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GEOME 2
Manuscript received: August 28, 2017
Accepted: October19, 2017

Geologica Macedonica, Vol. **31**, No. 2, pp. 143–156 (2017)
In print: ISSN 0352 – 1206
On line: ISSN 1857 – 8586
UDC: 631.416.83/.88(497.714)
582.929:546.3/.8(497.714)
582.711:546.3/.8(497.714)
Original scientific paper

BIOACCUMULATION AND TRANSLOCATION FACTOR OF HEAVY METALS IN THE PLANTS *LINARIA* SP., *MORICANDIA* SP. AND *VIOLA LUTEA* HUDS FROM THE ALŠAR LOCALITY – REPUBLIC OF MACEDONIA

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A b s t r a c t: This work was conducted in order to assess the distribution of metal and metalloids in soil and plants (*Linaria* sp., *Moricandia* sp. and *Viola lutea* Huds) which appears at the Alšar locality – Republic of Macedonia, as well as bioaccumulation and translocation of the metals in different parts of the plant. For this purpose, the contents of metals in soil and parts (root, tree, leaf and flowers) of the plants were digested and then analyzed by ICP-MS. Obtained results showed that studied soil and plants are enriched with thallium and arsenic. The contents of these elements in soil exceeded the intervention value for soils (The New Dutch list). According to New Dutch list, natural background content of Tl is 1.0 mg/kg, while interventional value is 15 mg/kg. In this work, the total Tl content in the soil from Alšar site is 561 mg/kg. The content of arsenic in unpolluted soils presented in other papers ranges from 4 to 150 mg/kg. Elevated As content, up to 732 mg/kg, is reported for Slovakian soils. Natural background content for As is 29 mg/kg, and intervention value is 55 mg/kg reported by the New Dutch list. In soil from Alšar content of As is 5376 mg/kg. Bioconcentration factor (BCF), bioaccumulation coefficient (BAC) and translocation factor (TF) also were determined. *Linaria* sp. has the highest TF for Tl, Rb and Cs. TF for Tl is: steam/root – 1.39, leaf/root – 7.54, and flower/root – 1.52. TF for Rb and Cs is 1.0 and 4.4 leaf/root, respectively. BAC has the highest value for Tl steam/soil – 10.19, leaf/soil – 55.45 and flower/soil – 11.16. BCF is the highest for Tl root/soil – 7.35. *Moricandia* sp. has TF values > 1, in leaf/root for Cr (1.44), Co (1.01), As (1.23), Ni (1.0), Rb (2.0), Cs (1.1), Ba (1.0) and Cd (1.2), flower/root for Rb (3.8), Cs (4.5), Cu (1.3) and Cd (1.7). *Viola lutea* Huds has TF values > 1 in steam/root for Cu (1.57), Zn (1.5), Tl (1.47), Mo (1.1), Sb (2.42) and Cd (1.3), and in leaf/root for Cr (3.04), Co (3.87), Ni (5.86), Zn (1.23), Pb (7.09), Tl (10.63), As (6.21) and Mo (4.48). *Moricandia* sp. and *Viola lutea* Huds for BAC and BCF have values < 1. Considering the BCF, BAC and TF values for *Linaria* sp., this plant for Tl, Cs and Rb possessed the characteristics of hyperaccumulator. Because *Linaria* sp., *Moricandia* sp. and *Viola lutea* Huds from Alšar locality have high TF values, could be useful for phytostabilization of contaminated soils.

Key words: arsen; thallium; *Linaria* sp.; *Moricandia* sp.; *Viola lutea* Huds; BAC; TF; BCF

INTRODUCTION

Metals are present in the soil in different chemical forms, which influence their reactivity and hence their mobility. The impact of these metals in soils is their possible transfer into water or plants, which is defined by the term "bioavailability". On the other hand, specific properties of plants are very significant in determining the bioavailability of trace elements and are quite variable with changing soil and plant conditions. Different genotypes of the same species uptake different amounts of the trace elements. Many plants that accumulate >1000 or >10000 mg kg^{-1} of trace metals are categorized as metals hyperaccumulators (Ma et al., 2001; Rosselli et al., 2003; Yoon et al., 2006; Sinegani et al., 2007). Accumulation of heavy metals varied greatly among plants species. Uptake of elements by a plant is primarily dependent on the plant species, its inherent controls, and the soil quality. The metal and plant interact in a specific way, which depends on several factors such as soil type, plant, growth conditions and the presence of other ions. (Baker and Brooks, 1989).

At present, there are four standards to judge hyperaccumulators, including the threshold value standard (the metal concentration in dry shoots >1000 mg/kg for As), the bioconcentrations factor standard (the ratio of metal content in a plant to soil is greater than 1.0, sometimes up to 50–100), the translocation factor standard (the ratio of metal concentration in aerial parts of plant to roots is greater than 1.0) and the strong metal-endurance standard (high capability to tolerate the toxicity of metals) (Sun Yuebing, 2007). Therefore, the purpose of this paper was to examine some plants (*Linaria* sp., *Moricandia* sp. and *Viola lutea* Huds) which appears at the Alšar locality – Republic of Macedonia, like potentia hyperaccumulator plants.

Hyperaccumulator plants can play a key role in the fate of the pollutants of contaminated matrices via their root systems.

MATERIAL AND METHODS

Description of the sampling site

Alšar locality is situated in the Vardar zone near the Macedonian–Greek border. From the geological point of view, the surroundings of the Alšar locality consist of several geological formations arranged in five stratigraphic complexes: complex of Precambrian metamorphic rocks; complex of Mezozoic (Triassic-Jurassic) rocks; complex of Upper Cretaceous sediment rocks; complex of Pliocene sediments, pyroclastites and volcanic rocks; complex of Quaternary sediments. The locality Alšar is a unique deposit with a number of minerals of thallium which are sulphates of thallium with arsenic, antimony, iron and mercury. As a result of this mineralization the entire space of a few square kilometers is enriched with arsenic, thallium and antimony. Also there are increased concentrations of these elements in the soils, water and plants.

The mine mineralization has been presented with As-Tl minerals, Fe-sulfides, Sb and Pb sulphates, cinabarite, sulfur and gold (Janković 1997; Boev 1993).

Sample collection and preparation

Soil and plant samples were collected from three sampling sites, where this community is present is the nearby of the village of Majden, on the locations of Alšar. All plant samples were collected in three species, taking a representative sample of root, tree, leaf and flowers and stored in polyethylene bags in the field, and transferred to the laboratory as soon as possible for analysis. Root, tree, leaf and flowers of plants were thoroughly washed with tap water to remove dust and other particles, followed by Mili-Q water. The samples were dried in an oven for 48 h at 80°C, and finally ground to powder for chemical analysis.

Soil and plant analysis

The <500 μm fraction soil samples were used to determine the maximal environmentally available trace element in soil. Soil samples were digested with a mixture of HCl (37%) and HNO_3 (70%), in a ratio of 3:1 (v/v), at room temperature for 16 h and after at 130 °C for 2 h, under reflux conditions (ISO 11466).

Plant samples were digested in 5 ml acid mixture of $HClO_4$ (Merck) (60%) and HNO_3 (Merck) (85%, s.g. 1.42) (15:85 v/v) overnight. Digestion was completed by gradual increase of temperature from 60 to 195 °C (60 °C, 3 h; 100 °C, 1 h; 120 °C, 1 h; 150 °C, 30 min; 175 °C, 30 min; 195 °C, 2.5 h). After cooling, HCl (20%, 2.5 ml) was added, whirl mixed and warmed to 80°C for 30 min. The final volume was brought to 10 ml with double distilled water and re-warmed to 80°C for another 30 min. Samples were filtered through cellulose filter (0.2 μM) (Barazani et al., 2004). Standard materials were included for assurance control.

The soil and plant digested solutions were cooled to room temperature, filtered, transferred quantitatively to 50 and 25 ml volumetric flasks, respectively, made up to volume with distilled water, and kept in clean plastic vials before metal analysis. Trace metal content in soil and plant extracts were determined by atomic emission spectrometry with ICP-MS (Inductive Coupled Plasma Mass Spectrometry).

All chemicals were declared proanalysis, and all solutions were prepared with double-distilled water. Standard working solutions were prepared from original certified stock solutions (MERCK), concentration 1000 mg/l in 1% (v/v) HNO_3. All samples were analyzed at the Analytical laboratory of the University "Goce Delčev" in Štip. The quality control procedure consisted of reagent blanks, replicated samples and certified standard material (PS-3, COOMET № 0001-1999 BG, COD № 310a 98). (Zapranova et al., 2006) The average values and standard deviations (SD) were calculated using Microsoft Office Excel 2003 in a computer.

RESULTS AND DISCUSSION

According to the New Dutch list (New Dutch list, 2012), natural background content of Tl is 1.0 mg/kg, while interventional value is 15 mg/kg. The total content of Tl in uncontaminated soils from different countries is reported as follows: <0.04–0.52 mg/kg in Sweden (Erikson. 2001); 0.01–0.41 in Poland (Lukaszewicz et al., 1996); 0.39–0.59 in Japan (Takeda et al., 2004). In this work, the total Tl content in the soil from Alšar site is 561 mg/kg. The content of arsenic in unpolluted soils presented in other papers ranges from 4 to 150 mg/kg (Wang and Mulligan, 2006). Elevated As content, up to 732 mg/kg, is reported for Slovakian soils from some locations (Čurlík and Šefčík, 1999). Natural background content for As is 29 mg/kg, and intervention value is 55 mg/kg reported by the New Dutch list (Figure 1). In soil from Alšar content of As is 5376 mg/kg (Table 1).

Table 1

Content of metals and metalloids in soil collected from Alšar locality

Mean content ±SD, $n = 3$			
M (soil)	mg/kg	M (soil)	mg/kg
Na	7175 ± 123	Co	15 ± 2.1
Mg	26650 ± 152	Ni	41 ± 7.8
Al	54577 ± 165	Cu	28 ± 1.7
P	1354 ± 87	Zn	57 ± 4.4
K	21714 ± 221	As	5376 ± 114
Ca	39562 ± 188	Pb	15 ± 6.1
Ti	2629 ± 76	Rb	95 ± 14
Mn	1813 ± 45	Sr	223 ± 16
Fe	28626 ± 112	Mo	10.2 ± 4.0
Cr	55 ± 12	Sb	8.3 ± 2.7
Ba	583 ± 43	Cs	34.1 ± 5.8
Tl	561 ± 49	Cd	1.0 ± 0.7
V	57 ± 8.0	Sn	9.0 ± 2.4

The average natural level of zinc in the earth's crust is 70 mg/kg (dry weight), ranging between 10 and 300 mg/kg (Singh, 2005). Elevated Zn content of natural sources is at the range of 126–683 mg/kg (Davies et al., 2003). The content, of Zn in non-contaminated soils reported for several European countries varies between 7 and 89 mg/kg (Kabata-Pendias, 2001). In this work, the total Zn content in the soil from Alšar locality is 57.13 mg/kg.

The copper content of world soils ranges from 2 to 100 mg/kg with an average value of about 30 mg/kg. Most of this is in unavailable mineral form. According the New Dutch list, natural background content of copper in nature is 36 mg/kg, while interventional value is 190 mg/kg (Figure 1). Normal copper concentration in plants is within the range from 2 to 50 mg/kg (Kabata Pendias, 2011). In the sampling point of Alšar locality, the total Cu content is 28.1 mg/kg (Table 1).

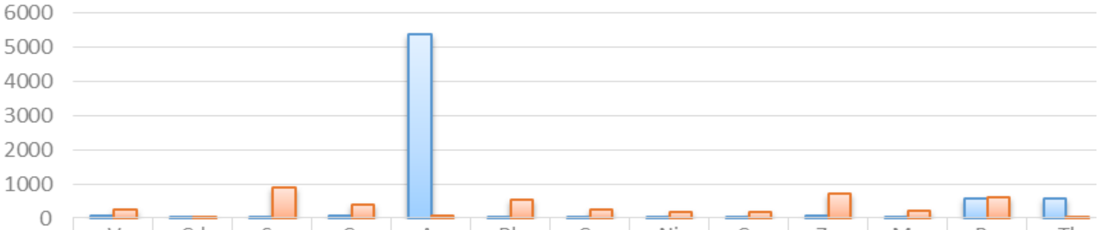
The reported Ba average range for soils on the world scale is from 362 to 580 mg/kg (Faucon et al., 2007). Monitoring on 218 surface-soil samples from Poland gave the Ba range of 20–130 mg/kg (Terelak et al., 2002). Swedish soils contain Ba in the range from 383 to 778 mg/kg (Erikson, 2001). In soil from Alšar site content of Ba is 583 mg/kg (Table 1).

Rubidium content in soils is largely inherited from the parent rocks, as is indicated by the highest mean Rb content, 100–120 mg/kg, in soils over granites and gneisses. The lowest contents of Rb, 30–50 mg/kg, are in sandy and organic soils, 1–10 mg/kg (Market and Lieth, 1987). In soil from Alšar side the mean content of Rb is 95 mg/kg.

The Co content in soils is inherited mainly from parent materials. The worldwide mean value of Co in surface soils is calculated as 10 mg/kg. Higher Co contents in surface soils are found in arid and semiarid regions; for example, Egyptian soils contain Co from 16.5 to 26.8 mg/kg (Nassem and Abdalla, 2003). Swedish arable soils contain Co in the range of 0.4–14 mg/kg, at the average value of 7.1 mg/kg (Kabata-Pendias, 2011).

Naturally high Co contents are observed in soils over serpentine rocks, up to 520 mg/kg, and in soils around ore deposits, up to 85 mg/kg (Kabata-Pendias, 2011). Content of Co in some parts of the Alšar locality is 15 mg/kg (Table 1).

Content of Ni in soil from Alšar is 41 mg/kg. The natural background content of Ni in soil according to the New Dutch list is 35 mg/kg. Content of Pb in soil from Alšar is 15 mg/kg, and well below natural background content is 85 mg/kg. The mean values for V, Cd, Sn, Cr, As, Pb, Co, Ni, Cu, Zn, Mo, Ba and Tl in soils (CS) in part of the Alšar site are shown on Figure 1.



	V	Cd	Sn	Cr	As	Pb	Co	Ni	Cu	Zn	Mo	Ba	Tl
CS	57	1	9	55	5376	15	15	41	28	57	10	583	561
IV	250	12	900	380	55	530	240	190	190	720	200	625	15

Fig. 1. Mean values for V, Cd, Sn, Cr, As, Pb, Co, Ni, Cu, Zn, Mo, Ba and Tl in soils (CS) in part of the Alšar locality (ICP-MS method, in mg/kg), and intervention values (IV) in agricultural soils in some European countries (The New Dutch list)

Distribution of metals and metalloids in different parts of the plants Linaria sp., Moricandia sp. and Viola lutea Huds from the Alšar locality

The uptake of metals from the soil depends on different factors such as their soluble content, soil pH, plant growth stages, types of species, etc. (Mench et al., 1994; Hammer and Keller, 2002; Marschner, 2002).

Different plant parts accumulate different levels of these metals. For example, higher metal concentrations were observed in the edible and inedible parts of vegetable species as reported by many authors (Overesch et al., 2007; Alloway, 2012; Ismail et al., 2014).

The specific properties of plants are very significant in determining the bioavailability of trace elements and are quite variable with changing soil and plant conditions.

Images from investigated plants (*Linaria* sp., *Moricandia* sp. and *Viola lutea* Huds) are given in Figs. 2, 3 and 4, respectively.

Fig. 2. *Linaria* sp. (K1) from Alšar locality

Fig. 3. *Moricandia* sp. (K2) from Alšar locality

Fig.4. *Viola lutea* Huds (K3) from Alšar locality

The total contents of the analyzed elements in the soil were analyzed in order to characterize possible soil pollution and to determine the transfer efficiency from the soil.

The contents of different elements in plants are highly associated with the chemical composition of growth media and plant species. Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport and crosses the plasma membrane of root epidermal cells. Under normal growing conditions, plants can potentially accumulate some metal ions in order of magnitude greater than the surrounding medium (Kim et al., 2003; Jung and Thornton 1996).

The total contents and distribution of metals and metalloids accumulated in investigation plants: *Linaria* sp, *Moricandia* sp. and *Viola lutea* Huds, are shown in Table 2.

Correlations between the elements contents in the investigated plants were established using bivariate statistics. The significant correlations are marked in bold in Tables 3 and 4.

T a b l e 2

The presence of metals and metalloids (mg/kg) in root, stem, leaf and flower of the plants Linaria sp. (K1), Moricandia sp. (K2) and Viola lutea Huds (K3) from Alšar locality

	Mean content ±SD, *n* = 3											
	K1 root	K1 stem	K1 leaf	K1 flower	K2 root	K2 stem	K2 leaf	K2 flower	K3 root	K3 stem	K3 leaf	K3 flower
Na	101	62.0	66.0	49.0	92.0	53.1	82.1	54.1	21.0	23.1	33.0	29.0
	± 32	± 21	± 19	± 12	± 18	± 13	± 15	± 13	± 75	± 5.0	± 6.0	± 5.0
Mg	2875	1947	3365	1367	585	336	1096	1045	291	603	1494	700
	± 65	± 58	± 76	± 79	± 46	± 28	± 54	± 38	± 21	± 32	± 38	± 43
Al	3246	376	507	308	637	163	1104	213	196	67	760	155
	± 57	± 24	± 36	± 38	± 42	± 28	± 51	± 28	± 18	± 15	± 31	± 21
P	882	1215	957	1229	730	1238	1865	1817	478	1084	945	1592
	± 44	± 65	± 33	± 45	± 23	± 45	± 46	± 32	± 23	± 65	± 28	± 76
K	20518	25522	13346	10581	17510	27210	24214	27388	11916	36576	17909	21487
	± 421	± 555	± 267	± 286	± 411	± 167	± 176	± 211	± 322	± 387	± 432	± 312
Ca	18540	9439	13769	3731	8791	4305	18934	5716	2764	3254	13641	2584
	± 512	± 321	± 454	± 366	± 657	± 132	± 566	± 654	± 165	± 122	± 321	± 176
Ti	79.0	36.0	53.0	15.0	36.1	17.1	77.0	23.0	12.0	13.0	55.0	11.0
	± 16	± 12	± 13	± 5.0	± 11	± 7.0	± 16	± 6.0	± 4.0	± 4.0	± 14.0	± 3.0
V	2.26	0.3	0.46	0.26	2.46	0.1	2.27	0.17	0.21	0.06	1.78	0.17
	± 0.44	± 0.03	± 0.05	± 0.03	± 0.04	± 0.7	± 0.7	± 0.04	± 0.06	± 0.01	± 0.05	± 0.04
Sn	0.14	0.11	0.14	0.08	0.06	0.08	0.07	0.06	0.06	0.11	0.08	0.08
	± 0.03	± 0.03	± 0.02	± 0.02	± 0.03	± 0.02	± 0.02	± 0.01	± 0.01	± 0.01	± 0.02	± 0.02
Cr	2.48	0.76	0.71	0.47	0.72	0.25	1.0	0.37	0.27	0.15	0.83	0.29
	± 0.34	± 0.21	± 0.12	± 0.11	± 0.07	± 0.3	± 0.1	± 0.2	± 0.1	± 0.2	± 0.2	± 0.1
Co	0.71	0.11	0.22	0.09	0.29	0.06	0.3	0.07	0.13	0.05	0.52	0.12
	± 0.2	± 0.02	± 0.03	± 0.01	± 0.03	± 0.01	± 0.1	± 0.02	± 0.0	± 0.01	± 0.1	± 0.02
Ni	8.0	2.0	2.0	1.0	2.0	0.7	2.0	0.89	0.85	0.77	5.0	2.0
	± 2.5	± 0.5	± 0.6	± 0.2	± 0.6	± 0.4	± 0.4	± 0.14	± 0.21	± 0.22	± 2.1	± 0.8
Cu	3.0	2.0	2.0	2.0	3.0	3.0	1.0	4.0	0.44	0.7	0.32	2.0
	± 0.6	± 0.4	± 0.4	± 0.3	± 0.3	± 0.6	± 0.3	± 1.4	± 0.2	± 0.4	± 0.06	± 0.04
Zn	13.0	8.0	10.0	5.0	8.0	4.0	7.0	7.0	2.0	3.0	3.0	10.0
	± 2.1	± 1.6	± 3.2	± 0.6	± 1.6	± 2.1	± 1.6	± 1.7	± 0.4	± 0.3	± 0.3	± 2.1
As	471	30.0	75.0	44.0	190	29.0	219	33.013	70.0	17.0	433	62.0
	± 36	± 14	± 32	± 12	± 24	± 13	± 32	±	± 16	± 4.6	± 23	± 12
Pb	0.87	0.22	0.36	0.21	0.95	0.13	0.89	0.11	0.13	0.08	0.95	0.16
	± 0.04	± 0.01	± 0.04	± 0.01	± 0.02	± 0.01	± 0.02	± 0.01	± 0.01	± 0.01	± 0.02	± 0.01
Rb	73	110	75.0	83.0	47.0	82.0	94.0	178	24.0	35.0	28.0	34.0
	± 13	± 32	± 16	± 20	± 15	± 25	± 21	± 32	± 9.0	± 8.0	± 5.0	± 6.0
Sr	15	7.0	7.0	1.0	16.0	4.0	11.0	2.0	11.0	12.0	27.0	3.00.7
	± 2.0	± 2.0	± 2.0	± 0.2	± 2.4	± 0.6	± 2.3	± 0.4	± 1.2	± 1.2	± 2.1	±
Sb	0.33	0.04	0.07	0.05	0.1	0.03	0.08	0.03	0.07	0.17	0.05	0.26
	± 0.04	± 0.01	± 0.01	± 0.01	± 0.02	± 0.01	± 0.02	± 0.01	± 0.02	± 0.04	± 0.01	± 0.02
Cs	5.0	2.0	22.0	5.0	2.0	0.22	2.0	9.0	12.0	14.0	3.0	8.0
	± 0.8	± 0.2	± 2.5	± 0.6	± 0.2	± 0.03	± 0.4	± 0.7	± 0.6	± 0.6	± 0.4	± 1.2
Ba	109	54.0	17.0	3.0	60	65	60	12.0	6.0	3.0	8.0	0.5
	± 21	± 12	± 2.9	± 0.7	± 17	± 21	± 18	± 2.0	± 1.0	± 0.6	± 1.2	± 0.06
Tl	4127	5718	31122	6265	184	52	91	146	185	272	1967	99.0
	± 64	± 121	± 413	± 254	± 35	± 12	± 21	± 32	± 33	± 43	± 65	± 21
Cd	2.0	0.13	0.38	1.0	0.06	1.0	0.7	0.1	0.07	0.09	0.07	0.04
	± 0.4	± 0.02	± 0.03	± 0.02	± 0.01	± 0.1	± 0.1	± 0.01	± 0.01	± 0.02	± 0.02	± 0.01

Table 3

Matrix of correlation coefficients between the elements contents in the investigated plants from Alšar locality

	Li	Be	B	Na	Mg	Al	Ca	Ti	V	Cr
Li	**1.000**									
Be	**0.986**	**1.000**								
B	0.560	0.463	**1.000**							
Na	**0.698**	**0.720**	**0.653**	**1.000**						
Mg	**0.613**	0.512	**0.692**	0.480	**1.000**					
Al	**0.995**	**0.989**	0.535	**0.690**	0.559	**1.000**				
Ca	**0.754**	**0.719**	0.599	**0.713**	**0.659**	**0.750**	**1.000**			
Ti	**0.784**	**0.755**	0.593	**0.721**	**0.642**	**0.784**	**0.998**	**1.000**		
V	**0.669**	**0.738**	0.199	**0.714**	0.208	**0.689**	**0.773**	**0.794**	**1.000**	
Cr	**0.971**	**0.953**	**0.649**	**0.759**	**0.612**	**0.974**	**0.855**	**0.881**	**0.743**	**1.000**
Mn	**0.823**	**0.830**	0.482	**0.772**	0.555	**0.827**	**0.951**	**0.963**	**0.898**	**0.896**
Fe	**0.975**	**0.983**	0.447	**0.695**	0.548	**0.981**	**0.803**	**0.834**	**0.791**	**0.966**
Co	**0.876**	**0.892**	0.249	0.536	0.514	**0.883**	**0.790**	**0.816**	**0.811**	**0.870**
Ni	**0.897**	**0.878**	0.374	0.446	0.610	**0.895**	0.686	**0.714**	0.594	**0.862**
Cu	0.211	0.230	0.431	**0.645**	0.274	0.203	0.075	0.074	0.057	0.203
Zn	**0.643**	0.614	**0.728**	**0.684**	**0.654**	0.625	0.502	0.512	0.303	0.653
As	**0.791**	**0.819**	0.128	0.444	0.379	**0.811**	**0.745**	**0.772**	**0.820**	**0.799**
Tl	0.102	0.008	0.387	0.179	0.780	0.032	0.295	0.256	–0.136	0.087
Pb	**0.632**	0.687	0.158	0.637	0.278	0.646	**0.800**	**0.814**	0.978	0.709

Table 4

Matrix of correlation coefficients between the elements contents in the investigated plants from Alšar locality

	Mn	Fe	Co	Ni	Cu	Zn	As	Tl	Pb
Mn	**1.000**								
Fe	**0.891**	**1.000**							
Co	**0.874**	**0.951**	**1.000**						
Ni	**0.726**	**0.915**	**0.931**	**1.000**					
Cu	0.105	0.150	–0.044	0.010	**1.000**				
Zn	0.513	0.574	0.417	0.545	0.593	**1.000**			
As	**0.829**	**0.897**	**0.979**	**0.888**	–0.131	0.276	**1.000**		
Tl	0.188	0.038	0.049	0.076	0.176	0.351	–0.086	**1.000**	
Pb	**0.898**	0.771	0.843	0.628	–0.025	0.253	**0.863**	–0.043	**1.000**

Tables 3 and 4 show significant correlations between: a) V, Ti, Cr, Mn, Fe, Co, Ni; b) Li, Be, Na, Al, Ca. Correlation coefficients between other elements are not siginificant. Basic statistics for elements contents in the plants *Linaria* sp. (K1), *Moricandia* sp. (K2) and *Viola lutea* Huds (K3) from Alšar locality are given in Table 5.

T a b l e 5

Basic statistics for elements contents in the plants Linaria sp. (K1), Moricandia sp. (K2) and Viola lutea Huds (K3) from the Alšar locality (contents are given in mg kg^{-1} of dried matter)

	Mean	Median	Range	Minimum	Maximum
Li (lithium)	0.047	0.025	0.272	0.0003	0.272
Be (beryllium)	0.018	0.006	0.098	0.0003	0.098
B (boron)	780	695	755	432	1187
Na (natrium)	55	53	80	21	101
Mg (magnesium)	1309	1071	3074	291	3365
Al (aluminium)	644	342	3179	67	3246
P (phosphorus)	1169	1150	1387	478	1865
K (potassium)	21181	21003	25995	10581	36576
Ca (calcium)	8789	7254	16350	2584	18934
Ti (titanium)	36	29	68	11	79
V (vanadium)	0,88	0,28	2,407	0,058	2,46
Cr (chromium)	0.723	0.593	2.321	0.156	2.477
Mn (manganese)	39,06	23,79	82	10	92
Fe (iron)	284	133	1144	33	1178
Co (cobalt)	0.224	0.129	0.652	0.0554	0.707
Ni (nickel)	2.345	1.799	7.556	0.702	8.258
Cu (copper)	1.895	2.234	3.209	0.318	3.526
Zn (zinc)	6,46	6,972	10	2,181	13
Ga (gallium)	1.355	0.539	3.798	0.248	4.046
Ge (germanium)	0.024	0.013	0.084	0.003	0.088
As (arsenic)	140	66.0	454	17.3	471
Se (selenium)	0.009	0.008	0.02	0.002	0.018
Rb (rubidium)	72.0	73.8	154	23.8	178
Sr (strontium)	9.54	8.61	25.18	1.36	26.54
Mo (molybdenum)	0.428	0.325	0.764	0.164	0.928
Pd (palladium)	0.092	0.019	0.611	0.012	0.623
Ag (silver)	1.72	0.007	20.5	0.002	20.5
Cd (cadmium)	0.4	0.094	1.612	0.042	1.655
Sn (tin)	0.091	0.08	0.087	0.058	0.145
Sb (antimony)	0.107	0.071	0.301	0.028	0.329
Cs (caesium)	12.04	6.45	63	0.227	3
Ba (barium)	32.96	14.72	108	0.507	109
Tl (thallium)	4186	228	31069	52	31121
Pb (lead)	0.423	0.212	0.874	0.081	0.954
Bi (bismuth)	0.009	0.002	0.097	-0.005	0.093
Th (tehnicium)	0.077	0.02	0.494	0.005	0.499
U (uranium)	0.006	0.003	0.016	0.001	0.018

Unlike the macroelements whose distribution depends on the type of the plant, trace elements mainly are concentrated in the leaves and root of plants in all the investigated species of herbs. The contents of Cr, Co, Ni, Pb, Sr, Ba and Cd in selected plants did not differ significantly among the different species of plants (Table 2). In terms of metals distribution in the plants *Linaria* sp. and *Moricandia* sp. average contents of these elements decrease in the order: root > steam > leaf > flower, with the exception of the plant *Viola lutea* Huds where the contents of these elements in the leaf were higher than those in the root (Figure 5).

The highest content of As is measured in roots of *Linaria* sp. (471 mg/kg) and *Moricandia* sp. (190 mg/kg) and in leaf of *Viola lutea* Huds (433 mg/kg). The contents of Rb, Cs and As in plants differ for selected species and for parts of plants. Increased levels of Rb are reported for plants from industrial regions, 3–150 mg/kg (Kosla et al., 2001). Cs distribution in plants is similar to K, which suggests that this metal can compete with K uptake and K binding sites in cells. On the basis of a few compiled data, Cs common range in various plants can be presented as < 0.1–3 mg/kg (Isaure et al., 2006). In this work, the highest content of Cs and Rb is measured in leaf of *Viola lutea* Huds (22 mg/kg and 75 mg/kg) and in flower of *Moricandia* sp. (9 mg/kg and 178 mg/kg respectively).

Distribution of chemical elements in soils and stream sediments in the area of Alšar mine was determinated by Bačeva et al., 2014. The Tl content in plants seems to be a function of Tl concentrations in soils. There is an assumption that some plants, especially of the Cruciferae and Gramineae families, can serve as hyperaccumulators for phytoremediation of contaminated soils. McGrath (1998) reported that there are programs for screening plants (e.g., *Brassica* sp.) for their ability to phytoextract Tl in plants from soils impacted by a cement factory. The content of Tl in plants from the Alšar area is very high (31.122 mg/kg), especially in *Linaria* sp. It decrease in order: leaf > flower > steam > root for *Linaria* sp., root > flower > leaf > stem for *Moricandia* sp., and leaf > stem > root > flower for *Viola lutea* Huds. The highest content of Tl is measured in all parts of *Linaria* sp.

The average contents of Zn decrease in the order: root > leaf > stem > flower for *Linaria* sp., root > leaf > flower > stem for *Moricandia* sp., and flower > stem > leaf > root for *Viola lutea* Huds. Zinc contents in plants vary considerably, reflecting the different factors of various ecosystems and of the genotypes (Kabata-Pendias, 2001).

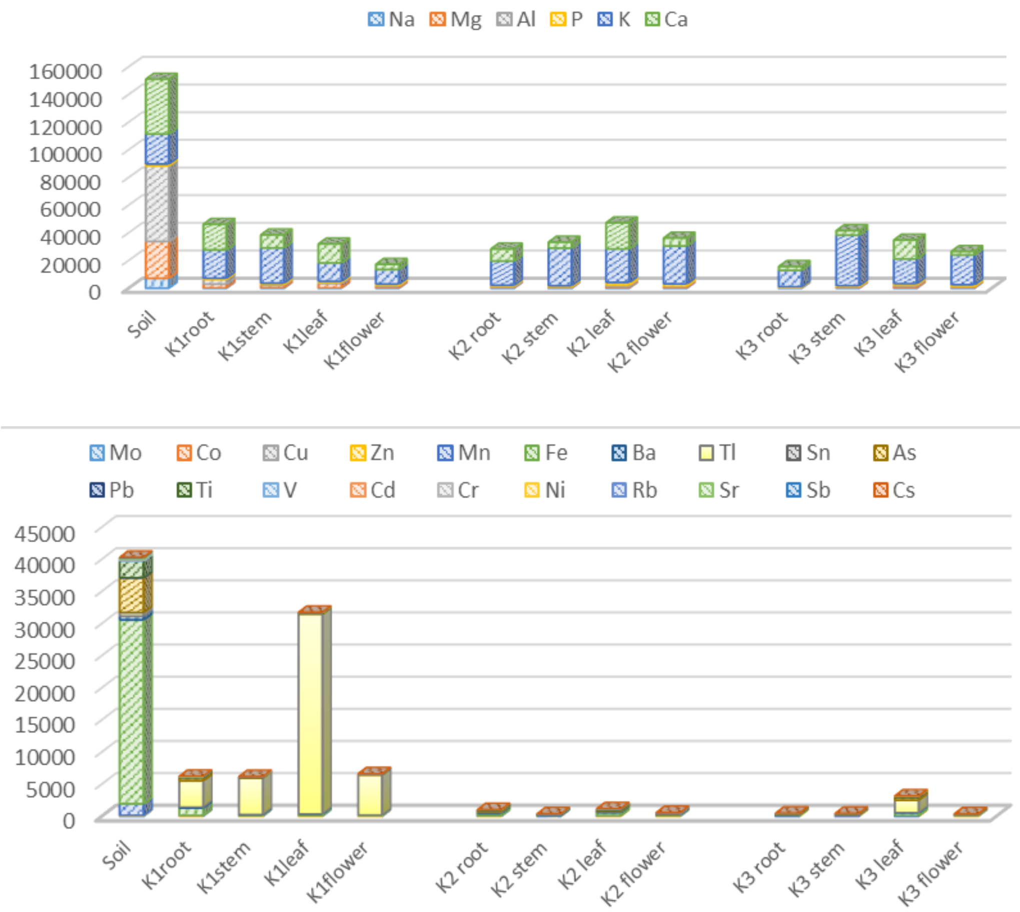


Fig. 5. Content of metals and metalloids in soil and parts of the plant (root, stem, leaf and flower) in investigated plants *Linaria* sp. (K1), *Moricandia* sp. (K2) and *Viola lutea* Huds (K3) from the Alšar locality

In plants from Alšar, the content of zinc is equally distributed between the organs of the plants. Translocation factor (TF), bioaccumulation coefficient and bioconcentration factor were described. The translocation factor was described as ratio of heavy metals in aerial part of the plants to that in plant root (equation 1) (Cui et al.,, 2007; Li et al., 2007). Plant responses to TF in soils depend on several factors, however there are some general trends, expressed by plant/soil. Translocation factor (TF) can be presented as generalized values: 10 (Cd); 1 (B, Br, Cs, Rb); 10^{-1} (Ag, Co, Cu, Ge, Hg, Mo, Pb, Sr, Te, Zn); 10^{-2} (Be, As, Li, F, I, Mn, Ni, Sb); 10^{-3} (Ba, Bi, Ga, Fe, Se, V, Tl, Zr) (Kabata-Pendias, 2001). Translocation factor was calculated by:

$$TF = \text{metals (aerial part)} / \text{metals (roots)}, \quad (1)$$

where metals (aerial part) and metals (roots) are metals contents in the aerial part of the plant ($mgkg^{-1}$) and in the root of the plant ($mgkg^{-1}$), respectively. TF > 1 represents that translocation of metals effectively was made to the shoot from root (Malik et al., 2010; Moffat, 1995; Yoon et al., 2006).

Biological accumulation coefficient (BAC) was calculated as ratio of heavy metals in aerial part of the plants to that in the soil (equation 2) (Cui et al., 2007; Li et al., 2007):

$$BAC = \text{metals (aerial part)} / \text{metals (soil)}. \quad (2)$$

Metals (aerial part) and metals (soil) are metals contents in the aerial part of the plant ($mgkg^{-1}$) and in the soil ($mgkg^{-1}$), respectively. BAC >1 indicates that the plant species has the ability to store metals from the soil into the aerial part of the plant (Baker and Brooks, 1989; Baker et al., 2000).

A plant's ability to accumulate metals from soils can be estimated using the BCF (biological concentration factor), which is defined as the ratio of metals content in the roots of plant to that in the soil. It is calculated as follows (equation 3) (Yoon et al., 2006):

BCF = metals (roots) / metals (soil). (3)

BCF, BAC and TF values >1 had been used to evaluate the potential of plant species for phyto-extraction and phytostabilization of metals in soil. By comparing BCF, BAC and TF we can compare the ability of different plants in taking up metals from soils and translocating them to the aerial part of the plant. Bioaccumulation coefficient (BAC), concentration factor (BCF) and translocation factor (TF) of Cr, Co, Ni, Cu, Zn, As, Pb, Rb, Cs, Sr, Sb, Ba, Tl and Cd in K1 (*Linaria* sp.), K2 (*Moricandia* sp.) and K3 (*Viola lutea* Huds) are given in Tables 6, 7 and 8 such as in Figures 6, 7 and 8, respectively.

Bioaccumulation of heavy metals in two endemic *Viola* species (*Viola allcharensis* G. Beck, *Viola arsenica* G. Beck) and one Balkan endemic species (*Viola macedonica* Boiss. & Heldr.) from the soil in the vicinity of the As-Sb-Tl mine Alšar, Republic of Macedonia, was determined by Bačeva et al., 2014. It was found that the accumulation of As, Sb, and Tl in these endemic species is significantly high.

Table 6

TF, BAC and BCF for Linaria sp. from the Alšar locality

	TF Translocation factor			BAC Bioaccumulation coefficient			BCF Bioconcentration factor
	steam/root	leaf/root	flower/root	steam/soil	leaf/soil	flower/soil	root/soil
Cr	0.307	0.288	0.191	0.014	0.013	0.009	0.045
Mn	0.295	0.585	0.212	0.015	0.030	0.011	0.051
Fe	0.121	0.215	0.104	0.005	0.009	0.004	0.041
Co	0.156	0.315	0.132	0.007	0.015	0.006	0.047
Ni	0.270	0.265	0.155	0.054	0.053	0.031	0.202
Cu	0.793	0.848	0.873	0.077	0.082	0.085	0.097
Zn	0.595	0.741	0.402	0.133	0.166	0.090	0.224
Pb	0.247	0.416	0.239	0.014	0.024	0.014	0.058
Tl	**1.386**	**7.542**	**1.518**	**10.189**	**55.453**	**11.163**	**7.353**
As	0.063	0.160	0.094	0.005	0.014	0.008	0.088
Mo	0.373	**1.228**	0.270	0.029	0.095	0.021	0.077
Sb	0.125	0.219	0.159	0.005	0.005	0.007	0.043
Rb	**1.513**	**1.021**	**1.138**	**1.160**	0.784	0.872	0.768
Cs	0.465	**4.358**	0.951	0.070	0.656	0.146	0.151
Sr	0.477	0.436	0.091	0.030	0.029	0.006	0.067
Cd	0.079	0.229	0.632	0.232	0.673	**1.858**	**2.939**

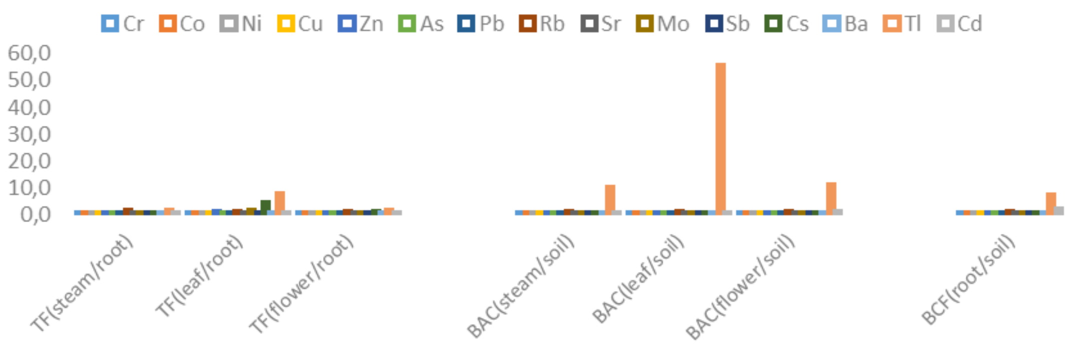


Fig. 6. TF, BAC and BCF for *Linaria* sp. from the Alšar locality

Table 7

TF, BAC and BCF for Moricandia sp. from the Alšar locality

	TF Translocation factor			BAC Bioaccumulation coefficient			BCF Bioconcentration factor
	steam/root	leaf/root	flower/root	steam/soil	leaf/soil	flower/soil	root/soil
Cr	0.341	**1.883**	0.503	0.005	0.025	0.007	0.013
Mn	0.281	**1.424**	0.358	0.009	0.045	0.011	0.031
Fe	0.191	**1.227**	0.257	0.003	0.016	0.003	0.013
Co	0.188	**1.017**	0.240	0.004	0.020	0.005	0.020
Ni	0.445	**1.282**	0.564	0.017	0.049	0.022	0.039
Cu	0.846	0.466	**1.216**	0.087	0.048	0.125	0.103
Zn	0.629	**1.060**	**1.076**	0.075	0.126	0.128	0.118
Pb	0.141	0.944	0.120	0.009	0.060	0.008	0.063
Tl	0.284	0.497	0.792	0.093	0.041	0.260	0.328
As	0.150	**1.151**	0.176	0.005	0.163	0.006	0.035
Mo	0.577	**1.798**	0.936	0.023	0.072	0.038	0.040
Sb	0.296	0.849	0.280	0.004	0.011	0.004	0.013
Rb	**1.736**	**1.984**	**3.759**	0.864	0.988	**1.871**	0.499
Cs	0.142	**1.259**	**5.885**	0.007	0.059	0.278	0.047
Sr	0.226	0.640	0.113	0.017	0.047	0.008	0.074
Cd	**19.189**	**1.189**	**1.774**	**1.948**	0.121	0.180	0.102

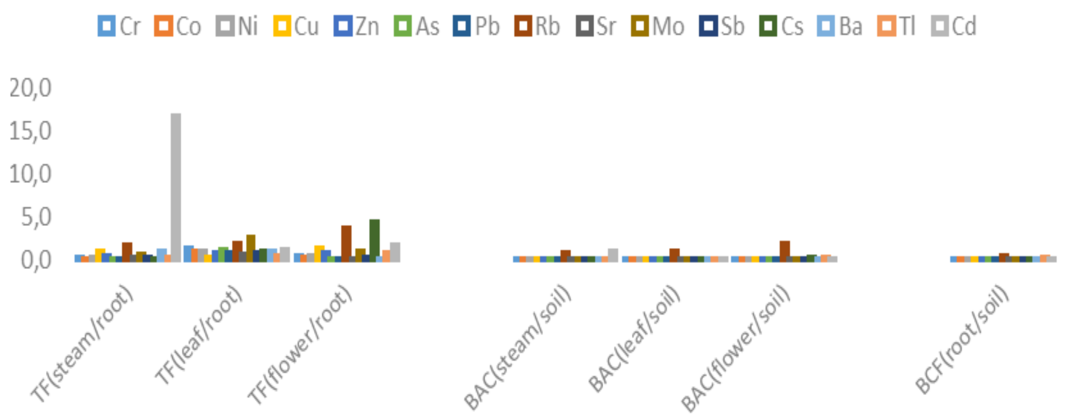


Fig 7. TF, BAC and BCF for *Moricandia* sp. from the Alšar locality

T a b l e 8

TF, BAC and BCF for Viola lutea Huds from the Alšar locality

	TF Translocation factor			BAC Bioaccumulation coefficient			BCF Bioconcentration factor
	steam/root	leaf/root	flower/root	steam/soil	leaf/soil	flower/soil	root/soil
Cr	0.574	**3.038**	**1.076**	0.003	0.015	0.005	0.005
Mn	0.517	**2.977**	0.747	0.006	0.032	0.008	0.011
Fe	0.352	**5.003**	0.974	0.001	0.017	0.003	0.003
Co	0.429	**3.871**	0.924	0.004	0.035	0.008	0.009
Ni	0.912	**5.860**	**2.776**	0.019	0.122	0.058	0.021
Cu	**1.568**	0.715	**3.317**	0.025	0.011	0.052	0.016
Zn	**1.155**	**1.232**	**4.371**	0.044	0.047	0.167	0.038
Pb	0.598	**7.095**	**1.175**	0.005	0.064	0.011	0.009
Tl	**1.468**	**10.629**	0.529	0.484	**3.506**	0.174	0.330
As	0.248	**6.213**	0.893	0.003	0.081	0.012	0.013
Mo	**1.105**	**4.476**	**1.161**	0.019	0.075	0.020	0.017
Sb	**2.421**	0.769	**3.692**	0.022	0.007	0.034	0.009
Rb	**1.482**	**1.187**	**1.412**	0.370	0.296	0.352	0.250
Cs	**1.124**	**5.108**	0.634	0.408	**1.855**	0.230	0.363
Sr	**1.045**	**2.321**	0.216	0.054	0.119	0.011	0.051
Cd	**1.304**	**1.093**	0.643	0.152	0.128	0.075	0.117

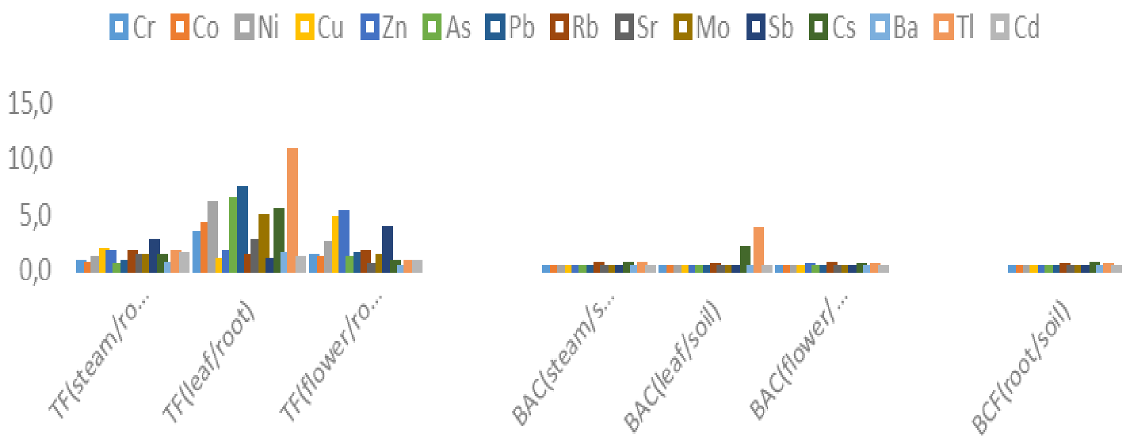


Fig. 8. TF, BAC and BCF for *Viola lutea* Huds from the Alšar locality

The accumulating species are characterized by a ratio of content of metals in the aerial part of the plant to the content in the soil greater than 1 (BAC > 1); species known as "excluder" having a ratio below 1 (Baker and Whiting, 2002). This agrees with Rotkittikhun et al. (2006), who suggested that accumulation occurs when the content of an element in the aerial part of a plant is higher than in the soil or when the content ratio of leaf/soil should be greater than 1 (BAC). This test reveals the ability of a species to accumulate metals. Secondly, the content of an element in the aerial parts of a plant should be 10 to 500 times higher than normal levels, and thirdly, the content ratio leaves/roots must be greater than 1 (TF > 1). The BCF values in the range of 0.1 to 0.50 (as potential phytostabilization) and values from 0.5 to 1.0 (as partial phytostabilization) should not be neglected. The species that show values of TF and especially BCF < 1 are inadequate to the phytoextraction (Fitz and Wenzel, 2002). In this work *Linaria* sp. has the highest TF for Tl. TF for Tl is 1.39 (steam/root), 7.54 (leaf/root) and 1.52 (flower/root). BAC has the highest values for Tl – 10.19 (steam/ soil), 55.45 (leaf/soil) and 11.16 (flower/soil); Rb 1.16 (steam/soil), Cd 1.86 (flower/soil). *Linaria* sp. has the highest BCF values for Tl – 7.35 (root/soil) and Cd (2.94). *Moricandia* sp. has TF values > 1 in steam/root for Rb (1.74) and Cd (19.19); in leaf/root for Cr (1.88), Co (1.02), As (1.15), Ni (1.28), Rb (1.98), Mn (1.42), Fe (1.23), Zn (1.06), Cs (1.26), Mo (1.80), Cd (1.19); in flower/root for Rb (3.76), Cs (5.89), Cu (1.21), Zn (1.08) and Cd (1.77). BAC is >1 for Rb (1.95) (steam soil); Cd (1.87) flower/soil. *Viola lutea* Huds has TF values > 1 in steam/root for Cu (1.57), Zn (1.16), Tl (1.47), Mo (1.11), Sb (2.42), Rb (1.48), Cs (1.12), Sr (1.05) and Cd (1.30); in leaf/root for Cr (3.04), Mn (2.98), Co (3.87), Ni (5.86), Zn (1.23), Pb (7.10), Tl (10.63), As (6.21), Mo (4.48), Rb (1.19), Cs (5.11), Sr (2.32) and Cd (1.09); in flower/root for Cr (1.08), Ni (2.78), Cu (3.32), Zn (4.37), Pb (1.18), Mo (1.16), Sb (3.69) and Rb (1.41).

CONCLUSION

After summarizing the data collected in this research, we can confirm that accumulation of selected metals varied among the plants *Linaria* sp., *Moricandia* sp., *Viola lutea* Huds and uptake of an element by a plant is primarily dependent on the plant species and the soil quality. Significant correlation is between: a) V, Ti, Cr, Mn, Fe, Co, Ni; b) Li, Be, Na, Al, Ca. Correlation coefficient between other elements is not siginificant. *Linaria* sp. (K1) has the highest content of elements in the root, while in *Moricandia* sp. (K2) and *Viola lutea* Huds (K3) the highest content of elements is in leaves. The highest values for bioaccumulation factor were obtained in case of Tl in steam, leaf and flower from *Linaria* sp. and *Moricandia* sp. *Linaria* sp. has the highest TF for Tl, Cs and Rb. *Moricandia* sp. and *Viola lutea* Huds for BAC and BCF have values < 1. Considering the BCF, BAC and TF values for *Linaria* sp. this plant for Tl, Cs and Rb possessed the characteristics of hyperaccumulator. Because *Linaria* sp., *Moricandia* sp. and *Viola lutea* Huds from the Alšar locality have high TF values for As, Tl, Sb, Ni, Cu, Zn, Cs, Rb, Co, Mo, Cr and Pb, could be useful for phytostabilization of soils contaminated with these elements.

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Р е з и м е

БИОАКУМУЛАЦИОНЕН И ТРАНСЛОКАЦИОНЕН ФАКТОР НА ТЕШКИ МЕТАЛИ ВО РАСТЕНИЈАТА *LINARIA* SP., *MORICANDIA* SP. И *VIOLA LUTEA* HUDS ОД ЛОКАЛИТЕТОТ АЛШАР – РЕПУБЛИКА МАКЕДОНИЈА

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Клучни зборови: арсен; талиум; *Linaria* sp.; *Moricandia* sp.; *Viola lutea* Huds; BAC; TF; BCF

Целта на овој труд е да се оцени дистрибуцијата на металите и металоидите во почвата и растенијата *Linaria* sp., *Moricandia* sp. и *Viola lutea* Huds од локалитетот Алшар – Република Македонија. Врз основа на добиените резултати се одредени факторот на биоконцентрација (BCF), коефициентот на биоакумулација (BAC) и факторот на транслокација (TF) во различните делови на растенијата. Содржината на металите во почвата и во деловите на растенијата (корен, стебло, лист и цвет) беше одредена со ICP-MS. Врз основа на добиените резултати беше утврдено дека *Linaria* sp. има највисок TF за Tl, Rb и Cs. TF за Tl е следниот: стебло/корен – 1,39; лист/корен – 7,54 и цвет/корен – 1,52. TF за Rb е 1,0 (лист/ корен) и за Cs е 4,4 (лист/корен). BAC има највисока вредност за Tl (стебло/почва – 10,19, лист/почва – 55,45 и цвет/почва – 11,16). BCF исто така е највисок за Tl (корен/почва – 7,35). *Moricandia* sp. има вредностите на TF > 1 во лист/корен за Cr (1,44), Co (1,01), As (1,23), Ni (1,0), Rb (2,0), Cs (1,1), Ba (1,0) и Cd (1,2), во цвет/корен за Rb (3,8), Cs (4,5), Cu (1,3) и Cd (1,7). *Viola lutea* Huds вредностите на TF има > 1 во стебло/корен за Cu (1,57), Zn (1,5), Tl (1,47), Mo (1,1), Sb (2,42) и Cd (1,3). во лист/корен за Cr (3,04), Co (3,87), Ni (5,86), Zn (1,23), Pb (7,09), Tl (10,63), As (6,21) и Mo (4,48). *Moricandia* sp. и *Viola lutea* Huds за BAC и BCF имаат вредности < 1. Според вредностите на BCF, BAC и TF за *Linaria* sp., ова растение за Tl, Cs и Rb ги поседува карактеристиките на хиперакумулатор. Испитувањата покажаа дека проучуваната почва и растенијата се богати со талиум и арсен. Поради тоа што *Linaria* sp., *Moricandia* sp. и *Viola lutea* Huds, имаат високи вредности на TF, тие би можеле да бидат користени за фитостабилизација на почви контаминирани со овие елементи.