (WHO 2007; Kulhari et al. 2013; Stanojković-Sebić et al. 2017; Atta et al. 2023).

It is important to note that WHO toxicity limits primarily refer to hexavalent chromium ( $\text{Cr}^{6+}$ ), which is highly toxic even at very low concentrations ( $\approx 0.02$  mg/kg). The presence of  $\text{Cr}^{6+}$  in plant material is therefore considered undesirable (Oladeji et al. 2024). However, chromium in plant tissues generally occurs predominantly in the trivalent form ( $\text{Cr}^{3+}$ ), which is less mobile and poorly absorbed by plant roots. Chromium is not considered an essential element for plants, and under unpolluted environmental conditions its concentration rarely exceeds 1 mg/kg (Stanojković-Sebić et al. 2017).

Consequently, the elevated chromium concentrations observed in the present study (2.88–7.23 mg/kg) strongly suggest environmental contamination. Similar chromium levels have been reported in medicinal plants growing near thermal power plants and industrial zones in Serbia and parts of Africa (Stanojković-Sebić et al. 2017; Oladeji et al. 2024). These findings support the hypothesis that chromium contamination in the study area is primarily associated with traffic-related emissions and resuspended roadside dust, rather than species-specific accumulation alone.

Although the chromium concentrations detected did not result in acute toxicological risk for consumers, as indicated by the EDI, THQ, and HI values (Table 8), they nevertheless point to environmental pollution. Given chromium's ability to bioaccumulate in the human body and its association with dermatological, respiratory, and carcinogenic effects under long-term exposure (Oladeji et al. 2024), careful selection of harvesting sites for medicinal plants remains essential.

**Copper** concentrations ranged from 4.67 mg/kg in *Convolvulus arvensis* to 6.88 mg/kg in *Silybum marianum* (Table 3). These values fall within the typical concentration range reported for medicinal plants (3–15 mg/kg) (Stanojković-Sebić et al. 2017). As all plants were collected from the same locality, the observed interspecific differences likely reflect species-specific physiological requirements rather than environmental variability. For example, *S. marianum* and *A. millefolium* may exhibit higher copper demand due to its role as an enzymatic cofactor.

Literature reports indicate that copper concentrations in *A. millefolium* may reach 14–16 mg/kg under normal conditions and up to 20 mg/kg in polluted or mining-affected areas (Hlihor et al. 2022), approaching phytotoxic levels. In the present study, none of the analyzed samples exceeded the plant toxicity threshold for copper.

From a human health perspective, the detected copper concentrations pose no concern. For instance, consumption of 10 g of plant material containing 10 mg/kg Cu would result in an intake of only 0.1 mg Cu, which is negligible relative to safe daily intake levels. Although certain plant species (e.g., *Cynodon dactylon*) are known copper accumulators in mining regions (Alvarez et al. 2023), no such hyperaccumulation behavior was observed in the investigated species.

**Iron** concentrations varied widely among the studied species, ranging from 281.83 mg/kg in *Achillea millefolium* to 790.67 mg/kg in *Convolvulus arvensis*, with *Silybum marianum* also showing elevated values (713.96 mg/kg) (Table 3). These concentrations exceed the typical range reported for plants (50–250 mg/kg) and, in the case of *S. marianum* and *C. arvensis*, surpass the WHO/FAO toxic reference value ( $>500$  mg/kg).

Elevated iron concentrations of this magnitude have been reported previously in plants growing in polluted or traffic-affected environments (Stanojković-Sebić et al. 2017). Since iron is an essential nutrient, its accumulation in plant tissues largely depends on soil chemistry, redox conditions, and rhizosphere interactions. The observed interspecific differences (e.g., *C. arvensis*  $>$  *S. marianum*  $>>$  *A. millefolium*) may reflect differences in root architecture and iron availability at microscale soil patches.

From a nutritional perspective, iron deficiency is common in human populations, and these plants may represent supplementary dietary sources when consumed as infusions. However, iron bioavailability from plant matrices is typically low. Despite elevated iron concentrations, the health risk assessment based on EDI, THQ and HI values indicates no significant non-carcinogenic risk under the assumed consumption scenario.

**Manganese** concentrations ranged from 43.11 mg/kg in *Silybum marianum* to 64.07 mg/kg in *Convolvulus arvensis* (Table 3). These values fall within the typical range reported for medicinal herbs (50–100 mg/kg) (Stanojković-Sebić et al. 2017). Literature data indicate that medicinal plants may accumulate between 20 and 300 mg/kg

Mn depending on species and soil properties (Hlihor et al. 2022).

According to WHO guidelines, manganese concentrations up to approximately 500 mg/kg are considered safe in medicinal herbs (Abebe et al. 2024), while plant toxicity thresholds are reported around 400 mg/kg (Stanojković-Sebić et al. 2017). Therefore, manganese does not represent a toxicological concern in the present study.

Although excessive manganese exposure in humans may cause neurological disorders, the levels detected in the investigated plants are negligible compared to industrial exposure. Interspecific differences likely reflect physiological requirements, as manganese plays a key role in photosynthesis and enzymatic activation.

**Nickel** concentrations ranged from 3.16 mg/kg in *Achillea millefolium* to 5.17 mg/kg in *Convolvulus arvensis*, with *Silybum marianum* showing intermediate values (3.99 mg/kg) (Table 3). While *C. arvensis* slightly exceeded the upper limit of the normal range (0.1–5 mg/kg), all values remained well below the WHO/FAO toxic concentration threshold of 30 mg/kg.

Comparable nickel concentrations have been reported for medicinal plants growing in rural and moderately polluted environments, whereas substantially higher values occur in Ni-enriched soils or industrial areas (Stanojković-Sebić et al. 2017; Hlihor et al. 2022). The slightly elevated nickel concentration in *C. arvensis* may reflect greater uptake capacity or localized environmental enrichment, potentially linked to ultrabasic parent material or traffic-derived dust.

Although nickel may induce allergic reactions and chronic effects at high exposure levels (Nordberg et al. 2015; Milaimi et al. 2017), the calculated THQ values remained far below 1 for all species (Table 8), indicating low non-carcinogenic risk.

**Lead** concentrations were low across all samples, ranging from 1.29 mg/kg in *A. millefolium* to 1.84 mg/kg in *S. marianum* (Table 3). These values are well below the reported plant toxicity threshold (10 mg/kg) (Kulhari et al. 2013), suggesting minimal environmental lead contamination or limited uptake capacity of the studied species.

In contrast, literature reports from polluted areas indicate lead concentrations of 5–20 mg/kg, with values as high as 15.9 mg/kg reported for *A. millefolium* near traffic-intensive zones (Stanojković-Sebić et al. 2017). In the present study, THQ values for lead remained well below 1 (Table 8), confirming negligible health risk under the assumed consumption scenario. Nevertheless, secondary contamination during drying and processing cannot be excluded, and continuous monitoring is recommended due to lead's cumulative toxicity.

**Zinc** concentrations ranged from 20.97 mg/kg in *C. arvensis* to 41.78 mg/kg in *S. marianum*, with *A. millefolium* showing intermediate values (Table 3). These concentrations are typical for medicinal plants and fall below the WHO desirable limit of 50 mg/kg (WHO 2007). Similar

values have been reported for *A. millefolium* collected from non-polluted areas (Hlihor et al. 2022).

Elevated zinc concentrations (>100 mg/kg) are generally associated with polluted environments and may approach phytotoxic levels (>200 mg/kg) (Stanojković-Sebić et al. 2017). The zinc concentrations observed in this study do not pose toxicological concern for plants or consumers. Given the recommended daily zinc intake (10–15 mg/day), the contribution from typical herbal consumption is minimal.

The relatively higher zinc concentration in *S. marianum* may reflect fertilizer inputs or adaptive physiological mechanisms. Interestingly, *S. marianum* also exhibited the highest lead concentration, which may suggest a protective role of zinc in mitigating metal stress (Milaimi et al. 2017). However, all zinc values remained far below levels associated with adverse effects.

Overall, comparison with literature data indicates that heavy metal concentrations in the investigated medicinal plants generally fall within permissible limits, reflecting low-level environmental contamination. Differences among species appear to be driven by both environmental factors and species-specific uptake mechanisms. While chromium and, to a lesser extent, nickel indicate traffic-related contamination, the calculated risk indices confirm that consumption of these plants under typical usage scenarios does not pose significant health risks.

### Correlation analyses of heavy metal and minerals in medicinal plants

Detailed Pearson correlation matrices for each species are presented in Table 6a–c. Table 6 presents the Pearson correlation coefficients ( $r$ ) describing the relationships between essential minerals and heavy metals in the investigated medicinal plant species. Overall, the correlation patterns indicate strong interactions between macrolelements and potentially toxic metals, reflecting both shared uptake pathways and competitive absorption mechanisms.

Potassium (K) exhibited a strong positive correlation with chromium (Cr) in *Silybum marianum* ( $r = 0.998$ ) and *Convolvulus arvensis* ( $r = 0.968$ ), suggesting a possible co-uptake or common environmental source, such as traffic-derived dust or soil particles enriched in multiple elements. In contrast, potassium showed a strong negative correlation with copper (Cu) in *S. marianum* ( $r = -0.920$ ), indicating potential antagonistic behavior during uptake or internal translocation.

Magnesium (Mg) was positively correlated with iron (Fe) across all investigated species ( $r = 1.000$ ), reflecting their shared involvement in fundamental physiological processes and possible co-regulation within plant tissues.

Conversely, magnesium displayed negative correlations with nickel (Ni) in *S. marianum* ( $r = -0.664$ ) and *Achillea millefolium* ( $r = -0.568$ ), suggesting a competitive interaction that may limit nickel accumulation in the presence of higher magnesium availability.

**Table 6** Pearson correlation coefficients (r) between minerals and heavy metals in the investigated medicinal plant species.

<b>Table 6a</b> <i>Silybum marianum</i> (Pearson r).											
	Ca	K	Mg	Na	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ca	1	0.931*	0.263*	0.999*	0.950*	-0.620*	0.280*	-0.636*	-0.896*	-0.874*	0.987*
K		1	0.596*	0.916*	0.998*	-0.920*	0.611*	-0.311*	-0.996*	-0.638*	0.978*
Mg			1	0.223*	0.551*	0.594*	1.000*	0.577*	-0.664*	-0.679*	0.417*
Na				1	0.936*	-0.652*	0.567*	-0.363*	-0.877*	-0.894*	0.979*
Cr					1	-0.344*	0.567*	1.000*	0.990*	-0.679*	0.988*
Cu						1	0.578*	1.000*	0.207*	0.923*	-0.484*
Fe							1	0.562*	-0.680*	0.220*	0.434*
Mn								1	0.227*	0.931*	-0.501*
Ni									1	0.568*	-0.956*
Pb										1	-0.783*
Zn											1

<b>Table 6b</b> <i>Achillea millefolium</i> (Pearson r).											
	Ca	K	Mg	Na	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ca	1	0.966*	0.793*	0.999*	-0.441*	0.843*	0.135*	0.990*	0.952*	0.885*	0.407*
K		1	0.609*	0.974*	-0.196*	0.953*	0.385*	0.993*	0.999*	0.975*	0.628*
Mg			1	0.772*	-0.897*	0.339*	-0.497*	0.969*	0.568*	0.417*	-0.234*
Na				1	-0.411*	0.860*	0.168*	0.994*	0.962*	0.900*	0.438*
Cr					1	0.112*	0.830*	-0.307*	-0.145*	-0.020*	0.640*
Cu						1	0.647*	0.911*	0.976*	0.996*	0.835*
Fe							1	0.277*	0.432*	0.581*	0.960*
Mn								1	0.986*	0.943*	0.535*
Ni									1	0.985*	0.668*
Pb										1	0.786*
Zn											1

<b>Table 6c</b> <i>Convolvulus arvensis</i> (Pearson r)											
	Ca	K	Mg	Na	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ca	1	1.000*	0.984*	0.649*	0.973*	-0.717*	0.862*	0.867*	0.884*	0.246*	0.910*
K		1	0.981*	0.663*	0.968*	-0.730*	0.956*	0.852*	0.875*	0.228*	0.917*
Mg			1	0.504*	0.998*	-0.581*	1.000*	0.942*	0.953*	0.414*	0.822*
Na				1	0.455*	0.455*	0.174*	0.183*	0.217*	-0.578*	0.906*
Cr					1	-0.536*	0.956*	0.959*	0.968*	0.464*	0.789*
Cu						1	-0.264*	-0.274*	-0.307*	-0.500*	-0.941*
Fe							1	1.000*	0.999*	0.703*	0.574*
Mn								1	0.999*	0.666*	0.582*
Ni									1	0.671*	0.610*
Pb										1	-0.179*
Zn											1

Note: Correlation is significant at the 0.01 level (2-tailed); Correlation is significant at the 0.05 level (2-tailed).

In *S. marianum*, sodium (Na) showed strong negative correlations with several potentially toxic metals, including lead (Pb;  $r = -0.894$ ), copper (Cu;  $r = -0.652$ ), and nickel (Ni;  $r = -0.887$ ). These relationships may indicate a protective role of sodium in reducing heavy metal up-

take or translocation, possibly through ionic competition at root uptake sites.

A pronounced positive correlation between chromium (Cr) and nickel (Ni) was observed in all studied species, particularly in *S. marianum* ( $r = 1.000$ ) and *C.*

*arvensis* ( $r = 0.998$ ). This consistent association strongly suggests a common source of contamination, most likely related to traffic emissions or soil material enriched with multiple metals. In *S. marianum*, chromium also exhibited a negative correlation with lead (Pb;  $r = -0.679$ ), implying differential mobility or selective uptake mechanisms for these metals.

Copper (Cu) showed a strong positive correlation with manganese (Mn) in *S. marianum* ( $r = 0.911$ ), a pattern also evident in *A. millefolium* and *C. arvensis*. This relationship may reflect their joint involvement in enzymatic systems related to photosynthesis and oxidative stress regulation. In contrast, copper was negatively correlated with zinc (Zn) in *S. marianum* ( $r = -0.484$ ), suggesting competitive interactions between these two micronutrients.

A significant positive correlation between manganese (Mn) and nickel (Ni) was detected in *A. millefolium* ( $r = 0.986$ ) and *C. arvensis* ( $r = 0.999$ ), indicating that species accumulating higher manganese levels also tend to accumulate more nickel, possibly due to shared transport pathways or similar soil bioavailability.

Finally, a strong negative correlation between lead (Pb) and zinc (Zn) was observed in *S. marianum* ( $r = -0.783$ ), which may indicate a protective or antagonistic interaction, where zinc accumulation limits lead uptake or vice versa.

Overall, the correlation analysis highlights complex interactions between essential nutrients and toxic metals, supporting the interpretation that both environmental factors (e.g., traffic-related pollution) and species-specific physiological mechanisms govern metal accumulation patterns in the studied medicinal plants.

The results presented in Table 6 demonstrate that calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) exhibit strong associations with the analyzed heavy metals, highlighting complex interactions between essential nutrients and potentially toxic elements. In particular, calcium and sodium showed significant positive and negative correlations with lead (Pb) and nickel (Ni), indicating possible competitive or antagonistic interactions during uptake and translocation within plant tissues.

Magnesium (Mg) was positively correlated with iron (Fe), reflecting their shared involvement in fundamental physiological processes, while both Mg and Na displayed negative correlations with several toxic metals. This pattern suggests a potential protective role of these macroelements, whereby higher availability of essential nutrients may limit the accumulation of certain heavy metals through competitive absorption mechanisms or physiological regulation.

A particularly strong correlation was observed between chromium (Cr) and nickel (Ni), supporting the hypothesis of a common source or shared pathway of entry into the plant system. Such associations are frequently reported in environments influenced by anthropogenic activities, especially traffic-related emissions and resus-

suspended roadside dust (Ayinde et al. 2020). Although the absolute concentrations of these metals remain relatively low, their consistent co-occurrence across all investigated species suggests an environmental signal indicative of low-intensity but persistent contamination.

Metal accumulation in plants is strongly influenced by multiple interacting factors, including the total concentration and chemical speciation of metals in the soil. Elements such as nickel (Ni) and chromium (Cr) may originate from both natural sources (e.g., ultrabasic parent material) and anthropogenic inputs, including industrial activities and vehicular emissions (Papadimou et al. 2024). Soil properties such as pH and organic matter content further regulate metal bioavailability; for example, acidic conditions are known to enhance the mobility and uptake of certain metals, including cadmium (Cd) (Papadimou et al. 2024).

Plant species-specific traits also play a critical role in determining metal accumulation patterns. Genetic and physiological mechanisms control not only the efficiency of metal uptake but also their internal distribution among plant organs. For instance, *Silybum marianum* has been reported to accumulate metals such as cadmium predominantly in roots while restricting their transport to aerial parts, thereby reducing potential toxic effects in reproductive tissues (Papadimou et al. 2024). In general, metals tend to accumulate more strongly in vegetative organs (leaves and stems) than in flowers or seeds, a pattern that is consistent with the findings of the present study.

In addition to biological factors, both anthropogenic and geological sources contribute to metal enrichment in plants. Identifying the origin of contamination is essential for interpreting observed concentration patterns. Lead (Pb) is commonly associated with traffic emissions, whereas nickel (Ni) and chromium (Cr) may reflect inputs from metallurgical activities or naturally metal-rich substrates.

Within this context, *Achillea millefolium* and *Convolvulus arvensis* primarily mirrored the prevailing environmental conditions, with toxic metals detected mainly at trace levels, suggesting a lightly impacted environment. In contrast, *Silybum marianum* appeared to exhibit a more pronounced exclusion or retention mechanism, as reflected by comparatively lower heavy metal accumulation in its aerial parts. This observation is consistent with previous studies highlighting the potential of *S. marianum* for phytoremediation in metal-contaminated soils, where metals are preferentially immobilized in roots or non-harvested tissues (Papadimou et al. 2024).

### Assessment of Daily Mineral Intake fulfillment from the investigated plants

Table 7 summarizes the estimated mineral intake derived from the consumption of 100 g dry weight (DW) of the investigated plant species and compares these values with established Recommended Dietary Allowance (RDA) reference values for adults. This scenario repre-

**Table 7** Estimated mineral intake (mg) from 100 g dry weight (DW) of the investigated plant species, with recommended dietary allowance (RDA) reference values for adults.

Species	Ca (mg/100 g)	Cu (mg/100 g)	Fe (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	Mn (mg/100 g)	Na (mg/100 g)	Zn (mg/100 g)
<i>Silybum marianum</i>	142.16	76.48	892.45	29.54	71.64	187.45	9.95	37.98
<i>Achillea millefolium</i>	59.46	55.70	352.29	26.87	29.99	275.91	8.68	27.72
<i>Convolvulus arvensis</i>	56.42	51.96	988.35	23.87	41.48	278.58	8.34	19.06

RDA reference values (adult): Ca = 1000 mg/day; Cu = 0.9 mg/day; Fe = 8 mg/day; K = 4700 mg/day; Mg = 420 mg/day; Mn = 2.3 mg/day; Na = 1500 mg/day; Zn = 11 mg/day.

Note: Mineral values are expressed as mg per 100 g DW for standardized comparison among species; typical daily intake of medicinal plants as herbal tea is considerably lower (commonly 2–10 g/day).

sents a theoretical maximum intake and is used to evaluate the potential contribution of medicinal plants to daily mineral supply rather than actual consumption patterns.

The results indicate that *Silybum marianum* is particularly rich in several essential minerals. Consumption of 100 g DW would provide approximately 142 mg of calcium (Ca), 71.6 mg of magnesium (Mg), and nearly 892 mg of iron (Fe). Notably, the calculated iron and copper contents substantially exceed daily nutritional requirements, reflecting the naturally high mineral density of the plant material rather than realistic dietary exposure. Potassium (K) and sodium (Na) contributions were comparatively low, accounting for only minor fractions of the recommended daily intake.

In *Achillea millefolium*, mineral contributions followed a similar pattern but at generally lower levels for most elements. Calcium (59.5 mg), magnesium (30.0 mg), and iron (352 mg) were present in moderate amounts, while manganese (Mn) showed particularly elevated values, consistent with the known tendency of this species to accumulate Mn. As with *S. marianum*, potassium and sodium intake remained negligible in relation to daily dietary needs.

*Convolvulus arvensis* exhibited the highest estimated iron intake (approximately 988 mg per 100 g DW), along with moderate levels of magnesium and manganese. Zinc concentrations were comparatively lower than in the other two species. Despite these elevated mineral contents, it is important to emphasize that medicinal plants are typically consumed in small quantities (e.g., as infusions or extracts), and thus actual dietary intake would be substantially lower than the values calculated under this hypothetical scenario.

Overall, the data presented in Table 7 demonstrate that the investigated medicinal plants can serve as concentrated sources of essential minerals, particularly iron, magnesium, and manganese. However, due to the unrealistically high consumption level assumed (100 g DW), these values should be interpreted with caution and regarded as indicators of mineral richness rather than direct dietary recommendations.

#### Assessment of risk factors (EDI, THQ and HI) in the study plants

Table 8 presents the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) for heavy metals such as chromium (Cr), nickel (Ni), and lead (Pb) in adults consuming the four-plant species included in the study. These values are evaluated for a 70 kg adult person and represent potential non-carcinogenic risks associated with plant consumption.

*S. marianum* – The EDI for chromium (Cr) was 1.97E-02 mg/kg/day, and the THQ value was 9.85E-03, both of which are below the threshold of 1, indicating acceptable non-carcinogenic risk. The EDI for nickel (Ni) was 1.95E-02 mg/kg/day, and the THQ was 1.30E-02, also below the threshold of 1. The EDI for lead (Pb) was 5.94E-03 mg/kg/day, and the THQ value was 5.94E-04, again below 1.

The sum of the THQs for the three metals resulted in an HI value of 2.34E-02, which is also below the threshold of 1, indicating no significant risk.

*A. millefolium* – The EDI and THQ values for all three metals (Cr, Ni, Pb) were below the threshold of 1, suggesting low risk. Specifically, the EDI for chromium was 2.08E-02 mg/kg/day and THQ was 1.04E-02, for nickel 1.54E-02 mg/kg/day and THQ 1.03E-02, and for lead 4.84E-03 mg/kg/day with a THQ of 4.84E-04.

**Table 8** Estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI) values for adults associated with the consumption of investigated medicinal plant species.

Species	Cr EDI (mg kg <sup>-1</sup> day <sup>-1</sup> )	Cr THQ	Ni EDI (mg kg <sup>-1</sup> day <sup>-1</sup> )	Ni THQ	Pb EDI (mg kg <sup>-1</sup> day <sup>-1</sup> )	Pb THQ	HI
<i>Silybum marianum</i>	1.97E-02	9.85E-03	1.95E-02	1.30E-02	5.94E-03	5.94E-04	2.34E-02
<i>Achillea millefolium</i>	2.08E-02	1.04E-02	1.54E-02	1.03E-02	4.84E-03	4.84E-04	2.12E-02
<i>Convolvulus arvensis</i>	4.95E-02	2.47E-02	2.53E-02	1.68E-02	4.16E-03	4.16E-04	4.20E-02

Note: EDI = estimated daily intake; THQ = target hazard quotient; HI = hazard index (sum of THQs). Values were calculated for a 70 kg adult.

The total HI value for this plant was  $2.12E-02$ , which is also below the threshold of 1.

*C. arvensis* – The EDI for chromium was  $4.95E-02$  mg/kg/day, and the THQ was  $2.47E-02$ , both of which are above the values seen in the other plants but still below the threshold of 1. The EDI for nickel was  $2.53E-02$  mg/kg/day, with a THQ value of  $1.68E-02$ , and for lead, the EDI was  $4.16E-03$  mg/kg/day with a THQ of  $4.16E-04$ .

The HI value for *C. arvensis* was  $4.20E-02$ , which is higher than the other plants but still below the critical threshold of 1.

The results from the analysis suggest that all plant species (*S. marianum*, *A. millefolium*, *C. arvensis*) have heavy metal EDI, THQ, and HI values below the threshold of 1, indicating no significant risk for non-carcinogenic effects from their consumption. However, *C. arvensis* exhibited slightly higher values, particularly for chromium and nickel, compared to the other species. Therefore, while all plants are considered safe based on these criteria, *C. arvensis* should be monitored more closely for potential exposure to higher levels of heavy metals.

The health risk assessment yields favorable results but advises caution regarding long-term exposure. The analysis was based on the consumption of 5–10 g of dried herbs per day by a person weighing approximately 70 kg. The Target Hazard Quotient (THQ) for the metals (Ni, Cr, and Pb) and the combined Hazard Index (HI) were calculated.

The THQ values for chromium (Cr), nickel (Ni), and lead (Pb) were very low for all plants (Cr THQ up to  $2.47E-02$ ; Ni THQ up to  $1.68E-02$ ; Pb THQ up to  $5.94E-04$ ), all of which are well below the value of 1. The THQ values remained completely under 0.1, indicating an acceptable non-carcinogenic risk under the assumed exposure scenario. These findings are consistent with other reports on medicinal herbs where exceedances (THQ > 1) are typically driven by Pb, Cd, As, or Hg, whereas Cu and Zn rarely exceed the threshold (Luo et al. 2021).

The Hazard Index (HI) values were all below 1 for each plant, indicating that if a person were to consume any of the plants regularly, the risk of adverse effects from the toxic metals would be low. In general, HI ranged from nearly 0.0212 for *A. millefolium* (which had lower concentrations of toxic metals) to approximately 0.0420 for *C. arvensis*, which was affected by Ni and Pb.

However, these values are still below the threshold of 1 and are thus considered acceptable (Luo et al. 2021). This outcome was expected, as none of the metals were present at concerning levels. Consequently, their combined effect resulted in a value well below the threshold of 1, indicating that their overall toxicological effect remains within acceptable tolerance level (Ozyigit et al. 2023).

According to the literature, certain heavy metals such as chromium (VI), nickel (Ni), cadmium (Cd), and lead (Pb) are classified as carcinogenic following long-term exposure. However, in our study, the levels of these metals were found to be very low, which significantly reduces

the carcinogenic risk. Long-term exposure (over years) is typically required for the concentrations of these metals to reach levels where the carcinogenic risk exceeds 1 (Luo et al. 2021). Therefore, the carcinogenic risk in our case is negligible.

Based on these findings, it can be concluded that the primary concern with heavy metals in the plants studied is not carcinogenicity but rather chronic non-carcinogenic toxicity. This could lead to health issues such as kidney damage from Ni (Milaimi et al. 2017) or nerve damage from Pb, among other potential effects. The THQ/HI data indicate that the consumption of these plants does not pose a significant health risk for an average user (e.g., a cup of tea, rather than consuming tens of grams per day). Therefore, the use of these plants in moderate amounts, without long-term continuous consumption, is considered safe.

Even though the HI values are below 1, it is important to minimize unnecessary exposure to heavy metals. This is particularly crucial as heavy metals have the tendency to bioaccumulate in the body over time. For instance, Pb has a very low renal excretion rate (Singh and Kalamdhad 2011), meaning that even small, daily doses can accumulate in bones and other tissues (Milaimi et al. 2017). This bioaccumulation is of particular concern for sensitive groups, such as children, pregnant women, and individuals with chronic illnesses, as they are more vulnerable to the neurotoxic effects of Pb. The WHO suggests that even low chronic exposure to Pb can impair mental development in children (Luo et al. 2021).

Thus, maintaining HI levels well below 1 should be a primary objective when developing herbal medicinal products. It is important to note that not all of the metal content in plants transfers to tea during brewing, as some metals remain bound to plant fibers and do not dissolve completely in water, reducing consumer exposure. However, commercial herbal preparations present a higher risk, as they could deliver the entire amount of metal to the consumer. Therefore, strict testing of commercial medicinal plant products for heavy metals, as recommended by the WHO, is essential. Supporting this concern, authors Dghaim et al. (2015) found exceedances of the permissible limits for Pb, Cd, and other metals in 81 spice/herb samples in the United Arab Emirates.

In our study, the primary risk is environmental, as the plants grow in natural habitats that are exposed to pollutants. The aim was to assess whether the environment in Prizren is polluted and evaluate the safety of plants sold in street markets, considering the additional exposure to metal dust from traffic. In Kosovo, most plants, including those in our study, are commonly found in street markets rather than processed outlets, increasing the potential risk of contamination. Beyond the environmental exposure, another risk lies in the correct identification of the plants. Misidentification can further compromise the safety of these plants.

Research by Stanojković-Sebić et al. (2017) indicates that plants collected near roads, particularly those near industrial activities, may contain toxic levels of chromium (Cr) and copper (Cu). Specifically, species like *Taraxacum officinale* L. and *Trifolium pratense* L. have been shown to accumulate dangerous concentrations of these metals, making them unsafe for human use.

Medicinal herbs have a known ability to accumulate heavy metals, and some are even used for soil remediation. In our study, while none of the plants demonstrated high metal accumulation, *S. marianum* stood out as having higher levels of Cu, Pb, and Zn compared to the others. This finding is consistent with Papadimou et al. (2024), who noted that *S. marianum* can thrive in contaminated soils, particularly those with high levels of cadmium (Cd). Interestingly, *S. marianum* accumulates Cd in the roots and stems without transporting it to the seeds, indicating that its seeds, when harvested from contaminated soil, remain safe for consumption. This characteristic makes *S. marianum* a promising candidate for phytoremediation, where its roots and stems could be discarded, and its main products (e.g., oil and seed extract) could remain free from contaminants.

A similar case can be made for *A. millefolium*, which is not a prominent heavy metal accumulator, but it shows tolerance to polluted soils, growing without significant damage despite not accumulating metals in large amounts (Murtiç et al. 2021). Additionally, *C. arvensis*, a wild grass resistant to harsh growing conditions, has been recommended in some countries for phytoremediation purposes, especially for Pb and Hg, alongside other species like *Convolvulus prostratus* Forssk. and *Convolvulus pluricaulis* in India (Kulhari et al. 2013).

From a public health perspective, the plants studied in our research present a low risk of heavy metal exposure. However, it is critical to ensure the control of heavy metals in phytotherapeutic products. As symptoms of heavy metal toxicity typically arise only after prolonged exposure, many countries have established rigorous control measures for medicinal plants. These measures include mandatory testing for heavy metals with each shipment, as highlighted by Dghaim et al. (2015). This underscores the importance of sourcing plants from clean areas, and performing regular heavy metal analysis in accordance with WHO guidelines for permissible limits (Kulhari et al. 2013).

## Conclusions and Recommendations

The present study assessed the concentrations of selected heavy metals and essential minerals in *Silybum marianum*, *Achillea millefolium*, and *Convolvulus arvensis* collected from a roadside environment. Overall, the results indicate low to moderate environmental contamination, with most measured elements occurring within ranges commonly reported for medicinal plants. The

calculated risk indicators (EDI, THQ and HI) remained consistently below the critical threshold value of 1, indicating no significant non-carcinogenic health risk for adults under the assumed consumption scenario.

Although certain elements, particularly chromium and iron, exceeded guideline or typical background or guideline values in specific species, these elevations did not translate into increased health risk according to the applied risk assessment framework. Lead, nickel, manganese, zinc and copper concentrations were generally within acceptable limits, supporting the safe use of the plants investigated for phytotherapeutic or culinary purposes, provided that harvesting and processing practices are properly controlled. The observed interspecific differences in metal accumulation – such as higher Cu, Pb and Zn concentrations in *S. marianum* and elevated Cr, Fe, Mn and Ni levels in *C. arvensis* – highlight species-specific uptake patterns and suggest differing physiological responses to environmental metal availability.

Based on these findings, several practical recommendations can be proposed:

- Medicinal plants should be collected and cultivated in environmentally safe areas, away from intensive traffic and potential industrial sources, to minimize the risk of metal contamination.
- Species showing higher accumulation capacities, particularly *S. marianum* and *C. arvensis*, may be further explored for phytoremediation purposes in soils moderately contaminated with heavy metals, provided that their use for human consumption is avoided in such contexts.
- Public awareness should be raised regarding the importance of proper plant identification and controlled harvesting, especially when plants are collected from wild or roadside habitats.
- Regular environmental and toxicological monitoring of medicinal plants is recommended to ensure product quality and protect public health.

The results of this study provide valuable information for environmental monitoring, public health protection and sustainable management of medicinal plant resources. They contribute to interdisciplinary fields including environmental science, toxicology, pharmacology and sustainable agriculture, and support the development of regulatory measures for the safe and sustainable use of medicinal plants in Kosovo and comparable regions.

Despite its contributions, this study has several limitations. It was conducted at a single sampling location, included a limited number of plant species, and did not incorporate soil or detailed environmental parameter analyses. In addition, the absence of long-term exposure data restricts conclusions regarding potential chronic health effects. Future research should address these limitations by integrating soil physicochemical properties (e.g. pH, texture, organic matter and elemental composition), biological factors (such as plant genotype and soil microorganisms), and multi-site sampling designs to

better elucidate the mechanisms governing metal uptake and accumulation in medicinal plants.

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