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Heavy metals content and health risk assessment of selected leafy plants consumed in Bosnia and Herzegovina

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Abstract: Today, there is widespread concern about the potential health effects on populations from consuming contaminated leafy plants and vegetables. In this study, heavy metal content is present in commonly consumed leafy plants (*Atriplex hortensis*, *Spinacia oleracea*, *Urtica dioica*, *Beta vulgaris*, and *Brassica oleracea*) from the mining area near Tuzla in Bosnia and Herzegovina was determined. After the preparation of the samples by wet digestion with HNO_3, the flame and graphite furnace atomic absorption spectrometry was used. According to the results, the lowest concentration in leafy plants was obtained for Cr 0.08 mg/kg (*Brassica oleracea*) and the highest for Fe 539.15 mg/kg (*Spinacia oleracea*). The novelty of this study was to estimate health risk assessment for selected leafy plants. The estimated daily intake (EDI) of Pb, Mn, Zn, and Cd from consuming leafy plants was higher than the maximum tolerated daily intake. For adults, the total target hazard quotient (THQ) calculated based on EDI of the heavy metals was found to be > 1 for Pb and Cd due to all leafy plant consumption and for the children risk level of THQ was observed for most heavy metals. The hazard index due to the intake of toxic metals from ingesting leafy plants was much > 1. According to the total carcinogenic risk index for adults and children, the carcinogenic risks for all samples were higher than the tolerable range. Based on the results of this study, there is a significant non-carcinogenic and carcinogenic health risk to the population associated with the consumption of leafy plants cultivated in the mining area.

Keywords: hazard elements; non-carcinogenic and carcinogenic analysis; food safety

Leafy plants and vegetables, which are grown and consumed on almost every continent, are vital to the human diet. It is well known that these plants mainly provide trace elements, vitamins, minerals, and fibres (Mateos-Maces et al. 2020, Sarkar et al. 2022). In addition to the nutritional benefits of leafy plants, most of them accumulate higher amounts of heavy metals because they absorb metals in their edible parts (Saleem et al. 2020).

Heavy metal accumulation in plants depends on soil and metal types, soil conditions, plant species, growth stages, weather, and environmental factors (Zhang et al. 2020). Leafy plants grown in contaminated soils have a greater chance of accumulating heavy metals than plants grown in uncontaminated soil (Proshad et al. 2020). Anthropogenic activities such as industrial processing and mining are the primary causes of environmental heavy metal pollution. Furthermore, the widespread use of agrochemicals such as metal-based pesticides and fertilisers contributes significantly to the contamination of various plants and vegetables (Briffa et al. 2020, Aloud et al. 2022). Therefore, several studies are directed toward examining heavy metals in leafy plants and their impact on human health. According to numerous toxicological studies, daily consumption of heavy metal-polluted leafy plants can have an adverse effect on the food chain and may have a negative impact on human health, including anaemia, hypertension, cardiovascular diseases, metabolic problems, psychological disor-

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Table 1. Description of leafy plants examined in this study

Plant species	Family	English name	Part analysed
Atriplex hortensis L.	Amaranthaceae	Garden orache	leaves
Spinacia oleracea L.	Amaranthaceae	Spinach	leaves
Urtica dioica L.	Urticaceae	Nettle	leaves
Beta vulgaris L.	Amaranthaceae	Swiss chard	leaves
Brassica oleracea L.	Brassicaceae	Collard Green	leaves

ders, etc. (Ahmed et al. 2022). The consumption of *Atriplex hortensis*, *Spinacia oleracea*, *Urtica dioica*, *Beta vulgaris*, and *Brassica oleracea* by the population in Mramor near Tuzla may become a possible route for human exposure to heavy metal pollution from the populated mining area. The Tuzla Canton of Bosnia and Herzegovina (B&H) has recently drawn some attention due to studies that found high levels of heavy metals in agricultural and industrial soils, and this poses a significant health risk (Huremović et al. 2017). Tuzla Canton is the region of B&H with the highest population density, is home to many polluting industries, such as several large salt and coal mines, and most agricultural land is located nearby (Husejnović et al. 2021).

Metals analysed in this study, such as Cu, Co, Fe, Ni, Cr, Mn and Zn, are commonly known as essential for various physiological and biochemical activities in humans, while their consumption at higher concentrations is toxic and may interrupt protein metabolism and cause a diverse illness (Engwa et al. 2019, Barboiu et al. 2020). Besides, the ingesting and use of leafy plants that contain Cd, Cr, and Pb in small amounts led to acute and chronic toxicities in humans, which can be associated with the development of tumour disorders.

This study aims to determine the content of heavy metals in leafy plants grown in the inhabited mining area. Furthermore, the use of the obtained data for the calculation of the estimated daily intake (EDI), target hazard quotient (THQ), carcinogenic health risk index (CRI), total carcinogenic health risk (TCRI) and hazard index (HI) for non-carcinogenic health risk in the presence of heavy metals in leafy plants for children and adults is an innovation in this study.

MATERIAL AND METHODS

Leafy plants collection. Samples of organically grown leafy plants (Table 1) were collected in an inhabited mining area Mramor (coordinate: 44°35'32.33''N, 18°33'55.66''E) near Tuzla city. The sampling area is distant fifteen kilometres from Tuzla city, which lies in the northeastern part of Bosnia, settled just underneath the Majevica mountain range (Figure 1).

According to the Federal Institute of Agropedology, Sarajevo, B&H, the physical and chemical characteristics of the soil (Vertisol) from the sampling area are as follows: pH_{KCl} value = 5.45–7.02, C_{org} (%) = 0.27–1.71, $CaCO_3$ (%) = 0.15–8.87, N_{org} (%) = 0–0.19, P (mg/kg) = 0.13–0.44, and K (mg/kg) 7.97–17.02.

Leafy plants *Atriplex hortensis*, *Spinacia oleracea*, *Urtica dioica*, *Beta vulgaris*, and *Brassica oleracea* were collected. Selected leafy plants are grown and consumed in B&H in relatively considerable amounts.

All plants were sampled directly from the land. After collection, the plant samples were cleaned of impurities and damaged parts and then subjected

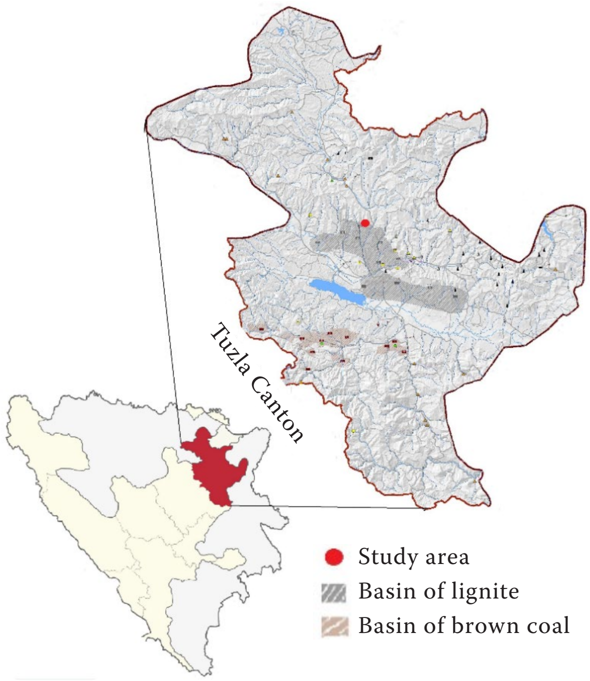

Figure 1. Location of the study area and sampling site Mramor (Tuzla Canton, Bosnia and Herzegovina)

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to drying. The Laboratory for Plant Systematics at the University of Sarajevo – Faculty of Science used keys and iconographies to verify the authenticity of plant samples (Hayek 1927, Trinajstić 1975–1986, Jávorka and Csapody 1991, Domac 1994).

Digestion of samples. The leaves of samples were cut into small pieces after thoroughly washing with tap water and deionised water. Drying was carried out in a dark and airy place at room temperature. After drying, samples were milled and sieved (1 mm stainless-steel mesh) for wet digestion. 1.00 g of the dried sample was transferred into polytetrafluoroethylene (PTFE) vessels, and then 30 mL of 65% HNO_3 was added. After evaporating the nitrogen oxides, the vessels were closed and allowed to react for 18 h at 90 °C. The digest was cool to room temperature, filtered, transferred to a volumetric flask of 50 mL, and filled up with redistilled water up to the mark. The digestion samples and blank were made in triplicate. A blank procedural probe was also prepared following all steps except adding of the samples.

Heavy metals analysis. A flame atomic absorption spectrometry (Varian AA240FS, Santa Clara, USA) was used to measure the concentrations of Mn, Fe, Pb, and Zn, and a graphite furnace (Varian AA240Z, Santa Clara, USA) was used to measure the concentrations of Cd, Co, Cr, Cu, and Ni. The standard solutions were prepared by dilution of 1 000 mg/L stock single-element atomic absorption standard solutions (Certipur Grade, Merck, Darmstadt, Germany) and used to develop calibration curves. The analysis was based on analytical-grade chemicals and reagents. Chemicals were purchased from Merck (Darmstadt, Germany) and used for sample preparation. The mean value was obtained by subtracting the value of the blank procedural probe. The accuracy and precision of the analysis were evaluated using the standard reference material (1570A, National Institute of Standards and Technology USA), and the obtained results were in the range of the reference material.

Health risk assessment. The USEPA model was used to calculate the health risks related to heavy metals ingested through leafy plants (USEPA 2011, 2012, 2017, Liu et al. 2021). To assess the human health risk of heavy metals, it is necessary to calculate the estimated daily intake (EDI) according to the Eq. 1 (Liu et al. 2021, Salihović et al. 2021):

$$\mathrm{EDI} = \frac{c_{hm} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{ET} \times \mathrm{BW}} \qquad (1)$$

where: c_{hm} – mean concentrations of heavy metals in plant samples (mg/kg); IR – daily consumption of leafy plants for adults and children is 2.44E-01 and 1.86E-01 kg/person/day, respectively (Liu et al. 2021); EF – exposure frequency (365 days/year); ED – exposure duration for adults 30 years, and for children 7 years; ET – exposure time is calculated as 365 × ED; BW – body weight (70 kg for adults and 26 kg for children) (Liu et al. 2021, Pazalja et al. 2022).

The target hazard quotient (THQ) was used to assess the non-carcinogenic risk caused by consuming heavy metals contaminated leafy plants. Furthermore, hazard in the index (HI) was calculated as the sum of the THQ of each heavy metal analysed in plants, representing the total non-carcinogenic risk. THQ and HI were calculated by following Eqs. 2 and 3 (USEPA 2011, 2012, 2017):

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{R_fD}} \qquad (2)$$

where: R_fD – oral reference consumption dose of the heavy metals (mg/kg/day) (USEPA 2012).

$$\mathrm{HI} = \sum_{n=1}^{i} \mathrm{THQ_n};\ \mathrm{i} = 1, 2, 3, \dots, n \qquad (3)$$

The carcinogenic risk index (CRI) was calculated by multiplying the estimated daily intake by the oral cancer slope factor (CSF) for corresponding heavy metals. As described by Li et al. (2021), Eq. 4 was used to calculate the CRI:

$$\mathrm{CRI} = \mathrm{EDI} \times \mathrm{CSF} \qquad (4)$$

The CRI was calculated for Pb, Cr, Ni and Cd. The values for CSF for Pb, Cr, Ni and Cd were defined by USEPA (2012, 2017) and Li et al. (2021). The CSF values for Pb, Cr, Ni and Cd are 8.50E-03, 0.50, 0.84 and 0.38 mg/kg/day, respectively.

The total carcinogenic risk index (TCRI) of potential carcinogens is the sum of potential carcinogenic risk of each heavy metal. The following Eq. 5 was used for calculation of the TCRI (Li et al. 2021):

$$\mathrm{TCRI} = \sum_{n=1}^{i} \mathrm{CRI};\ \mathrm{i} = 1, 2, 3, \dots, n \qquad (5)$$

Statistical analyses. Results were evaluated using IBM SPSS Statistics for Windows, Version 24.0. (IBM Corp, 2016, Armonk, USA). *P*-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Levels of heavy metals in leafy plants. The levels of heavy metals in leafy plants cultivated and usually consumed in B&H has been investigated. The result represents the mean values of three replicate

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determinations and is presented in Table 2. The total concentration of nine tested metals was expressed as the sum of the concentration of the metals in the leafy plants. The total concentration of heavy metals ranged from 93.27 mg/kg DW (dry weight) (*Brassica oleracea*) to 737.33 mg/kg DW (*Spinacia oleracea*). The mean concentration of heavy metals in analysed plants decreases as follows Fe > Zn > Mn > Pb > Cu > Co > Ni > Cd > Cr. In all samples, except for *Atriplex hortensis*, the highest Fe content was found among all metals. Therefore, leafy plants are a valuable source of iron and can increase the bioavailability of iron in the daily diet, which will help reduce the occurrence of iron deficiency anemia.

The level of Mn and Fe in leafy plants ranged from 11.63 to 58.57 mg/kg DW and from 41.97 to 539.15 mg/kg DW, respectively. The lowest concentration of Mn and Fe was found for *Brassica oleracea* and the highest for *Spinacia oleracea*. The results showed that the obtained concentrations for Mn in *Spinacia oleracea*, *Beta vulgaris*, and *Brassica oleracea* are higher than those reported by Mahmood and Malik (2014).

The levels of Zn and Cu were found to be from 10.40 to 99.48 mg/kg DW and from 5.85 to 15.68 mg/kg DW, respectively. The lowest concentration of Zn and Cu was found in the analysed sample of *Brassica oleracea*, and the highest Zn content was recorded in *Spinacia oleracea*, while the highest Cu content was verified in *Urtica dioica*. The Cu content in the analysed *Urtica dioica* leaves is three times higher than the content published by Nica et al. (2013). The accumulation of copper in soils and plants is mainly due to anthropogenic origins, such as mining or industrial activities. Agricultural use of copper-containing products is also widespread, especially in pesticides used in orchards and vineyards (Tóth et al. 2016).

The Ni concentration in plant samples was found in the range of 1.02 (*Brassica oleracea*) to 5.08 mg/kg DW (*Urtica dioica*). The Co concentration ranged from 0.38 (*Beta vulgaris*) to 8.15 mg/kg DW (*Urtica dioica*). The content of Ni and Co in the *Urtica dioica* is higher than in the study carried out by Barboiu et al. (2020) and Nica et al. (2013). The increased concentration of Ni and Co, and other metals can be related to the fact that *Urtica dioica* is a wild plant that often grows in landfills and can accumulate heavy metals. Consequently, this plant has great potential as a photo indicator of environmental pollution. However, the information about heavy metal content in *Urtica dioica* collected from the investigated area is missing.

The level of Pb and Cd in leafy plants ranged from 9.90 to 18.92 mg/kg DW and from 0.77 to 1.93 mg/kg DW, respectively. The lowest concentration for Pb and Cd was found for *Beta vulgaris* and the highest for *Spinacia oleracea*. Pb and Cd are examples of heavy metals that can accumulate in various parts of plants and living beings despite not being essential components or playing any role in cell metabolism (Okereafor et al. 2020). The levels of Pb and Cd obtained in this study were found to exceed the allowable limit value for leafy vegetables defined by the Food Safety Agency of B&H (Food Act, Official Gazette B&H No. 50/04). According to the food act defined by the Food Safety Agency of B&H, the limit values for Pb and Cd in fresh samples of leafy plants are 0.30 and 0.20 mg/kg.

The content of Cr in leafy plants ranged between 0.08 mg/kg DW (*Brassica oleracea*) to 0.33 mg/kg DW (*Beta vulgaris*). Although chromium is important for hormone and enzyme activity, increased concen-

Table 2. Levels of heavy metals (mg/kg dry weight) in selected leafy plants consumed in Bosnia and Herzegovina

Metal	*Atriplex hortensis*	*Spinacia oleracea*	*Urtica dioica*	*Beta vulgaris*	*Brassica oleracea*	Mean	*P*-value
Mn	18.28 ± 0.08	58.57 ± 0.31	25.29 ± 0.33	22.05 ± 0.13	11.63 ± 0.19	27.16	0.029
Fe	85.17 ± 0.33	539.15 ± 0.77	51.30 ± 0.18	217.17 ± 0.08	41.97 ± 0.08	186.95	0.116
Co	8.08 ± 0.28	0.55 ± 0.05	8.15 ± 0.20	0.38 ± 0.03	5.32 ± 0.21	4.50	0.059
Ni	3.13 ± 0.13	53.72 ± 0.15	5.08 ± 0.28	3.00 ± 0.15	1.02 ± 0.13	3.19	0.008
Zn	92.32 ± 0.51	99.48 ± 0.58	20.18 ± 0.37	22.85 ± 0.36	10.40 ± 0.36	49.05	0.064
Cu	12.43 ± 0.35	14.84 ± 0.29	15.68 ± 0.28	11.02 ± 0.28	5.85 ± 0.39	11.96	0.002
Cd	1.32 ± 0.15	1.93 ± 0.16	1.45 ± 0.20	0.77 ± 0.08	1.33 ± 0.10	1.36	0.002
Pb	17.00 ± 0.10	18.92 ± 0.33	12.12 ± 0.16	9.90 ± 0.33	15.75 ± 0.20	14.74	0.001
Cr	0.30 ± 0.05	0.18 ± 0.03	0.12 ± 0.06	0.33 ± 0.03	0.08 ± 0.03	0.20	0.015
Total	238.03	737.33	139.37	287.46	93.27	299.12	–

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trations are toxic to living organisms. Permissible limits for Cr in vegetables and food set by European Union (2006) have a value of 1.00 mg/kg DW. The values obtained for the analysed leafy plants in our study are significantly lower than the permissible values, which is significant because the chromium content is often not reduced by the cooking process (Saraiva et al. 2021).

The total content of heavy metals in analysed leafy plants decreases as follows *Spinacia oleracea* > *Beta vulgaris* > *Atriplex hortensis* > *Urtica dioica* > *Brassica oleracea*. The highest total metal content was recorded for plants of the *Amaranthacea* family, and the plants of this family can be used for phytoremediation to clean heavy metal-contaminated areas (Devi and Swapna 2022).

Obtained results were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and all data showed a normal distribution, and the one sample *T*-test was used for data analysis, and the difference at the $P < 0.05$ level was considered statistically significant. As shown in Table 2, a statistically significant difference was found in the content of Mn, Ni, Cu, Cd, Pb, and Cr ($P < 0.05$). The content of the mentioned heavy metals differs statistically significantly in these five different plants that were analysed.

For comparison, the literature minimum and maximum values of heavy metals content in similar investigations of edible leafy plants are presented in Table 3. Analysing the results from Table 3, the heavy metals studied in leafy plants consumed in B&H are relatively high. Similar research results have been published, showing that green leafy plants generally accumulate heavy metals more than non-leafy plants (Cai et al. 2015, Ahmed et al. 2022). The study area's high concentrations of heavy metals in the plant samples are most likely the result of anthropogenic activities like mining and car exhaust pollution. Other authors also reported high levels of heavy metals in various vegetables and crops grown in the mining areas. Musilová et al. (2021, 2022) reported elevated heavy metal levels in the soils of the Middle Spiš and Gemer region (Slovakia), which has a degraded environment due to centuries of mining activities. This is reflected in their accumulated home-grown vegetables, especially potatoes, in the Middle Spiš region. The Gamer area is characterised by high concentrations of cadmium, lead, and mercury, either in bioavailable or pseudo-total form. Nevertheless, agricultural crops in this area do not show elevated accumulation of risk elements, and, in the case of cadmium, the levels were below the detection limit. The availability of heavy metals from the soil to plants is affected by many factors, including pH, macronutrient content, redox state, availability, temperature and water content (Kowalska 2021).

The accumulation of metals in the leaves of plants could directly impact the health of nearby inhabitants. The leaves of plants growing in the area under study are consumed mainly locally. Thus, we assessed the potential health risk of heavy metals in leafy plants the local inhabitants consume.

Table 3. Comparison of obtained values of heavy metals in analysed leafy plants with similar studies

Leafy plants	Mn	Fe	Co	Ni	Zn	Cu	Cd	Pb	Cr	References
Atriplex hortensis	–	1.43	–	–	3.70 –214	3.55 –10.7	2.08 –4.9	0.23 –40	–	Prisacaru et al. (2017), Lăcătușu (2014)
Spinacia oleracea	0.14 –0.71	1 220 –3050	–	3.13 –5.49	14.51 –36.24	4.03 –4.12	0.013 –0.89	56.38 –204.11	2.03 –168.99	Ahmed et al. (2022)
Urtica dioica	–	375.67 –1436.36	0.42 –6.00	4.36 –6.18	31.80	7.47 –9.71	0.65 –1.26	0.25 –2.77	0.90	Murtić et al. (2021), Prisacaru et al. (2017), Barboiu et al. (2020), Bislimi et al. (2021)
Beta vulgaris	3.91 –10.45	–	1.57 –6.23	1.78 –6.03	0.2 –5.83	0.55 –3.97	0.27 –0.69	0.11 –0.64	0.55 –2.17	Mahmood and Malik (2014)
Brassica oleracea	3.76 –8.18	–	2.18 –6.16	1.92 –4.97	1.11 –7.02	0.12 –1.87	0.01 –0.46	0.01 –0.38	0.20 –1.98	Mahmood and Malik (2014)
This study	11.63 –58.57	41.97 –539.15	0.38 –8.15	1.02 –5.08	10.40 –99.48	5.85 –15.68	0.77 –1.93	9.90 –18.92	0.08 –0.33	–

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Health risk assessment

Estimated daily intake of heavy metals. The EDI is calculated to determine the effects of heavy metals in leafy plants on adults and children's health. The EDI of non-carcinogenic and carcinogenic heavy metals for adults and children is listed in Table 4. The results for Mn, Cd, Pb, Cu, and Zn were above the R_fD value in all plants for adults and children. The intake of Ni, Cr, and Co for adults, and Cr for children from the consumption of studied plants were below the R_fD limit (USEPA 2012). A higher intake of Pb, Cd, and Mn from the consumption of all samples was found, whereas a higher intake of Zn for Amaranthaceae plants. Therefore, the health risk for heavy metals through the consummation of leafy plants could be a great concern for the local population.

Non-carcinogenic health risk. The non-carcinogenic health risk (THQ) of the analysed heavy metals through the consumption of leafy plants for adults and children inhabitants of the study area were determined and presented in Table 4. For adults risk level of target hazard quotient (THQ > 1) was observed for Cd and Pb in all leafy plants, Mn for *Spinacia oleracea*, Zn for *Atriplex hortensis* and *Spinacia oleracea* and Co for *Atriplex hortensis* and *Urtica dioica*. For all samples, the values for Cu were relatively close to the maximum limit. A similar pattern of THQ, with Cu and Pb dominance in mining areas, was reported by other authors (Gebeyehu and Bayissa 2020, Manea et al. 2020). Due to chromium's high R_fD, the health risk was the lowest for adults and children when compared to other investigated metals. For children risk level of THQ was observed for most heavy metals; the exception is chromium. Similar observations have been reported previously by Sultana et al. (2017).

Further, the non-carcinogenic risks for leafy plants were expressed as the hazard index, the sum of individual metal THQ (Figure 2). All leafy plants have non-cancerogenic HI > 1 for adults and children. The lowest and highest HI values were found in the present study for *Beta vulgaris* and *Spinacia oleracea*, respectively. When the HI exceeds 1, it is considered a significant risk and potential health hazard (Sultana et al. 2017). Further, HI showed Pb and Cd as the dominant contaminants in leafy plants. It implies that the population from the sampling area

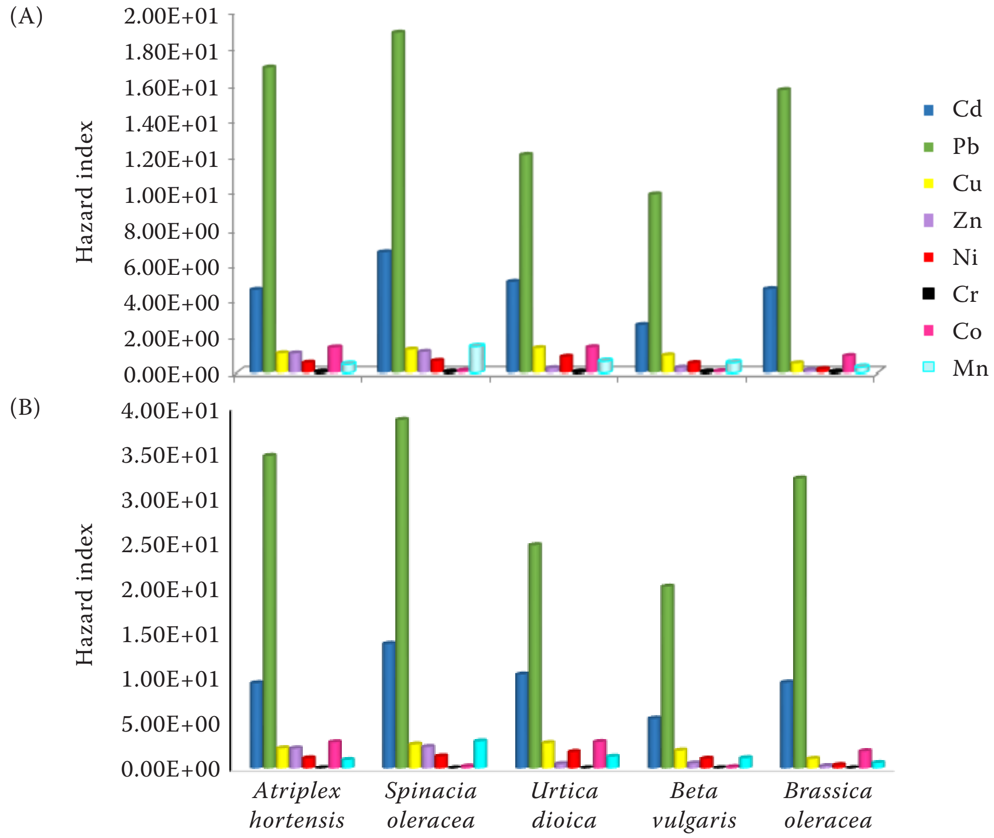

Figure 2. Hazard index for non-carcinogenic risk for (A) adults and (B) children due to heavy metals intake *via* consumption of leafy plants

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Table 4. Estimated daily intake (EDI), target hazard quotient (THQ) values of heavy metals *via* consumption of leafy plants for adults and children

Metal	EDI (mg/kg) for adults					THQ for adults				
	Atriplex hortensis	*Spinacia oleracea*	*Urtica dioica*	*Beta vulgaris*	*Brassica oleracea*	*Atriplex hortensis*	*Spinacia oleracea*	*Urtica dioica*	*Beta vulgaris*	*Brassica oleracea*
Mn	6.37E-02	2.04E-01	8.82E-02	7.69E-02	4.05E-02	4.55E-01	1.46E+00	6.30E-01	5.49E-01	2.90E-01
Cd	4.60E-03	6.73E-03	5.05E-03	2.68E-03	4.64E-03	4.60E+00	6.73E+00	5.05E+00	2.68E+00	4.64E+00
Pb	5.93E-02	6.59E-02	4.22E-02	3.45E-02	5.49E-02	1.69E+01	1.88E+01	1.21E+01	9.86E+00	1.57E+01
Cu	4.33E-02	5.17E-02	5.47E-02	3.84E-02	2.04E-02	1.08E+00	1.29E+00	1.37E+00	9.60E-01	5.10E-01
Zn	3.22E-01	3.47E-01	7.03E-02	7.96E-02	3.63E-02	1.07E+00	1.16E+00	2.34E-01	2.65E-01	1.21E-01
Ni	1.09E-02	1.30E-02	1.77E-02	1.05E-02	3.56E-03	5.46E-01	6.48E-01	8.85E-01	5.23E-01	1.78E-01
Cr	1.05E-03	6.27E-04	4.18E-04	1.15E-03	2.79E-04	3.49E-01	2.09E-01	1.39E-01	3.83E-01	9.30E-02
Co	2.82E-02	1.92E-03	2.84E-02	1.32E-03	1.85E-02	1.41E+00	9.59E-02	1.42E+00	6.62E-02	9.27E-01
	EDI (mg/kg) for children					THQ for children				
Mn	1.31E-01	4.19E-01	1.81E-01	1.58E-01	8.32E-02	9.34E-01	2.99E+00	1.29E+00	1.13E+00	5.94E-01
Cd	9.44E-03	1.38E-02	1.04E-02	5.51E-03	9.51E-03	9.44E+00	1.38E+01	1.04E+01	5.51E+00	9.51E+00
Pb	1.22E-01	1.35E-01	8.67E-02	7.08E-02	1.13E-01	3.47E+01	3.87E+01	2.48E+01	2.02E+01	3.22E+01
Cu	8.89E-02	1.06E-01	1.12E-01	7.88E-02	4.19E-02	2.22E+00	2.65E+00	2.80E+00	1.97E+00	1.05E+00
Zn	6.60E-01	7.12E-01	1.44E-01	1.63E-01	7.44E-02	2.20E+00	2.37E+00	4.81E-01	5.45E-01	2.48E-01
Ni	2.24E-02	2.66E-02	3.63E-02	2.15E-02	7.30E-03	1.12E+00	1.33E+00	1.82E+00	1.07E+00	3.65E-01
Cr	2.15E-03	1.29E-03	8.58E-04	2.36E-03	5.72E-04	7.15E-01	4.29E-01	2.86E-01	7.87E-01	1.91E-01
Co	5.78E-02	3.93E-03	5.83E-02	2.72E-03	3.81E-02	2.89E+00	1.97E-01	2.92E+00	1.36E-01	1.90E+00

Table 5. Carcinogenic risk index and total carcinogenic risk for heavy metals in leafy plants

Leafy plants	Adults					Children				
	Pb	Cr	Ni	Cd	TCRI	Pb	Cr	Ni	Cd	TCRI
Atriplex hortensis	5.04E-04	5.23E-04	9.16E-03	1.75E-03	1.19E-02	1.03E-03	1.07E-03	1.88E-02	3.59E-03	2.45E-02
Spinacia oleracea	5.61E-04	3.14E-04	1.09E-02	2.56E-03	1.43E-02	1.15E-03	6.44E-04	2.24E-02	5.25E-03	2.94E-02
Urtica dioica	3.59E-04	2.09E-04	1.49E-02	1.92E-03	1.74E-02	7.37E-04	4.29E-04	3.05E-02	3.94E-03	3.56E-02
Beta vulgaris	2.93E-04	5.75E-04	8.78E-03	1.02E-03	1.07E-02	6.02E-04	1.18E-03	1.80E-02	2.09E-03	2.19E-02
Brassica oleracea	4.67E-04	1.39E-04	2.99E-03	1.76E-03	5.35E-03	9.58E-04	2.86E-04	6.13E-03	3.62E-03	1.10E-02

TCRI – total carcinogenic risk index

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may be exposed to some potential health risks as a result of heavy metal intake. Therefore, it is crucial to take proper measures to reduce Pb, Cd, and other heavy metals pollution in this area to save the local population from non-carcinogenic risks.

Carcinogenic health risk. The obtained results of cancer risks for Pb, Cr, Ni, and Cd are listed in Table 5. The cancer risk for adults and children varied from minimum values 1.39E-04 and 2.06E-04 for Cr in *Brassica oleracea* to 1.49E-02 and 3.05E-02 for Ni in *Urtica dioica*, respectively. Compared to the other three metals (Cd, Cr, and Pb), Ni appeared to be the most dangerous contaminant, followed by Cd, Pb, and Cr. Table 5 shows the TCRI values calculated to assess the total carcinogenic health risk of heavy metals from the consumption of leafy plants from the mining area near Tuzla city.

A wide range of TCRI was calculated. For adults from 5.35E-03 to 1.74E-02 and children from 1.10E-02 to 3.56E-02. *Urtica dioica* had the highest values, while *Brassica oleracea* acquired the lowest values. All samples had carcinogenic risks that exceeded the tolerable range of 1E-06 to 1E-04 (USEPA 2012). Most scientists reported similar trends investigating the carcinogenic risk from heavy metals to the population through the consumption of different vegetables (Gebeyehu and Bayissa 2020, Proshad et al. 2020). Gebeyehu and Bayissa (2020) reported that in the Mojo area in central Ethiopia, the total cancer risk (TCR) of As, Cr, and Ni due to tomato consumption was larger than the maximum limit value. Similarly, TCR values for As, Cd, Cr, and Ni due to the consumption of cabbage also exceeded the maximum limit value. High TCRI values for analysed samples from Mramor near Tuzla indicated that the local population consuming leafy plants from the mining area suffered a significantly increased carcinogenic risk. Prompt action should be needed to remediate heavy metal contamination in soil and environment and to reduce metal content in edible leafy plants in this area, and their implications for human health should be identified urgently by in-depth studies.

REFERENCES

Ahmed S., Mahdi M.M., Nurnabi M., Alam M.Z., Choudhury T.R. (2022): Health risk assessment for heavy metal accumulation in leafy vegetables grown on tannery effluent contaminated soil. Toxicology Reports, 9: 346–355.

Aloud S.S., Alotaibi K.D., Almutairi K.F., Albarakah F.N. (2022): Assessment of heavy metals accumulation in soil and native plants in an industrial environment, Saudi Arabia. Sustainability, 14: 5993.

Barboiu G., Radulescu C., Popescu I., Dulama I., Bucurică I., Teodorescu S., Stirbescu R.M., Tanase N. (2020): Potential health risk assessment associated with heavy metal accumulation in native *Urtica dioica*. Romanian Reports in Physics, 72: 711.

Bislimi K., Halili J., Sahiti H., Bici M., Mazreku I. (2021): Effect of mining activity in accumulation of heavy metals in soil and plant (*Urtica dioica* L). Journal of Ecological Engineering, 22: 1–7.

Briffa J., Sinagra E., Blundell R. (2020): Heavy environmental metal pollution and their toxicological effects on humans. Heliyon, 6: e04691.

Cai L.M., Xu Z.C., Qi J.Y., Feng Z.Z., Xiang T.S. (2015): Assessment of exposure to heavy metals and health risks among residents near Tonglushan mine in Hubei, China. Chemosphere, 127: 127–135.

Devi C.M., Swapna T.S. (2022): Weed plants: a boon for remediation of heavy metal contaminated soil. In: Kumar V., Shah M., Shahi S. (eds.): Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water. Amsterdam, Elsevier, 127–141. ISBN: 9780323885485

Domac R. (1994): Flora of Croatia: Manual for Identification of Plants. Školska knjiga. Zagreb, 294–296.

Engwa G.A., Ferdinand P.U., Nwalo F.N., Unachukwu M.N. (2019): Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the Modern World-New Tricks for an Old Dog, 10: 70–90.

European Union (2006): Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 364: 5–24. Available at: https://eur-lex.europa.eu/eli/reg/2021/1317/oj

Gebeyehu H.R., Bayissa L.D. (2020): Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. PloS One, 15: e0227883.

Hayek A. (1927): Prodromus florae peninsulae Balcanicae. Repertorium Specierum Novarum Regni Vegetabilis, 30: 1–472.

Huremović J., Horvat M., Kotnik J., Kocman D., Žižek S., Ribeiro Guevara S., Muhić-Šarac T., Memić M. (2017): Characterization of mercury contamination surrounding a chloralkali production facility in Tuzla, Bosnia and Herzegovina. Analytical Letters, 50: 1049–1064.

Husejnović M.Š., Janković S., Nikolić D., Antonijević B. (2021): Human health risk assessment of lead, cadmium, and mercury co-exposure from agricultural soils in the Tuzla Canton (Bosnia and Herzegovina). Archives of Industrial Hygiene and Toxicology, 72: 268–279.

IBM Corp. Released (2016): IBM SPSS Statistics for Windows Version 24.0. IBM Corp, Armonk.

Javorka S., Csapody V. (1991): Iconographia Florae Partis Austro-Orientalis Europae Centralis. Budapest, Akadémiai Kiadó (Reprint), 576. (In Latin)

Kowalska G. (2021): The safety assessment of toxic metals in commonly used herbs, spices, tea, and coffee in Poland. International Journal of Environmental Research and Public Health, 18: 5779.

Lăcătuşu R. (2014): Contributions regarding heavy metals flow within soil-plant-animal system in polluted areas. Acta Metallomica-MEEMB, 1: 73–88.

Liu X.M., Gu S.B., Yang S.Y., Deng J.S., Xu J.M. (2021): Heavy metals in soil-vegetable system around E-waste site and the health risk assessment. Science of The Total Environment, 779: 146438.

Mahmood A., Malik R.N. (2014): Human health risk assessment of heavy metals *via* consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arabian Journal of Chemistry, 7: 91–99.

Manea D.N., Ienciu A.A., Ştef R., Şmuleac I.L., Gergen I.I., Nica D.V. (2020): Health risk assessment of dietary heavy metals intake from fruits and vegetables grown in selected old mining areas – a case study: the Banat Area of Southern Carpathians. International Journal of Environmental Research and Public Health, 17: 5172.

Mateos-Maces L., Chávez-Servia J.L., Vera-Guzmán A.M., Aquino-Bolaños E.N., Alba-Jiménez J.E., Villagómez-González B.B. (2020): Edible leafy plants from Mexico as sources of antioxidant compounds, and their nutritional, nutraceutical and antimicrobial potential: a review. Antioxidants, 9: 541.

Murtić S., Zahirović Ć., Čivić H., Sijahović E., Jurković J., Avdić J., Šahinović E., Podrug A. (2021): Phytoaccumulation of heavy metals in native plants growing on soils in the Spreča river valley, Bosnia and Herzegovina. Plant, Soil and Environment, 67: 533–540.

Musilová J., Harangozo Ľ., Franková H., Lidiková J., Vollmannová A., Tóth T. (2021): Hygienic quality of soil in the Gemer region (Slovakia) and the impact of risk elements contamination on cultivated agricultural products. Scientific Reports, 11: 14089.

Musilová J., Franková H., Lidiková J., Chlpík J., Vollmannová A., Árvay J., Harangozo L., Urminská J., Tóth T. (2022): Impact of old environmental burden in the Spiš region (Slovakia) on soil and home-grown vegetable contamination, and health effects of heavy metals. Scientific Reports, 12: 16371.

Nica D.V., Bordean D.M., Pet I., Pet E., Alda S., Gergen I. (2013): A novel exploratory chemometric approach to environmental monitorring by combining block clustering with Partial Least Square (PLS) analysis. Chemistry Central Journal, 7: 1–10.

Okereafor U., Makhatha M., Mekuto L., Uche-Okereafor N., Sebola T., Mavumengwana V. (2020): Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. International Journal of Environmental Research and Public Health, 17: 2204.

Pazalja M., Salihović M., Smajović A. (2022): Operator exposure to heavy metals from wood pellet ash-risk assessment. Journal of Chemists and Chemical Engineers, 71: 551–556.

Prisacaru A.E., Apostol L.C., Ropciuc S. (2017): Estimation of heavy metal levels in green leafy vegetables purchased from Suceava. Food and Environment Safety Journal, 16: 234–238.

Proshad R., Kormoker T., Islam Md.S., Chandra K. (2020): Potential health risk of heavy metals *via* consumption of rice and vegetables grown in the industrial areas of Bangladesh. Human and Ecological Risk Assessment: an International Journal, 26: 921–943.

Saleem M.H., Ali S., Rehman M., Hasanuzzaman M., Rizwan M., Irshad S., Shafiq F., Iqbal M., Alharbi B.M., Alnusaire T.S., Qari S.H. (2020): Jute: a potential candidate for phytoremediation of metals – a review. Plants, 9: 258.

Salihović M., Pazalja M., Šapčanin A., Dojčinović B.P., Špirtović-Halilović S. (2021): Element contents and health risk assessment in wild edible mushrooms of Bosnia and Herzegovina. Plant, Soil and Environment, 67: 668–677.

Saraiva M.A., Chekri R., Guérin T., Sloth J.J., Jitaru P. (2021): Chromium speciation analysis in raw and cooked milk and meat samples by species-specific isotope dilution and HPLC-ICP-MS. Food Additives and Contaminants: Part A, 38: 304–314.

Sarkar T., Salauddin M., Roy S., Chakraborty R., Rebezov M., Shariati M.A., Thiruvengadam M., Rengasamy K.R.R. (2022): Underutilized green leafy vegetables: frontier in fortified food development and nutrition. Critical Reviews in Food Science and Nutrition, 2022: 1–55.

Sultana M.S., Rana S., Yamazaki S., Aono T., Yoshida S., Kanan S. (2017): Health risk assessment for carcinogenic and non-carcinogenic heavy metal exposures from vegetables and fruits of Bangladesh. Cogent Environmental Science, 3: 1291107.

Tóth G., Hermann T., Da Silva M.R., Montanarella L.J.E.I. (2016): Heavy metals in agricultural soils of the European Union with implications for food safety. Environment International, 88: 299–309.

Trinajstić I. (ed.) (1975–1986): Analytical Flora of Yugoslavia 2 (1–4). University of Zagreb, University Publishing House Liber.

USEPA (2011): Exposure Factors Handbook. Final edition. EPA/600/R-09/052F,Washington, DC. Available at: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252 (accessed on 12. 11. 2022)

USEPA (2012): Integrated Risk Information System of the US Environmental Protection Agency. Washington, U.S. Environmental Protection Agency Available at: https://www.epa.gov/iris (accessed on 12. 12. 2022)

USEPA (2017): United States Environmental Protection Agency. IRIS Assessments. Washington, U.S. Environmental Protection Agency Available at: https://cfpub.epa.gov/ncea/iris2/atoz.cfm (accessed on 19. 12. 2022)

Zhang H.Y., Yuan X.Z., Xiong T., Wang H., Jiang L.B. (2020): Bioremediation of co-contaminated soil with heavy metals and pesticides: influence factors, mechanisms and evaluation methods. Chemical Engineering Journal, 398: 125657.

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