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Determination of potentially toxic elements in some wild edible and medicinal plants

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Abstract

The aim of this research was to determine the Cu, Ni, Zn, Cd, and Pb contents in the following wild plants: dandelion (*Taraxacum officinale* Web.), broad-leaved plantain (*Plantago major* L.), and ribwort plantain (*Plantago lanceolata* L.) collected from the natural environment at three different locations in the north of the Republic of Srpska, in the surroundings of Banja Luka (two localities) and Doboj (one locality). The examined species represent wild edible and medical plants, most commonly used in folk medicine. Plant material and associated soils were air-dried, acid digested, and analyzed by atomic absorption spectrometry (AAS). The results have shown the highest Zn contents in all examined plants, which were followed by the contents of other determined biogenic elements (Ni and Cu), while contents of toxic elements (Pb and Cd) were noticeably lower. The contents of potentially toxic elements determined in the plants were lower than recommended limits of toxicity, with the exception of the location L3-Stanari where elevated Ni contents were found in all examined plants and the associated soil. This implies an evident risk in case of consumption of the plants grown at this location and the need for further investigation in order to establish the exact degree of contamination and to implement proper activities.

Key words: heavy metals, wild edible plants, potential health risk.

Introduction

Using self-grown plants in the human diet, herbal medical therapies, and natural cosmetic production has been present throughout human history (Maiga et al., 2005; Luo et al., 2021). Wild edible plants (WEPs) include plant species that are neither cultivated nor domesticated, but are available from their wild natural habitat (Lulekal et al., 2011). These plants are also traditional foods that tend to be richer in micronutrients than cultivated crops (Hunter et al., 2019), which are embedded into traditional food knowledge as an integral part of local, ethnic nutrition systems (Aziz et al., 2020).

Although consumers of WEPs are trying to collect them from safe, unpolluted areas because of the widespread presence of potentially toxic elements (PTEs) in the environment, collected plants often include their residues (Luo et al., 2021). According to Kumar et al. (2018), the most often PTEs present in wild plants are iron (Fe), zinc (Zn), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and cadmium (Cd). Some of these elements are essentially micronutrients (Fe, Cu, Zn, Cr, Ni, etc.) important for normal growth and development of plants and animals, while their presence in excessive amounts produces various negative impacts. On the other hand, some PTEs (Hg, As, Pb, Cd) are toxic and harmful to all living beings even in small concentrations (Kabata-Pendias, 2011). The sources of these elements in the environment are natural (rock degradation, forest fires, volcanic eruptions, etc.) or connected with various human activities (anthropological) caused by urbanization and industrial development (Sparks, 2003).

Wild plants used as medicinal and edible herbs require special attention due to the lack of appropriate legislation in our country, their unlimited accessibility caused by spontaneous growth in meadows and usual consumption without determination of PTEs content. WEPs belonging to the genus *Taraxacum* and *Plantago* are the most common grassland and roadside plants used in traditional medicine and nutrition for centuries world-wide (Samuelsen et al., 2007; Martinez et al., 2015). Thus, the main aim of this research was to quantify several essential (Cu, Ni and Zn) and toxic (Pb, Cd) elements from the group of PTEs in three wild edible and medicinal plant species: dandelion- *Taraxacum officinale* Web., broad-leaved plantain- *Plantago major* L., and ribwort plantain- *Plantago lanceolata* L. and to estimate the degree of their contamination with the examined elements. During the research the pseudo-total and available contents of the same metals in the associated soils were also determined, as well as their main chemical properties that have a great deal of influence on the mobility and availability of metals to the plants (pH, cation exchange capacity (CEC), content of organic matter (OM). Finally, the estimate of the degree of contamination was done by comparison of the determined metal contents with the allowed maximums for unpolluted soil and plants.

Material and methods

The research was carried out in the north of the Republic of Srpska, where plains and lowlands dominate, with hills and lower mountains (Motajica, Vučjak, Ozren) rising above them. This area is characterized by a moderate continental climate, as well as developed agricultural and industrial activities. Samples of the plant material and soils were collected at three locations in the surroundings of the cities of Banja Luka and Doboj: L1-Aleksandrovac, L2- Čardačani, and L3-Stanari (Fig. 1) in June 2019. All locations were in the vicinity of the frequent, main traffic roads (less than 500 m from the nearby highway direction).

The L1-Aleksandrovac location (N 44°58´33.9", E17°18´11.6") was a barley field, placed in the middle part of the Vrbas River valley, primarily utilized for crop and vegetable production. This point is about 25 km downstream from Banjaluka. Dominant soils here are fluvisoils (Mojičević et al., 1976). The second sampling location, L2-Čardačani, was black chokeberry orchard (N 44°52´35.0", E 17°19´23.7"), situated 20 km east from Banjaluka, in the zone of hilly terrains formed on the geological bedrock of limestone, marl, sandy, and loamy sediments (Mojičević et al., 1976). Orcharding, wine growing, and vegetable production are primary branches of agricultural production in the vicinity of this location. The sampling location L3-Stanari (N 44°44´43.45", E 17°46´11.18") was a meadow. This point is situated 25 km east from Doboj, near the small river stream which flows between the serpentine rocks (Sofilj et al., 1984) and 1.5 km away from the Stanari coal mine, exploited since the middle of the 20^{th} century and a thermal power plant which started to work since 2016.

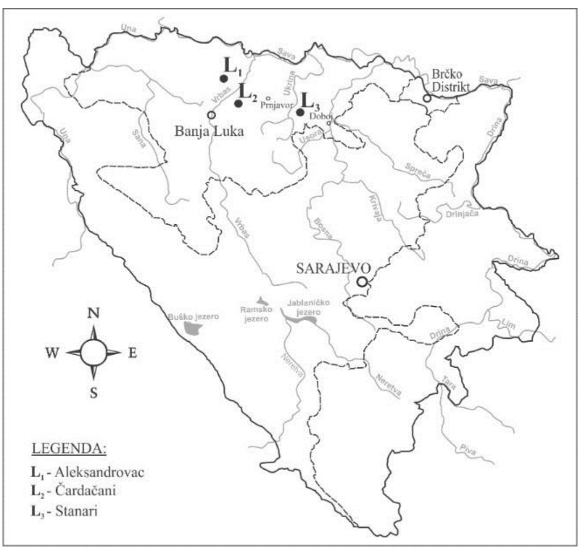

Fig. 1. Map of the research area with the sampling locations

During the process of sampling, plant material (aboveground parts: stems and leaves) were put in paper bags (min. 500 g of the raw material per species, taken at about 10 points within the same location) and transported to the laboratory on the same day for pre-treatment. The plants were thoroughly washed under tap water followed by deionized water and then air-dried to a constant mass. Dried plant material was ground to powder using an electric mill.

Soil sampling was done with an agrochemical probe, where each average sample (about 1 kg) was formed from several individual samples (10-15) taken from the top soil layer (depth 0-25 cm). Prior to the analyses, the average soil samples were air dried, then crushed in a mortar, and sieved through a 2 mm sieve. The main chemical properties of the soil were determined according to standard agrochemical methods: 1. Soil reaction (pH) in suspension of 1: 2.5 soil to KCl solution (c=1 mol/L) ratio using pH meter (Hanna pH 211R), 2. Organic matter (OM) content according to the Tjurin method (Tjurin, 1951). 3. Cations exchange capacity (CEC) by saturating the samples with NaOAc, at pH 7.0 (Chapman, 1965). 4. Content of phosphorus (P) and potassium (K) by the ammonium lactate method according to Egner et al. (1960).

The contents of PTEs in the soils were determined as available, by extraction with NH_4NO_3 c=1 mol/L (Deutsches Institut für Normung [DIN], 1993) and pseudo-total, by acid digestion. Quantification of metal contents was done by atomic absorption spectrophotometry (AAS). The soil samples were acid digested (concentrated HNO_3 + 33% H_2O_2) according to the slightly modified Method 3050B (United States Environmental Protection Agency [US EPA], 1996). However, during the acid digestion, crystal lattice of the silicate minerals is not degraded by nitric acid, thus, a certain proportion of the metals total content remains in the residuum. For this reason, the content of metals obtained after digestion with nitric acid is considered as pseudo-total content, but will be further regarded as total to simplify the presentation of the main research findings. Additionally, available contents of metals were a part of their total amount, which was mobile and, therefore, available to the plants.

Destruction of the plant tissues (shoots) was obtained with concentrated HNO_3 supplemented with 30% H_2O_2 and 70% $HClO_4$ (Pequerul et al., 1993). The extracts obtained, after the described procedures, were stored at 4°C and afterwards analyzed for Cd, Cu, Ni, Pb, and Zn. Metal determination was carried out with a Perkin Elmer AAnalyst 400 atomic absorption spectrophotometer.

Quality control of the processes of extraction and quantification of metals was carried out by the analyses of certified reference material (ERM®-CD218, rye grass) and (ERM®-CC141, loam soil). All analytical procedures were triplicated and made with acid-prewashed (10% HNO_3) glassware and plastic materials.

Results and discussion

Determined chemical characteristics of the tested soils are presented in Table 1. Soils at the L1-Aleksandrovac and L2-Čardačani locations were strongly acid and with low contents of organic matter (OM). The values for CEC determined in both soils indicate their low adsorption capacity. It was also found that these soils were well-provided with potassium (K), and very poorly provided with phosphorus (P).

Tab. 1. Chemical properties[a] of the tested soils at investigated localities in the top soil layer (0-25 cm)

Location	pH_{KCl}	OM %	CEC meq/100g	mgP_2O_5 /100g	mgK_2O/ 100g
L1 Aleksandrovac	4.26±0.04	1.97±0.20	12.50±0.25	13.70±0.36	23.80±0.35
L2 Čardačani	4.34±0.02	2.04±0.25	10.05±0.25	2.08±0.13	23.4±0.30
L3 Stanari	5.98±0.02	3.18±0.15	18.38±1.23	2.00±0.20	7.80±0.30

[a]Results presented as means ± SD of three replicates.

On the other hand, the soil at the Stanari (L3) location was very poor with both determined plant nutrients (K and P). This soil also had a weak acid reaction, small OM content, and medium adsorption capacity. This is in line with a well-known fact about a strong, positive correlation between the content of organic matter and soil adsorption capacity (Jordanoska et al., 2014).

Table 2 presents total and available metal contents determined in the tested soils, together with the allowed maximums for unpolluted soils according to the regulations in our country (Rulebook on permitted quantities of hazardous and harmful substances in agricultural soil and water for irrigation, and methods for their testing, 2016) and trigger values defined by DIN-standard (Bundes-Bodenschutz und Altlastenverordnung, 1999). The total contents of Ni in the soil exceeded the allowed maximum (50 mg Ni/kg) at the L3-Stanari (about 11 times) and L1-Aleksandrovac locations (slightly higher than allowed maximum). On the other hand, the contents of other determined essential elements (Cu and Zn) as well as toxic PETs (Cd, Pb) were within the allowed maximums.

Besides, the available contents of Ni, Cu, Zn, and Cd determined in all tested soils (extraction with NH_4NO_3, c=1 mol/L) were small and less than trigger values (Bundes-Bodenschutz und Altlastenverordnung, 1999). Available contents of PTEs in the soil higher than trigger values demand further investigation of possible harmful effects and soil pollution. Our results indicate that for Pb, because of the elevated available contents (> 0.1 mg Pb/kg) in all tested soils.

Tab. 2. Potentially toxic elements contents[a] (mg/kg) in the tested soils at investigated localities in the top soil layer (0-25 cm)

Total contents (conc. HNO_3+$HClO_4$)					
Location	Cd	Cu	Ni	Pb	Zn
L1 Aleksandrovac	0.36±0.01	23.16±0.25	51.77±1.59	25.43±0.23	49.20±2.29
L2 Čardačani	0.26±0.03	13.01±0.39	21.06±0.43	23.50±1.11	52.45±6.61
L3 Stanari	0.21±0.01	23.08±1.67	596.98±16.6	16.47±0.14	37.5±0.62
Allowed maximum	1	90	50	100	150
Available contents (NH_4NO_3, c= 1 mol/L)					
L1 Aleksandrovac	0.01±0.00	0.03±0.01	0.69±0.03	0.30±0.01	0.36±0.03
L2 Čardačani	0.01±0.00	0.06±0.02	0.10±0.01	0.24±0.02	0.20±0.02
L3 Stanari	<l.d.	0.03±0.00	0.66±0.00	0.40±0.03	0.02±0.01
Trigger values	-	1	1.5	0.1	2

[a]Results presented as means ± SD of three replicates.

Also, percentage of availability (share of available in total content) of Ni, Cu, Zn, and Cd was the lowest in the soil at L3-Stanari, which was probably caused by the determined higher pH value and adsorption capacity, and therefore lower mobility of metals compared to the soils at the L1 and L2 locations. Similar influence of soil acidity and adsorption capacity on availability of metals was reported by Mihajlović (2017) during the research done at the soils in the north-western part of the Republic of Srpska. On the other hand, Pb was the most available in the soil at the L3 location and the most independent in terms of availability, among the examined elements, from the soil characteristics. This was probably caused by its dominant origin from anthropological sources (perhaps the nearby traffic roads), but it needs to be examined in a new research at the same locations in order to get a clear conclusion.

Tab. 3. Content of potentially toxic elements (mg/kg) in the examined plants[a] at investigated localities

Location	Cd	Cu	Ni	Pb	Zn
Taraxacum officinale L.					
L1 Aleksandrovac	0.32±0.01	16.88±0.28	3.52±0.58	5.57±0.23	91.70±0.77
L2 Čardačani	0.21±0.03	13.75±0.31	4.23±0.08	5.78±0.13	36.71±3.34
L3 Stanari	0.02±0.01	16.51±0.36	140.25±1.14	7.21±0.36	40.33±0.71
Plantago major L.					
L1 Aleksandrovac	0.07±0.02	8.05±0.40	3.86±0.12	6.57±0.16	43.68±1.70
L2 Čardačani	0.03±0.01	4.77±0.24	3.23±0.25	5.51±0.32	21.88±0.97
L3 Stanari	<l.d.	6.98±0.04	60.17±0.95	6.54±0.10	27.08±1.82
Plantago lanceolata L.					
L1 Aleksandrovac	0.08±0.02	5.23±0.35	3.60±0.17	4.98±0.13	31.97±1.84
L2 Čardačani	0.06±0.01	6.18±0.12	4.55±0.21	5.58±0.40	25.00±0.45
L3 Stanari	<l.d.	5.31±0.27	34.21±0.88	5.48±0.39	21.89±0.47
Recommended maximum	0.3[b]	20-100[c]	10-50[c]	10[b]	100-400[c]

[a]Results presented as means ± SD of three replicates;
[b]WHO (2007); [c]toxic range (Kabata-Pendias, 2011).

The high total and low available contents of Ni determined in the tested soils were probably caused by the dominant origin of this element from natural, geochemical sources. Metals predominantly originating from geochemical sources were present in the soil in less available forms, bound in silicates, oxides, sulfides, etc. (Kabata-Pendias, 2011). In addition, serpentine rocks were found in the vicinity of the above-mentioned locations (L1, L3) with elevated total contents of Ni (Sofilj et al., 1984, Mojičević et al., 1976). Based on the well-known fact that soils formed on the serpentine rocks contain elevated Ni contents (Sparks, 2003; Tsadilas, 2019), it can be presumed that the presence of Ni in the examined soils was mainly caused by rock degradation and pedological processes, along with leaching by water flows.

The contents of metals determined in the examined plants are presented in Tab. 3 and Fig. 2. It is obvious that essential, beneficial elements (Zn, Ni, Cu) are present in the plants in higher amounts than toxic elements (Pb, Cd). Among the elements determined, Zn was the most present metal in the plants, which was probably caused by its biological role and more expressed mobility (Kabata-Pendias, 2011). The average contents of Zn determined in the plants varied from 21.88 to 91.70 mg/kg, followed by Cu (5.23-16.88 mg/kg), while the contents of Pb and Cd were noticeably lower (Fig.2). The average contents of Ni in the plants at the L1 and L2 locations (3.52-4.55 mg Ni/kg) were lower than mean values determined for content of the other two essential elements (Zn, Cu). On the other hand, at the L3-Stanari location the highest Ni contents were found in all plants. They were above the recommended range of toxicity (10-50 mg Ni/kg) in all three examined plants and in the following increasing order: *Plantago lanceolata* L. (34.21 mg Ni /kg*)* < *Plantago major* L. (60.17 mg Ni/kg) < 140.25 mg Ni/kg in *Taraxacum officinale* Web.

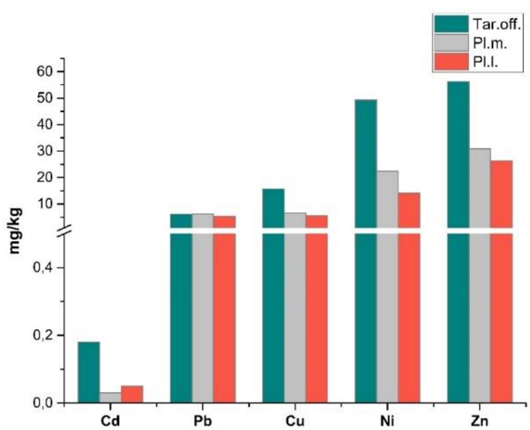

Fig 2. Average contents (mg/kg) of potentially toxic elements determined in the examined plants Tar.off.- *Taraxacum officinale* Web., Pl.m.-*Plantago major* L. and Pl.l.-*Plantago lancelota* L.

In addition, the contents of metals in the examined plants were generally within the permissible limits recommended by the World Health Organization (2007) in the case of toxic PTEs (Cd and Pb) and according to referent literature for Cu and Zn (Kabata-Pendias, 2011). On the other hand, the contents of Cu, Ni, and Zn indicate a good supply of these nutrients to the plants. As stated before, the contents of Ni were higher than the permitted level in all plants grown at the L3 location, which was most probably caused by very high total contents of Ni in the soil. The total Ni contents in the soil at this location were 11 times higher than the maximum allowed (50 mg Ni/kg; Rulebook on permitted quantities of hazardous and harmful substances in the agricultural soil and water for irrigation, and methods for their testing, 2016). The percentage of availability determined for Ni from the soil (share of available in the total contents) was very small here, while the Ni contents in the plants were 3-13 times higher than the recommended level of toxicity (10 mg Ni/kg; Kabata-Pendias, 2011). This could be explained by the fact that the contents of Ni in the most mobile and available forms in the soil were low, but its elevated contents in the tested plants were mainly caused by its high total content. All that implies an evident risk in case of consumption of the plants grown at this location. According to our knowledge, similar studies were not done at the same locality and its surroundings before. Since this is a preliminary research, it is necessary to conduct further, more detailed research in order to determine the exact degree of contamination of the soil and plants with Ni and to estimate the health risk caused by the consumption of the plants growing there.

Furthermore, the comparison between the mean content of PTEs in the plants, which were calculated as average of the mean elements content on the different locations in the same plant species, showed that the uptake of Ni, Zn, Cu, and Cd by *Taraxacum officinale* Web. (dandelion) was significantly greater than in the Plantago spp. (Fig. 2). Pb contents were slightly higher in *Plantago major* L. compared to the other two examined plant species. The Pb contents determined in all plants were in the narrow range, between 5-7 mg Pb/kg (Table 3) and therefore there were no significant differences between their average values.

Similar results were found by other authors (Djiganova and Kuleff, 1999; Stančić et al., 2015; Chizzola, 2012). During a research done in the green areas in the city Varaždin, Stančić et al. (2015) found higher contents of Ni, Cu, and Cd in *Taraxacum officinale* Web. than in Plantago spp. On the other hand, in *Plantago lancelota* L. (108 mg Zn/kg, 0.63 mg Pb/kg) higher average contents of Zn and Pb were found than in *Taraxacum officinale* Web. (96.4 mg Zn/kg, 0.3 mg Pb/kg) and *Plantago major* L (42.6 mg Zn/kg, 0.4 mg Pb/kg). Besides, higher Zn and Pb contents determined in all tested plant species in Varaždin compared to those in our research were most probably caused by the fact that the soils in Varaždin contained more Zn and Pb (the values determined were in the range between 55.8-154.9 mg Zn/kg and 50.48-155.60 mg Pb/kg).

In the study based on 30 different researches with the aim to determine various PTEs contents in medicinal plants from Europe and Mediterranean region Chizzola (2012) reported that *Taraxacum officinale* Web. compared to *Plantago lancelota L.* and *Plantago major* L. accumulated higher contents of metals, with decreasing order in all three plants as follows: Zn>Cu>Ni>Pb>Cd. As mentioned before, similar order of the metal contents was found in our research: Zn>Ni>Cu>Pb>Cd. Higher average Ni than Cu contents (Fig.2) found in the plants in our research were probably caused by elevated Ni contents in the soil.

Conclusion

Summarizing all the data, it can be concluded that the contents of PTEs in the examined plants depends on their total content in the soil, biological role, mobility, and plant species. The contents of metals were generally within the permissible limits, except at the L3-Stanari location with elevated Ni contents in all examined plants and the associated soil. This implies an evident risk of consumption of the plants grown at this location and a need for further investigation in terms of the soil and plant quality monitoring. Besides, the available contents of Pb determined in the tested soils were higher than trigger values (>0.1 mg Pb/kg) established by the DIN standard, but their contents in all plants were within the allowed maximum (10 mg Pb/kg), which finally indicates a low risk of environmental pollution with Pb from the soil. Elevated availability of Pb was probably caused by its dominant anthropological origin (from nearby traffic roads), but it needs to be further examined in order to arrive at clear conclusions.

Generally, the total contents of PTEs in tested soils can be arranged as follows Ni>Zn>Cu>Pb>Cd, while their mean contents in the examined plants, with the exception of the results at the L3 location, decreased in the following order Zn>Ni>Cu>Pb>Cd. It is evident that essential micronutrients (Zn, Cu, and Ni) are present in higher amounts than toxic elements (Pb, Cd). Among the examined plant species, *Taraxacum officinale* Web. showed significant greater accumulation ability for Zn, Ni, Cu, and Cd. On the other hand, the average Pb contents determined in all plants were in the same, narrow range which implies a similar level of intake by the plants.

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Одређивање садржаја потенцијално токсичних метала у самониклим и љековитим биљкама

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Сажетак

Циљ овог истраживања био је утврђивање садржаја Cd, Cu, Ni, Pb и Zn у самониклим биљкама: маслачку (*Taraxacum officinale*), широколисној боквици (*Plantago major*) и усколисној боквици (*Plantago lanceolata)*, узоркованим на природном станишту са три локације на сјеверу Републике Српске, у околини Бања Луке (2 локације) и Добоја (једна локација). Испитиване врсте представљају самоникле јестиве и љековите биљке, широко заступљене у традиционалној, народној медицини. Биљни материјал и земљиште су ваздушно сушени, разарани киселинском дигестијом и анализирани методом атомске апсорпционе спектрофотометрије (ААС). Резултати истраживања у испитиваним биљкама указују на највиши садржај Zn, а потом и друга два биогена елемената (Ni и Cu), док су утврђени садржаји токсичних елемената (Pb и Cd) знатно нижи. Утврђени садржаји метала у испитиваним биљкама су нижи од препоручених граница токсичности, са изузетком локације L3-Станари на којој је установљен повишени садржај Ni у биљкама и земљишту, што указује на постојање ризика од конзумације биљака раслих на овом локалитету и потребу за даљим истраживањем ради тачног утврђивања степена загађености и предузимања одговарајућих мјера.

Кључне ријечи: садржај тешких метала, самоникле биљке, потенцијални здравствени ризик.

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