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ENVIRONMENTAL ASSESSMENT OF SOME HEAVY METALS IN SELECTED MEDICINAL PLANT SPECIES ALONG A BUSY ROAD IN VRNJAČKA BANJA, SERBIA

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Abstract: This study reported the concentrations of Pb, Cd, Cr, Mn in parts of four medicinal plant species (*Cichorium intybus* L., *Mentha×piperita*L., *Plantago lanceolata*L., *Verbascum densiflorum* Bertol.) collected from two sites, one few meters away and the other 500 m away from a busy road in Vrnjačka Banja, Serbia. Heavy metals were determined by the atomic absorption method using atomic absorption spectrophotometer. Similar content of tested metals in the soil of the tested locations was recorded, only there was more manganese in the soil closer to the road. The highest concentrations of heavy metals were found in the roots of plants closer to the road. The leaves and stem contain smaller quantities of these metals than root, but there are differences between the studied plant species. In conclusion, all examined metals were present in higher concentrations in plants at the location closest to the road than at the location further away from the road.

Keywords: heavy metal; medicinal plant species; busy road; bioaccumulation

1. INTRODUCTION

The presence of toxic metals in the environment is a major problem (Briffa et al., 2020). The harmful effect of increased concentrations of heavy metals on the development of plants and other living organisms have been known before, but in recent decades it has received considerable attention due to the development of scientific and public awareness, as well as technical possibilities of accurate measurement of their concentrations. The content of heavy metals in soil may be affected by geological, geochemical and anthropogenic factors such as road traffic, railway vehicles andagricultural activities (Horasan et al., 2020). Considering traffic has become higher in the last few decades which is a very important source of heavy metals (Stanković et al., 2008). Emissions from heavy traffic contain potentially toxic metals such as lead, cadmium, zinc, copper. Metabolism of living organisms is based on enzyme systems that use about 20 essential elements, and some of the other elements (Pb, Hg, Cr, Cd, etc.) can be very toxic even at low concentrations and contamination of the food chains (Ali & Khan, 2019). However, these metals do not have to be associated with environmental pollution, toxicity or adverse effects on living organisms (Ali & Khan, 2018). Chronic exposure to heavy metals in the environment poses a real threat to living organisms (Wieczorek-Dąbrowska et al., 2013). Potentially toxic metals accumulation in plants depends upon plant species and many factors which include temperature, moisture, organic matter, pH and nutrient availability (Jiwan & Kalamdhad, 2011). Toxic metals mostly enter the food chain through plants and in a certain way they affect the ecological situation, suppressing the development and biological activity of many living organisms and human health (Jordao et al., 2006; Surucu et al., 2020).

Toxic metals act by blocking the essential functional groups of biomolecules, or by substituting essential metal ions by other ones (Küpper et al., 2002). The heavy metals influence on plant germination, growth and production which is mainly associated with the physiological, biochemical and genetic elements of the plant system (Sethy & Ghosh, 2013). Plant species have different strategies to cope with heavy metals (Maleki et al., 2017). Plant resistance to heavy metals boils down to absorption selectivity and detoxification at the cellular level (Hall, 2002). Lead blocks uptake of essential elements of chlorophyll such as Mg and Fe and inhibits chlorophyll synthesis (Haider et al., 2006), transpiration, respiration, enzymes inhibition (Van Asshe & Clijasters, 1990), inhibition of cell division (Eun et al., 2000). The distribution and accumulation of Cd depends on the chemical form in which this metal is present in the plant (Qiu et al., 2011), inhibits plant growth and development (DalCorso et al., 2008). Chromium is toxic even at lower concentrations because it is soluble, it affects the germination of seeds, growth stem and root length (Mallick et al., 2010). Manganese has several different roles in biological systems. It is an essential micronutrient in plant development, for vital plant functions, but it may have toxic effect when present in higher concentration that varies within a very wide range, depending on plant species, and environmental conditions (Ducic & Polle, 2005).

Excessive accumulation of these toxic metals can be toxic to most plants. Some plants have developed ability to tolerate elevated concentrations metals which have no known biological function and complex mechanisms that serve to control the uptake, accumulation and detoxification of metals (Benavides et al., 2005).

Environmental contamination due to heavy metals is a serious problem because of their non-degradable and persistent nature. The most of heavy metals can cause serious environmental and health problems even at very low concentrations (Cay et al., 2019). These metals can accumulate in the medicinal plants which people consume and have severe health hazards. Medicinal plants have found extensive use in prevention, disease treatment and great percentage world's population relying on traditional medicine as their primary form of healthcare (Annan et al., 2013). The aim of this study was to determine the content of heavy metals in selected medicinal plants that grown next to the busy road with heavy traffic in two places: one location was a few meters from the road, and the other location was at a distance of 500 m.

2. MATERIALS AND METHODS

The research was conducted in the municipality of Vrnjačka Banja (Serbia) in the month of July and August in 2020. The experiment has included four species medicinal plants, namely: chicory (*Cichorium intybus* L.), mint (*Mentha ×piperita* L.), ribwort plantain (*Plantago lanceolata* L.), denseflower mullein (*Verbascum densiflorum* Bertol.). For the experimental work, these plants were collected from two locations near a busy road (E-761): one was just a few meters away from the road (A) and the other was 500 meters away (B). Geographic coordinates of the sampling sites are: location A - 43°63'461''N and 20°93'844''E and location B - 43°63'492''N and 20°93'750''E.

Root, stem and leaf were collected from each plant species, and the content of four heavy metals - Pb, Cr, Cd and Mn was examined. One sample consists of parts of several individual plants. For one sample 5 grams of dry mass for each plant species and for each part were taken. Each sample was read in three replications.

Heavy metals were determined by the atomic absorption method using an atomic absorption spectrophotometer (Perkin-Elmer Model 1100 B) The plant materials were first washed with tap water, then washed with distilled water and dried in a dryer (Thermo Scientific "Heratherm") for 24 hours at 105°C. The dried plant materials were grounded in to powder with a mixer. Five grams of sample were placed in the dryer to be burned until a visible black ash was observed. After that, the sample a were scorched in a dryer for 10-12 hours at 450°C and two milliliters of concentrated HNO_3 and HCl were added to the cooled samples. The content was then evaporated to complete dryness and returned to the annealing oven for another 1 hour at 450°C. Five milliliters of 6 M HCl were added to different samples and warmed slightly. Further the samples were then filtered and quantitatively transferred to a 10 ml volumetric flask. Then the metal content (Pb, Cd, Cr, and Mn) was determined using an atomic absorption spectrophotometer - AAS, Perkin - Elmer 1100 B and their concentrations were expressed in mg kg^{-1}.

Soil samples were taken from the examined locations at a depth of 0.25 m. One gram of dried and finely powdered soil sample weight and annealed for 3 hours at a temperature of 500°C. After cooling, the rest of the sample was soaked in 1-2 ml of distilled water, 1 ml of concentrated H_2SO_4

and 20 ml of HF acid. Then, it was slightly heated to 120°C on the stove, until white SO_3 vapors appeared. After cooling, the solution was shaken well, and about 50 ml of solution was filtered into a volumetric flask and distilled water was added. The metal content was determined using an atomic absorption spectrophotometer - AAS, Perkin - Elmer 1100. In addition, using standard methods, pH reaction (potentiometric method), nitrogen content (Kjeldahl method), phosphorus and potassium (Egner-Riehm method) and humus (Tyurin method) were determined.

The results were processed using STATISTICS 10.0 program. The significance of the difference between the investigated probes was determined upon the variance analysis, i.e. Fisher's LSD test.

3. RESULTS AND DISCUSSION

The contents of Pb, Cr, Mn and Cd in soil samples from locations A and B were below the maximum allowed amount (Table 1). This soil has preserved its basic function and these metals are probably the product of pedogenetic processes.

The content of metals was found to be lower than the maximum allowable concentration in soil prescribed by the legislation according to decree on limit values of polluting, harmful and dangerous substances in soil in Republic of Serbia (30/2018 and 64/2019). The chromium concentration in the tested samples in the present study was relatively high, 97.31 and 95.31 mg kg^{-1}, although it is below the maximum allowed concentration for this metal (100 mg kg^{-1}). Cr binds very tightly in soils rich in clay and organic matter, so it is very poorly mobile and is adsorbed in the surface layer about 5–10 cm deep) and concentration depending on substrate characteristics (Rauf et al., 2020). Mn supply in soil is good at 699.43 and 805.18 mg kg^{-1}, while the worldwide average of manganese content in soil is 488 mg kg^{-1} (Kabata-Pendias & Pendias, 2001). The total manganese content in the soil is practically derived from the parent substrate. Manganese exhaust can also be a source of car exhaust because its organic compound methylcyclopentadienyl manganese tricarbonyl (MMT) is used as one of the alternatives to lead additives in gasoline. Metals are emitted from motor vehicles both during tire brake wear and in corrosion processes (Blok, 2005; Guney et al., 2010).

The plants represent a sensitive system for early diagnosis of environmental changes. The results showed that the content of metals varies depending on the sampling location, as well as on plant part and plant species. Ducic & Polle (2005) stated that the accumulation of heavy metals in certain organs, tissues and different organelles of cells of certain species is different. The accumulation of Pb in plants depends on the distance of plants from the center of emission, the coverage of the land with plants, the duration of vegetation, the direction and intensity of the wind, etc.

In our study, the roots have a higher content compared to the leaves and stems of the plants (Table 2). A statistically significant difference in Pb content was observed in the root of plants from location A, except *P. lanceolata* where Pb was observed in roots from location B. Similar trend was observed with stems and leaves, but in leaves of *C. intybus* there was no statistical difference in Pb content between plants from different locations. According to Winther & Slento (2010) the road transport emissions heavy metals

Table1. Chemical properties of the soils

Parametres Soil	pH		Total nitrogen %	P_2O_5 mg/100g	K_2O mg/100g	Humus %	$CaCO_3$	Pb mg kg^{-1}	Cr mg kg^{-1}	Cd mg kg^{-1}	Mn mg kg^{-1}
	H_2O	KCl									
A	6.77	5.86	0.298	3.2	26.11	3.04	-	36.75	97.31	0.36	699.43
B	6.57	5.66	0.254	8.7	22.44	3.36	-	43.78	95.31	0.53	805.18

Soil A was few meters away the road; soil B was 500 meters away the road

Table 2. Content of Pb (mg kg^{-1}) in plants parts

Plant species	Location	Root	Stem	Leaves
Cichorium intybus L.	A	0.91^{d}	0.50^{b}	0.49^{f}
	B	0.39^{f}	0.23^{d}	0.47^{f}
Mentha × piperita L.	A	3.08^{a}	1.03^{a}	1.26^{b}
	B	0.74^{e}	0.23^{d}	0.74^{c}
Plantago lanceolata L.	A	1.36^{c}	0.47^{b}	0.58^{e}
	B	1.50^{b}	0.24^{d}	0.36^{g}
Verbascum densiflorum Bertol.	A	0.90^{d}	0.49^{b}	1.65^{a}
	B	0.72^{e}	0.29^{c}	0.65^{d}

*Same letters in superscript point the absence of significant differences by Fisher's LSD test ($p < 0.05$)

including Cu, Zn, Cd, and Pb almost solely originate from brake lining, fuel and engine oil consumption, vehicle tire and brake wear and road abrasion. In contrast to the content Pb in the parts of medicinal plants in present research, Rohilla et al., (2021) found that the concentration of this metal in Indian black tea leaves varies from the lowest 61.0 µg kg^{-1} to the highest 2404.3 µg kg^{-1}, and the cadmium content ranges from 15.4 to 75.03 µg kg^{-1}. The people who living in areas where these plants grow, collect them locally for personal or family use. That why it is important that content metals in studied medicinal plant materials compared with World Health Organization standards WHO (1998) is in within the limits permitted so they use of these plants in traditional medicine is safe.

Most commonly found Cr was observed in the root (4.330 mg kg^{-1}) and leaf (0.313 mg kg^{-1}) of plantain from location A (Table 3). More Cr was present in the root, stem and leaves of the tested plants from location A, although this difference was not statistically in plants from location A and B, namely: in stem of the plantain and leaves chicory, as well as denseflower mullein from different locations (Table 3).

Cr adversely affects the metabolic processes and plant growth (Rahman et al., 2010). Most of the adopted chromium accumulated in the root, and a smaller part is distributed to the stem and leaves of plants (Oliveira, 2012). Studying phytotoxic effects of chromium on germination and seedlings growth of wheat (*Triticum aestivum* L.), Shaikh et al., (2013) reported that with increasing chromium concentration, seed germination decreases, and phytotoxicity of shoots and roots increases.

Cadmium is an element with a very toxic effect on plants, animals and humans. It is rapidly transported from the soil to the plant. The main cause of Cd toxicity is the high affinity of Cd for thiol groups (SH) in enzymes and other proteins.

The highest amount of Cd was in the root of denseflower mullein 0.52 mg kg^{-1}, plantain stem 0.30 mg kg^{-1} and chicory leaf 0.38 mg kg^{-1} (Table 4). More cadmium was accumulated in the roots of the tested plants than in the stem and leaves. In all parts of plants from the location A registered statistically significant higher concentration of Cd than in plants from location B, the only exception was noted in leaves *V. densiflorum* in which the difference has no statistical significance. Salt et al. (1995) reported that the primary point of entry for Cd into plants is through the roots. Plant roots are a barrier to metals, so the concentration of heavy metals in the root is usually higher than in stems and leaves (Yang et al., 2020). Similar to the present study results, Cd content in the analyzed *Medicago sativa* L. samples grown along the highway (E75 route section Belgrade-Leskovac, Republic of Serbia) is in the range of normal values, i.e. up to 10 mg kg^{-1}. However, in some of these samples the concentration Pb and Fe were above the toxic values or the maximum tolerant level for animal nutrition (Pivić et al., 2017). Xin et al., (2014) examined Cd uptake, translocation, and distribution in young seedlings of two hot pepper cultivars found that Cd was mostly accumulated in the roots.

Table 3. Content of Cr (mg kg^{-1}) in plants parts

Plant species	Location	Root	Stem	Leaves
Cichorium intybus L.	A	0.69 ^{d}	0.03 ^{b,c}	0.04 ^{d,e}
	B	0.35 ^{e}	0.01 ^{d}	0.01 ^{e}
Mentha × piperita L.	A	1.72 ^{b}	0.49 ^{a}	0.23 ^{b}
	B	0.35 ^{e}	0.01 ^{d}	0.01 ^{e}
Plantago lanceolata L.	A	4.33 ^{a}	0.03 ^{b,c}	0.31 ^{a}
	B	1.44 ^{c}	0.02 ^{c}	0.05 ^{c,d,e}
Verbascum densiflorum Bertol.	A	0.26	0.04^{b}	0.09^{c,d}
	B	0.13 ^{g}	0.01 ^{d}	0.10 ^{c}

*Same letters in superscript point the absence of significant differences by Fisher's LSD test ($p < 0.05$)

Table 4. Content of Cd (mg kg^{-1}) in plants parts

Plant species	Location	Root	Stem	Leaves
Cichorium intybus L.	A	0.32 ^{c}	0.25 ^{a,b}	0.38 ^{a}
	B	0.21 ^{d}	0.06 ^{c,d}	0.21 ^{b}
Mentha × piperita L.	A	0.32 ^{c}	0.14 ^{b,c}	0.12 ^{c}
	B	0.12 ^{e,f}	0.02 ^{e}	0.03 ^{e}
Plantago lanceolata L.	A	0.19 ^{d}	0.30 ^{a}	0.12 ^{c}
	B	0.10 ^{f}	0.05 ^{d}	0.04 ^{d,e}
Verbascum densiflorum Bertol	A	0.52 ^{a}	0.16 ^{b,c}	0.10 ^{c,d}
	B	0.15 ^{e}	0.05 ^{d}	0.07 ^{d}

*Same letters in superscript point the absence of significant differences by Fisher's LSD test ($p < 0.05$)

Table 5. Content of Mn (mg kg^{-1}) in plants parts

Plant species	Location	Root	Stem	Leaves
Cichorium intybus L.	A	42.19^{f}	41.23^{b}	19.30^{f}
	B	19.30^{h}	1.76^{f}	60.70^{c}
Mentha × piperita L.	A	75.51^{a}	19.12^{c}	49.88^{e}
	B	29.72^{g}	19.21^{c}	57.11^{d}
Plantago lanceolata L.	A	63.27^{b}	1.60^{f}	11.24^{g}
	B	44.53^{d}	60.30^{a}	6.02^{h}
Verbascum densiflorum Bertol.	A	53.77^{c}	8.35^{e}	77.27^{a}
	B	42.61^{e}	14.37^{d}	66.22^{b}

*Same letters in superscript point the absence of significant differences by Fisher's LSD test ($p < 0.05$)

Manganese is essential for chlorophyll biosynthesis. It is an important component of several enzymes, and its most important function, participation in various oxido-reduction processes and building resistance to abiotic and biotic stresses (Pittman, 2005). However, its elevated concentrations can pose a danger to plant health. In the present research results showed that in roots of tested plants from location A higher concentration of Mn was noted the exception of plantain, whose leaves from location A contain more Mn than the leaves of this plant from location B. In the stem, there was more Mn in plants from location B, while in *M.×piperita*, there was no statistical significance between plants from different locations (Table 5). However, in stem of *C. intybus* a higher content of Mn was recorded from location A. In leaves, the content Mn was statistically significant between the plants from locations A and B, but there was no clear trend. In C. *intybus and M. × piperita* a higher concentration of Mn was reported in plants from location B, while in the other two plant species a higher concentration of Mn was recorded in plants from location A.

Manganese primarily accumulates in the above ground parts, and less in the root (Millaleo et al., 2010). Concentrations Mn in terrestrial plants tend to range from 20 to 500 mg kg^{-1}(Stokes et al., 1988). Similar to present study Skrynetska et al., (2019) found that Mn content in the *P. lanceolata* plants grown along the road was in the range of 14-41 mg kg^{-1} and the Pb content was 0.4 to 4.0 mg kg^{-1}. Nadgórska-Socha et al., (2013) found that Mn content in the leaves of *Arabidopsis arenosa* L. vas is from 11.8 to 62.3 mg kg^{-1} and *Plantago lanceolata* from 6.1 to 22.7 mg kg^{-1}.

4. CONCLUSION

Based on results of the present research, it can be concluded that the amount and distribution Pb, Cr, Cd and Mn in plants depend on several factors: location, plant part and plant species. In the examined species, the content of heavy metals depends on the distance from the road in terms of establishing a decreasing gradient. The distribution of tested metals was highest in the roots of plants (root>stem> leaf) at both locations. Plant species have accumulated the metals differently. Mint plants accumulated the most Pb, the largest amount of Cr was recorded in plantain, most Cd was found in chicory, while Mn amount was found to be highest in the denseflower mullein and in mint than in the other two examined species. The contents of heavy metals in all the studied plant parts were within the permitted quantities according to the regulations WHO and recommendations may be put forward to use these plants as medicines. Also, it is necessary to conduct continuous monitoring of these locations as these locations are suitable for harvesting due to increasing traffic activities and utilization of chemical inputs in agricultural production.

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