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STUDY OF ESSENTIAL METALS IN SELECTED MEDICINAL PLANT FROM SERBIA

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In this paper, we have studied uptake and accumulation of essential major and trace metals present in the six selected medicinal plants from Serbia, and their transfer from soil to plant. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to analyze the metals: Na, K, Mg, Ca, Mn, Fe, Cu, and Zn in plant and soil samples. Selected plants accumulate a sufficient amount of studied metals, except Cu. They are not accumulator of Na, Ca and Fe, neither are they tolerant to Na and Mn, except Dittrichia graveolens, tolerant species to all examined metals. The tested plant species can be important in human diet as a source of the essential elements of importance for the optimal functioning of the human body.

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Key words: *essential metals, medicinal plant, ICP-OES*

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Introduction

Medicinal plant preparations have a significant role in the pharmaceutical markets and health care sector of the 21st century (1). Herbs do not only provide organic constituents of medicinal value, but also metals required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health (2). On the other hand, essential metals can produce toxic effects when the metal intake is in high concentration (3). The World Health Organization (WHO) recommends that medicinal plants, as the raw materials for the finished medicinal products should be checked for the presence of heavy metals. The ability of plants to selectively accumulate metals varies in different species and is subjected to certain geochemical characteristics depending on the type of soil (4). For these reasons, the study of the metal contents accumulation in medicinal plants from Serbia is very important.

Aim

The objective of the present study was to deter-mine the content of essential major and trace metals (Na, K, Mg, Ca, Mn, Fe, Cu, and Zn) present in the six selected medicinal plants from Serbia and their transfer from soil to plants.

Materials and Methods

Chemicals

All chemicals and reagents were of analytical reagent grade and were purchased from the Sigma–Aldrich Chemical Company. Standard solutions used in the ICP-OES techniques were purchased from Alfa Aesar, Germany. The certified reference material BCR-701 was purchased from the Community Bureau of Reference (5).

Sample collection

Throughout this work, abbreviations were used in tables to indicate the growing area of the different plant populations. Six different medicinal plant species were used in the study: Thymus glabrescens Willd. (Tg.), Thymus pulegioides L. (Tp.), Satureja kitaibelii Wierzb. ex Heuff. (Sk.), Libanotis montana Crantz subsp. leiocarpa (Heuff.) Soó. (Apiaceae) (Lm.), Peucedanum longifolium Waldst. & Kit. (Pl.) and Dittrichia graveolens (L.) Greuter (Dg.). A voucher specimens were deposited at the Her-barium of the Department of Botany, Faculty of Biology, University of Belgrade-Herbarium Code BEOU. The sampling areas were located in the east Serbia: Kravlje village

(1.)-Tg., Suva planina mountain (2.)-Tp., Sićevo gorge Nature Park (3.)-Sk., Vidlič mountain (4.)-Lm., Rtanj mountain (5.)-Pl. and Stara planina mountain (6.)-Dg. Samples from seven different specimens from each population and soil from the rhizosphere were collected and transported to the laboratory where they were submitted to analysis.

Soil and plant metal analysis

Soil samples were dried at 60 °C and sieved (≤ 2 mm). The total metal content was analyzed in the same soil fraction after grounding samples and submitting them to an acid digestion (HF, HNO3 and HCl). The soil bioavailable metal content was analyzed using 0.05 mol/dm3 EDTA (pH 7) solution, at a ratio of 1:25 (soil/solution). The samples were mixed for 5 h and centrifuged at 10,000 × g for 10 min.

The metal content in plant tissue was analyzed after washing the plants with distilled water and drying them at 60 °C for 48 h. The leaves were excised from the stems and submitted to extraction (HNO_3, H_2O_2 and HCl). Metals were quantified in ICP-OES (Thermo Scientific iCAP 6500 Duo ICP spectrometer). The certified reference material BCR-701 from the Community Bureau of Reference was treated by ICP-OES analysis (5).

The soil–plant transfer coefficient (TC) and bioconcentration factor (BF) were used to analyze the relationship between soil and plant metal content. TC was calculated as the concentration of metal in plant over that in soil (TC = [metal plant]/[metal soil]) and it represented the capacity of a species to accumulate an element. BF was based on the ratio of metal in plant to that in a bioavailable form in soil (BF = [metal plant]/[EDTA-extractable metal in soil]) and it represented the tolerance for a potential toxic element in the absence of toxicity symptoms. A plant could be considered to be an accumulator for a particular metal when TC > 1 and tolerant to certain metal when BF > 1 (6). The experimental results were expressed as mean ± standard deviation of three replicates.

Results

Metal contents of soils in total and EDTA-extractable forms were summarized in Table 1 and Table 2. Concentrations of studied metals were within the specified soil values (7). In the group of major metals, the largest number (four) of the highest concentration values (μg/g) was recorded in the locality of Sićevo gorge Nature Park: Ca 154250.35 in the total form and Na, 25.28; Mg, 606.23 and Ca, 78283.89 in the EDTA-extractable form. The six highest concentration values of studied trace metals (μg/g) were found in the locality of Vidlič mountain; in the total form: Fe, 15411.14; Cu, 17.05 and Zn, 54.53, and in the EDTA-extractable form: Mn, 305.35; Cu, 0.06 and Zn, 2.19. It was interesting that the highest level of Ca in both total and EDTA-extractable forms was in Sićevo gorge Nature Park. On the other hand, the highest concentrations of Cu and Zn in both total and EDTA-extractable forms were in the mountain Vidlič.

Table 1. Soils' total metal content (μg/g)

Metals	**Localities**					
	1.	**2.**	**3.**	**4.**	**5.**	**6.**
Na	2162.47±41.95	2410.86±46.77	718.69±13.94	815.47±15.82	1266.73±24.57	289.06±5.61
K	6880.97±56.42	7315.09±59.98	4030.65±33.05	6740.61±55.27	4997.30±40.98	10871.18±89.14
Mg	3229.80±53.29	2459.54±40.58	1332.23±21.98	1466.49±24.20	1159.37±19.12	1463.56±24.15
Ca	99821.98±1078.08	10251.92±110.73	154250.35±1665.90	22115.18±238.84	25586.59±276.33	14308.62±154.53
Mn	918.11±6.61	1280.25±9.21	736.86±5.31	1044.43±7.52	820.68±5.91	11.03±0.08
Fe	13516.51±227.08	14745.34±247.72	8156.82±137.03	15411.14±258.91	9669.43±162.44	4786.97±80.42
Cu	16.11±0.14	11.46±0.10	5.79±0.05	17.05±0.15	10.45±0.09	2.25±0.02
Zn	40.70±0.64	25.97±0.41	23.67±0.37	54.53±0.86	42.93±0.68	8.45±0.13

Table 2. Soils' metal content in EDTA-extractable forms (μg/g)

Metals	**Localities**					
	1.	**2.**	**3.**	**4.**	**5.**	**6.**
Na	18.71±0.36	10.64±0.21	25.28±0.49	12.19±0.24	12.69±0.25	5.59±0.11
K	313.81±2.57	73.73±0.60	282.40±2.32	232.78±1.91	188.04±1.54	108.88±0.89
Mg	446.67±7.37	223.25±3.68	606.23±10.01	509.63±8.41	257.91±4.26	103.89±1.71
Ca	76973.40±831.31	3762.50±40.64	78283.89±845.47	10463.60±113.01	13620.71±147.10	829.93±8.96
Mn	207.52±1.49	252.24±1.81	227.95±1.64	305.35±2.20	263.84±1.90	4.60±0.03
Fe	2.08±0.03	3.04±0.05	2.83±0.04	2.60±0.04	4.83±0.08	26.63±0.44
Cu	0.01±0.00	0.01±0.00	0.01±0.00	0.06±00	0.01±0.00	0.01±0.00
Zn	0.01±0.00	0.59±0.01	0.01±0.00	2.19±0.03	0.31±0.01	0.34±0.01

The result of the levels of examined metals in selected plants was presented in Table 3. Selected plants accumulated a sufficient amount of studied metals, except Cu. Also, it could be said that T. glabrescens and P. longifolium accumulated lower Na content in comparison with normal range described in plants (8). Plant species D. graveolens accumulated four highest contents, from eight investigated metals (μg/g): Na, 27.00; K, 12333.56; Mn, 50.85 and Fe, 132.76.

Table 3. Metal content in plant leaves (μg/g)

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	3.20±0.06	6.55±0.13	6.17±0.12	6.33±0.13	3.52±0.07	27.00±0.52
K	9848.73±80.76	7805.78±64.00	5789.57±47.47	8011.79±65.69	5891.94±48.31	12333.56±101.14
Mg	1624.03±26.80	3609.30±59.55	2446.64±40.37	2199.26±36.29	1314.96±21.70	1370.80±22.62
Ca	8755.53±94.56	9638.73±104.10	12292.58±132.76	10545.32±113.89	7010.00±75.71	11672.56±126.06
Mn	21.18±0.15	18.24±0.13	19.85±0.14	36.35±0.26	42.71±0.31	50.85±0.37
Fe	88.43±1.49	123.90±2.08	50.40±0.85	42.82±0.72	24.40±0.41	132.76±2.23
Cu	4.16±0.04	3.67±0.03	2.76±0.02	6.03±0.05	1.80±0.02	2.49±0.02
Zn	21.17±0.33	25.49±0.40	15.56±0.25	22.35±0.35	12.16±0.19	12.26±0.19

To estimate metal accumulation and tolerance of wild plant populations, the transfer coefficient (TC) and the bioconcentration factor (BF) were calculated as indicated in the previous section. As shown in Table 4, TC values revealed that the selected plant species was not an accumulator of Na, Ca and Fe. Curiously, TC values were dependent on studied populations. For instance, only D. graveolens was an accumulator for Mn, Cu and Zn. BF values (Table 5) suggested that studied plants were not tolerant to Na and Mn, except D. graveolens, tolerant species to all examined metals. Despite a lower tolerance of plants to Na and Mn, it should be noted that the adverse impact of these metals was not observed during their collection.

Table 4. Soil–plant transfer coefficient (TC) for selected plants growing in different soils

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	0.0015	0.0027	0.0086	0.0078	0.0028	0.0934
K	1.4313	1.0671	1.4364	1.1886	1.1790	1.1345
Mg	0.5028	1.4675	1.8365	1.4997	1.1342	0.9366
Ca	0.0877	0.9402	0.0797	0.4768	0.2740	0.8158
Mn	0.0231	0.0142	0.0269	0.0348	0.0520	4.6102
Fe	0.0065	0.0084	0.0062	0.0028	0.0025	0.0277
Cu	0.2582	0.3203	0.4764	0.3538	0.1722	1.1081
Zn	0.5201	0.9815	0.6575	0.4098	0.2831	1.4511

Table 5. Soil–plant bioconcentration factor (BF) for selected plants growing in different soils

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	0.17	0.62	0.24	0.52	0.28	4.83
K	31.38	105.87	20.50	34.42	31.33	113.28
Mg	3.64	16.17	4.04	4.32	5.10	13.19
Ca	0.11	2.56	0.16	1.01	0.51	14.06
Mn	0.10	0.07	0.09	0.12	0.16	11.05
Fe	42.52	40.76	17.81	16.47	5.05	4.99
Cu	415.89	367.10	276.00	100.51	180.03	249.01
Zn	2116.83	43.20	1556.35	10.20	39.21	36.06

Discussion

Selected medicinal plants from Serbia exhibit significant antibacterial activity (9-11). Environmental and health effects of metal exposure from soil depend on the mobility and availability of the elements. Total metal levels in soil reflect the soil's geological origin and weathering. Certainly, chemical forms of the metals are very important, which may affect their environmental mobility and availability to organisms (12). The metal content of the soils included in this study had a different origin. Soils from Vidlič mountain and Rtanj mountain had the highest chemical similarity in terms of total metal content. Metals after EDTA-extraction are considered the most mobile and potentially bioavailable forms present in soils, and may best capture the anthropogenic contribution of greatest possible concern for human exposure (13). Soils from Kravlje village and Sićevo gorge Nature Park had the highest chemical similarity in terms of EDTA-extractable metal content.

Leaves, organs with photosynthetic activity accumulate higher quantities of metals. As we said, investigated plants accumulated a sufficient amount of studied metals, except copper. The comparison of our results with the results of other authors showed a considerable agreement regarding the heavy metals content in the plants from the region of Southeast Serbia (14). Compared with published data, it is also important to note that in our study a significantly lower content of Na was established in the plant leaves (15).

High concentrations of Na, Ca and Mn in EDTA-extractable forms resulted in the reduction of tolerance of these metals (BF < 1), which may be manifested in their toxicity (12). D. graveolens may be considered as K, Mn, Cu and Zn accumulator and it is tolerant to these four metals, as deduced from TC and BF values obtained in this study. Moreover, D. graveolens is tolerant to all the metals examined. In addition to chemical and ecological aspects, the study of plant-soil interaction can help in the design of modern herbal medicinal products.

Conclusion

The present study gives a new perspective on the chemical composition of selected medicinal plants from Serbia and their corresponding soil. Within the group of major metals, the four highest concentration values were recorded in the locality of Sićevo gorge Nature Park, while the six highest concentration values of studied trace metals were found in the locality of Vidlič mountain. Selected plants accumulate a sufficient amount of studied metals, except copper. They are not accumulator of Na, Ca and Fe, neither are they tolerant to Na and Mn, except D. graveolens, a tolerant species to all examined metals. Analysis of the metal content in the plant material confirms that the tested plant species can be important in human diet as a source of the essential elements of importance for the optimal functioning of the human body at a biochemical level.

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PROUČAVANJE ESENCIJALNIH METALA U ODABRANIM MEDICINSKIM BILJKAMA IZ SRBIJE

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U radu je proučavano usvajanje i akumulacija esencijalnih makro i mikro elemenata u šest odabranih medicinskih biljaka iz Srbije i njihov transfer iz zemljišta u biljku. Za analizu metala: Na, K, Mg, Ca, Mn, Fe, Cu i Zn u uzorcima zemljišta i biljaka korišćena je optičko-emisiona spektroskopija sa induktivno-spregnutom plazmom (ICP-OES). Odabrane biljke akumuliraju dovoljnu količinu proučavanih metala, osim Cu. One nisu akumulatori Na, Ca i Fe, takođe, nisu tolerantne na Na i Mn, osim vrste Dittrichia graveolens, koja je tolerantna na sve ispitivane metale. Proučavane biljne vrste mogu biti značajne u ljudskoj ishrani kao izvor esencijalnih elemenata potrebnih za optimalno funkcionisanje ljudskog organizma.

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Ključne reči: *esencijalni metali, medicinska biljka, ICP-OES*