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Original scientific paper

BIOACCUMULATION OF SOME TOXIC ELEMENTS BY ENDEMIC PLANT SPECIES *ONOBRYCHIS DEGENII* DÖRFLER, *KNAUTIA CAROLI-RECHINGERI* MICEV. AND *CENTAUREA KAVADARENSIS* MICEV. FROM ALLCHAR LOCALITY, REPUBLIC OF MACEDONIA

Katerina Bačeva Andonovska[1], Trajče Stafilov[2], Vlado Matevski[1,3]

[1]*Research Center for Environment and Materials, Macedonian Academy of Sciences and Arts, 1000 Skopje, Republic of Macedonia*
[2]*Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, POB 162, Skopje, Republic of Macedonia*
[3]*Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, POB 162, Skopje, Republic of Macedonia*
kbaceva@manu.edu.mk; trajcest@pmf.ukim.mk

A b s t r a c t: Allchar mine, located in the southern part of the Republic of Macedonia, is a unique deposit within the world, due to the variety of its mineral composition especially in the high content of arsenic and thallium. The goal of this investigation was to establish the intensity of accumulation of various elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sr, Tl, V and Zn) with focus on As, Sb and Tl, in three endemic plant species from Allchar locality, *Onobrychis degenii* Dörfler, *Knautia caroli-rechingeri* Micev. and *Centaurea kavadarensis* Micev. Samples of different parts of these plants and corresponding soils were collected, prepared, digested and then analyzed by inductively coupled plasma – atomic emission spectrometry (ICP-AES). From this investigation it was found that the content of As and Tl in soil samples that were taken from the locations where the plants grow was high ranged from 102 mg/kg to 5288 mg/kg and from 4.45 mg/kg to 409 mg/kg, respectively. In comparison to the content of As and Tl in soil samples, the content of Sb was lower ranged from 8.4 mg/kg to 80.7 mg/kg. The results showed that the accumulation of As, Sb and Tl in the investigated endemic species is significant, indicating that they are able to hyperaccumulate toxic elements in their roots and shoots. Accumulation of these elements in the studied endemic species may be used as a valuable tools for bioindication, while, from the other hand, the accumulation of these toxic elements in the plants can cause high risk to the human and animal health.

Key words: accumulation; mobility; antimony; arsenic; thallium; endemic; plant; Allchar; BAF; BTF

INTRODUCTION

Contamination of soils with toxic elements represents a serious threat to environmental quality and human health. Although toxic elements can be incorporated into soils from natural sources (weathering of parent materials, emissions from volcanoes, mining, etc.), most of their inputs have an anthropogenic origin (Alloway, 1995). Soil toxicity has become a global concern in areas with the increasing contamination of soil, water and plants in many regions of the world.

Among the most toxic elements emitted by mining activities are antimony, arsenic and lead. These elements are frequently present together in ore minerals or in mineral associations. The exploittation of such deposits for the economic ore minerals provokes a simultaneous release of such toxic elements into the environment. None of them has a known biological function in animals or plants, being highly toxic, especially to mammals. Inorganic arsenic species are generally highly toxic to plants. In humans arsenic and antimony cause a great variety of adverse health effects, including mutagenic and carcinogenic effects, whereas Pb mainly causes hematological, gastrointestinal and neurological dysfunctions, with children being particularly sensible (Winship, 1987; Lockitch, 1993; Mandal and Suzuki, 2002; Sundar and Chakravarty, 2010). Arsenate acts as a phosphate analog and affects phosphate metabolism, whereas arsenite reacts with sulphydryl groups of enzymes and tissue proteins, leading to inhibition of cellular function and death (Meharg & Hartley-Whitaker, 2002). In As nonhyperaccumulating plants, resistance to environmental As has been shown to involve a decreased uptake of arsenate due to suppression of the high-affinity phosphate uptake system (Meharg & Macnair, 1991, 1992). The study of the environmental status of this kind of scenarios is, therefore, crucial to establish the current and eventual environmental and health impacts and to assess the right measures to be undertaken in order to minimize such impacts.

So far, a number of many studies have been carried out for the extraction and accumulation of heavy metals in many plants (Baroni et al., 2004; Antosiewicz et al., 2004; Chang et al., 2005). The extraction of heavy metals from the soil by the plant still depends on the presence and the solubility of the heavy metals in the soil where the plant grows and develops, the physical-chemical properties of the soil, the type of plants, the age of the plants, and the exposure time of these plants on such soils (Marin et al., 1992).

The main objectives of this work are: i) to carry out the environmental characterization of the soils impacted by the former exploitation of As–Sb–Tl deposit "Allchar", Republic of Macedonia, ii) to study the endemic plants species *Onobrychis degenii* Dörfler, *Knautia caroli-rechingeri* Micev. and *Centaurea kavadarensis* Micev. that spontaneously growing in this area, and iii) to evaluate the toxic elements phytoavailability and the consequent environmental and health implications.

MATERIALS AND METHODS

Study area

The locality of Allchar is volcanogenic hydrothermal deposit and is situated at the northwestern part of the Kožuf Mts. (Republic of Macedonia), close to the border between the Republic of Macedonia and Greece (Figure 1). From the geotectonic point of view, ore mineralization is related to a Pliocene volcano-intrusive complex (Janković et al., 1997; Volkov et al., 2006; Boev and Jelenković, 2012; Amthauer et al., 2012).

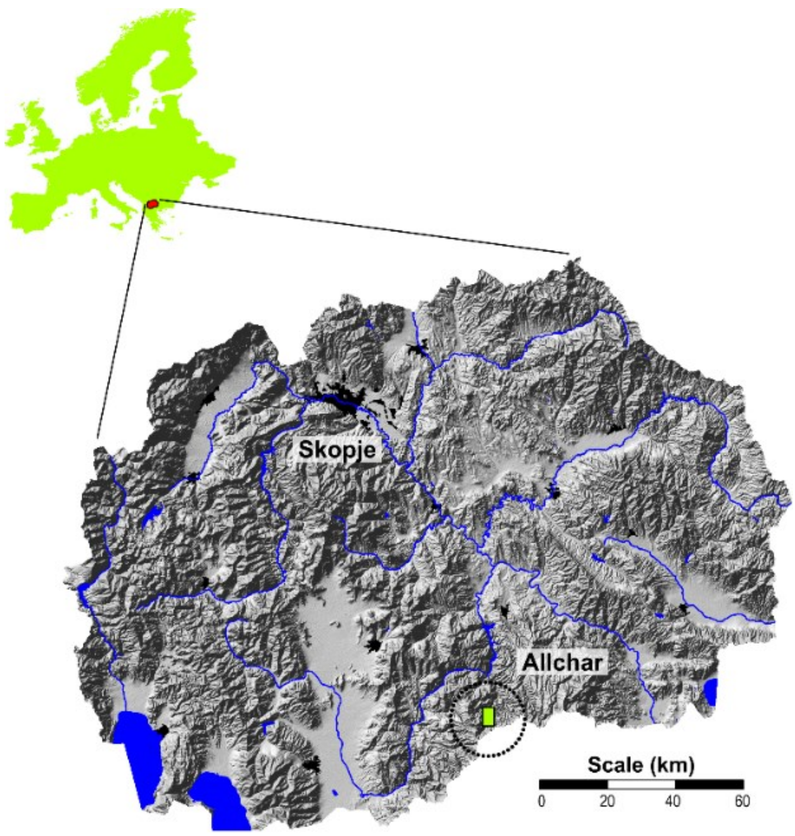

Fig. 1. The location of the Allchar mine

The Allchar locality consist several geological formations: complex of Precambrian metamorphic rocks; complex of Mezozoic rocks; complex of Upper Cretaceous sediment rocks; complex of Pliocene sediments, pyroclastites and volcanic rocks; complex of Quaternary sediments. Several ore bodies occur within a zone of 2 km in length and around 300–500 m wide. The most important ore minerals are Fe-sulphides, As- and Tl-minerals, cinnabarite, and As-, Pb- and Sb-sulphosalts (Balić Žunić et al., 1993; Boev et al., 1993, 2001–2002; Volkov et al., 2006; Jelenković and Boev, 2011). As a result of mineralization the entire area of a few square kilometers is enriched with arsenic, thallium and antimony.

Study species

In addition to our previously investigated endemic species, such as *Viola allchariensis* G. Beck, *Viola arsenica* G. Beck and *Viola macedonica* Boiss. & Heldr. (Bačeva et al., 2014b), *Thymus alsarensis* Ronniger (Bačeva et al., 2016) and *Centaurea leucomalla* Bornm. (Bačeva, 2013), that are grow in the area of Allchar locality, another three endemic plant species: *Onobrychis degenii, Knautia caroli-rechingeri* and *Centaurea kavadarensis* could be found.

Onobrychis degenii (Figure 2) is a significant plant species on the Red List of Threatened Plants of the IUCN (International Union for Conservation of Nature) (Walter & Gillet, 1997), which is of indefinite status. This species grows only at the Allchar locality, near the village Majdan. However, it could be a Macedonian (sub)endemic species, tough in floristic literature is also stated for Bulgaria (Kozhukharov, 1976, 1992).

Fig. 2. *Onobrychis degenii*

Knautia caroli-rechingeri (Figure 3) is a local Macedonian endemic species that is described by Micevski (1981), found only in the vicinity of the village Majdan, Allchar locality, and represents his *Locus classicus*. This plant occupies a very isolated position in relation to other species of the *Knautia* genus that are developed on the territory of the Republic of Macedonia, so that in the taxonomic relation it is closest to the *Knautia dinarica* species. For this time being, this species is known only for two sites located near the village of Majdan.

Fig. 3. *Knautia caroli-rechingeri*

Centaurea kavadarensis (Figure 4) belongs to the *Centaurea deusta* group and it is a local endemic species that can be found in the surroundings of the village Majdan, Allchar (Micevski, 1987).

Fig. 4. *Centaurea kavadarensis*

During the summer of 2011, soil samples and endemic plant specimens of these three endemic plant species were collected in the vicinity of the abandoned Allchar mine (Figure 5). The plant material was collected according to their presence in the area. In each of the locations, 5 to 10 subsoil samples are taken to the part where the plant is located. For the soil samples the possible organic layer was removed.

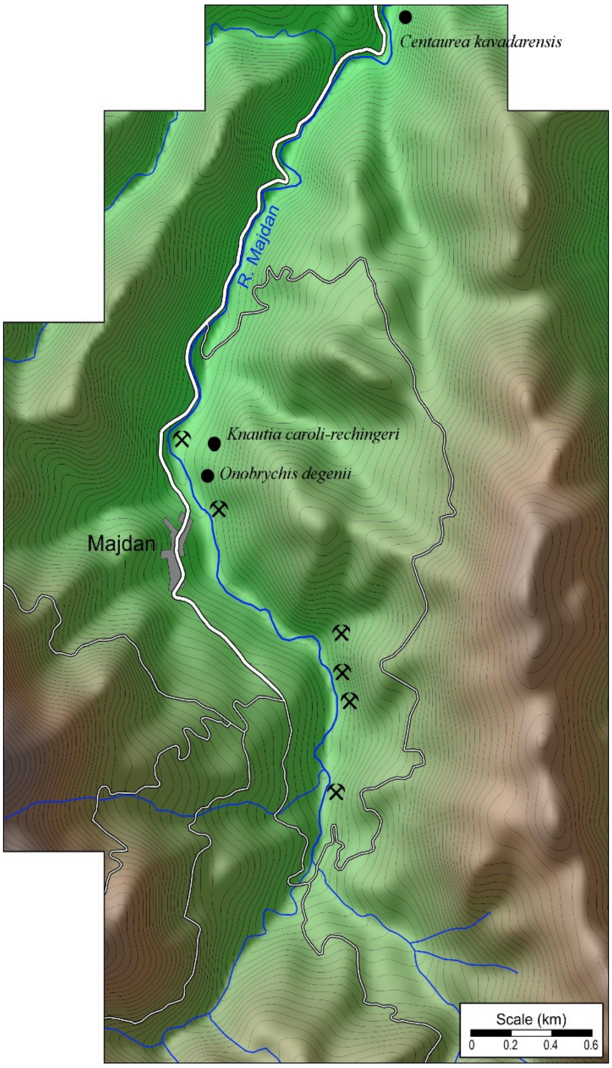

Fig. 5. Sampling locations in the Allchar locality

Cleaning, drying and preparation of soil samples

Collected soil samples were dried at room temperature for several days, and after extraneous material was removed, soil samples were sowing using a plastic sieve with a 2 mm mesh. A certain amount of the sieved material is grinded into an agate mill until a powder having a particle size of 0.125 mm is obtained (Salminen et al., 2005). Such prepared samples were collected in polyethylene bags ready for chemical analysis. Digestion of soil samples was performed by taking exact weighted sample (0.2500 g), placed in a PTFE digestion vessel and digested on a hot plate at about 100˚C. Digestion was performed in three steps. First, HNO_3 was added to remove all organic matter. Then, a mixture of HF and $HClO_4$ was added. To the end, HCl and redistilled water were added to dissolve the residue. The solution was transferred quantitatively into 25 ml volumetric flask and then analyzed.

Cleaning, drying and preparation of plant samples

Plant material was collected according to the presence of the plant of the endemic species (10–20 specimens of plants, represent composite material for one sample for analysis). After collecting the plants, the whole plant was very carefully washed with redistilled water in order to remove the accumulated impurities from the ground, and then split into the root, stem, leaf and flower. The plant material after drying at room temperature for 7–14 days to a constant mass is homogenized and prepared for complete digestion.

A specific mass (0.5000 g) of each plant sample is weighed into PTFE container to which 7 ml of concentrated HNO_3 and 2 ml H_2O_2 (30%, m/V) were added, then the PTFE vessels are placed in a microwave digestion system (CEM, Mars). In the first step of the previously established method, the temperature of 180°C is reached for 15 min and then heated at the same temperature for additional 20 min. Then, the PTFE vessels are removed from the microwave digestion system when the sample temperature is close to room temperature. The solutions obtained after the thermal decomposition of the plant tissues are quantitatively transferred into 25 ml calibrated flasks.

Extractions with various soil extraction plants

To determine the degree of extraction (bioavailability) of the analyzed soil elements, three different extraction solutions were used (Pansu and Gautheyrou, 2006):

– Extraction with water. With this extraction, the natural conditions for extraction of heavy metals from the soil were simulated. To determine which elements can be extracted from the soil if it rains in nature under normal conditions.

– Reagent for extraction of diethylenetriamine pentaacetic acid-calcium chloride-triethanol amine (DTPA-$CaCl_2$-TEA). This extraction solution is very often used to determine the extraction of trace elements or biogenic elements which the plant most often uses for its growth and development.

– Dissolve with low pH value is used to determine potentially accessible forms that are difficult to extract from the soil. In this case, 0.1 M HCl solution was used.

With the simulation of these extraction solutions, we managed to determine the level of available soil elements where plants are being developed.

Instrumentation

All analyzed elements were determined by the application of atomic emission spectrometry with inductively coupled plasma, ICP-AES (Varian, 715-ES) applying an ultrasonic nebulizer CETAC (ICP/U-5000AT^{+}) for better sensitivity. For this study, certified reference materials were used to validate the method for all considered elements and the difference between measured and certified values was satisfied ranging within 15%. Thus, the quality control was ensured by standard moss reference materials M2 and M3 (Steinnes et al., 1997) as well as JSAC 0401 soil reference material. The measured concentrations were in good agreement with the recommended values.

RESULTS AND DISCUSSION

After statistical process of the results for plant specimens of the species *Onobrychis degenii* Dörfler, *Knautia caroli-rechingeri* Micev. and *Centaurea kavadarensis* Micev., and the corresponding soil samples are presented in the Table 1. In Table 1 the contents of the 27 analyzed elements from different parts of the plant (root, stem, leaf and flower) that grow on the Allchar locality, are presented. Endemic species *Onobrychis degenii* and *Knautia caroli-rechingeri* were taken in the Crven Dol area, or a hydrothermal-modified rocks area, where the mineralization of the As and Tl is predominant, and opposite of them the plant *Centaurea kavadarensis* was taken 5.5 km from the hydrothermal altered stones along the river of Majdanska Reka. In parallel with the endemic plants, soil samples were collected from the same places where plants are taken. From the results that are presented in Table 1 it can be seen that plants that grow on soils rich with As and Tl show higher contents of these elements distributed in different parts of the plants. Similar to the other endemic plants that are collected from the same region of Crven Dol (Bačeva et al., 2014a, 2014b, 2016), it can be noted that the plants *O. degenii* and *K. caroli-rechingeri* have higher content of the As and Tl in their roots.

From the results shown in Table 1 it can be seen that the content of As in soil samples taken from the sites where *O. degenii, K. caroli-rechingeri* and *C. kavadarensis* grow are 5288 mg/kg, 4932 mg/kg and 102 mg/kg, respectively. The content of Tl in the soil samples is also high, for the soil sample taken from the *O. degenii* vicinity is 330 mg/kg, for soil from site of the *K. caroli-rechingeri* it is 409 mg/kg and for *C. kavadarensis* site the content of Tl in the soil is 4.45 mg/kg. Unlike As and Tl in soil samples taken from plant sites of *O. degenii, K. caroli-rechingeri* and *C. kavadarensis*, the content of Sb is low and it is 80.7 mg/kg, 30.9 mg/kg and 8.4 mg/kg, respectively. These plants exist on soils with high contents of As and Tl in the area of hydrothermal alterations in the immediate vicinity of the mine. The reason for the high content of these elements in the soil is due to the surface mineralization that has occurred in the past in this area as well as from the mining activities in the past (Stafilov et al., 1988; Janković, 1993; Frantz et al., 1994; Bačeva et al., 2013, 2014a, 2014b, 2016). Therefore, the contents of these elements are high, especially in the roots of these plants.

Thus, the content of As in the root of *O. degenii* is 114 mg/kg, and for *K. caroli-rechingeri* is 354 mg/kg (Figure 6). The content of As in the above-ground parts of the plant *O. degenii* is 4.03 mg/kg in the leaf and 3.49 mg/kg in the flower. For *K. caroli-rechingeri*, the content of As in the leaf is 3.78 mg/kg (Table 1, Figure 6). The content of Tl in the root of these plants is also high, ranges from 86.7 mg/kg in the roots of *O. degenii* to 216 mg/kg in the roots of *K. caroli-rechingeri* (Table 1, Figure 7). For the other parts of the plants (stem, leaf and flower), the content of Tl in *O. degenii* is 6.3 mg/kg, 6.8 mg/kg and 6.19 mg/kg, respectively, while for the *K. caroli-rechingeri* the content of Tl in the stem, leaf and flower is higher: 26.3 mg/kg, 10.4 mg/kg and 4.31 mg/kg, respectively (Table 1, Figure 7).

The content of Sb is significantly lower in different parts of the plants compared to the content of As and Tl (Table 1). The reason for this due to the low content of Sb in the part of Crven Dol locality and therefore its accumulation by the plants is much lower (Table 1).

From the results shown in Table 1 and Figures 6 and 7 it can be noticed that the contents of As, Sb and Tl in the soil sample collected from the site where *C. kavadarensis* samples are collected are much lower than in the soil samples from the sites of the other two plants (Bačeva et al., 2013, 2014a), and therefore the accumulation of these elements is much smaller in *C. kavadarensis* compared to *O. degenii* and *K. caroli-rechingeri*.

T a b l e 1

Contents of 27 analyzed elements in soil and different parts of plants of Onobrychis degenii, Knautia caroli-rechingeri and Centaurea kavadarensis

	Onobrychis degenii					*Knautia caroli-rechingeri*				*Centaurea kavadarensis*				
Element/Unit	Soil	Root	Stem	Leaf	Flower	Soil	Root	Stem	Leaf	Soil	Root	Stem	Leaf	Flower
As, mg/kg	5288	114	0.58	4.03	3.49	4932	354	1.66	3.78	102	<0.50	<0.50	<0.50	<0.50
Sb, mg/kg	80.7	<0.50	<0.50	<0.50	<0.50	30.9	0.68	<0.50	<0.50	8.40	<0.50	<0.50	<0.50	<0.50
Tl, mg/kg	330	86.7	6.34	6.83	6.19	409	216	26.3	10.4	4.45	4.31	4.13	5.38	3.18
Ag, mg/kg	<1	<0.02	0.02	<0.02	<0.02	<1	0.03	<0.02	<0.02	<1	0.03	<0.02	<0.02	<0.02
Al, mg/kg	50300	911	34.0	82.6	49.0	19600	890	11.3	76.0	44800	403	33.5	517	49.8
Ba, mg/kg	573	7.70	4.56	3.08	2.05	915	10.8	2.03	1.82	1117	14.1	3.69	11.1	6.25
Ca, %	5.83	2.42	0.79	1.30	1.15	0.80	2.36	0.90	1.03	4.82	0.42	0.32	2.07	0.96
Cd, mg/kg	3.53	0.14	<0.05	<0.05	<0.05	3.14	1.38	0.05	0.07	3.55	0.24	0.14	0.40	0.37
Co, mg/kg	<0.5	<0.05	<0.05	<0.05	<0.05	<0.5	0.40	<0.05	<0.05	<0.5	<0.05	<0.05	0.16	<0.05
Cr, mg/kg	81.4	1.19	0.22	0.49	0.34	38.1	0.75	0.15	0.80	387	5.48	0.52	6.16	0.90
Cu, mg/kg	35.3	4.00	5.48	4.43	4.14	45.3	13.1	8.14	5.59	38.7	6.39	2.89	5.30	9.09
Fe, mg/kg	30300	447	46.2	85.4	59	26700	681	17.9	85	30600	554	41.3	496	82.9
Ga, mg/kg	72.5	0.81	<0.5	<0.5	<0.5	73	0.87	<0.5	<0.5	103	1.40	<0.5	1.00	<0.5
K, %	1.60	0.76	1.66	0.99	1.18	2.06	1.42	2.59	1.58	2.56	0.95	0.99	1.92	1.41
Li, mg/kg	20.3	0.38	0.03	0.05	0.03	12.1	0.24	0.02	0.05	15.6	0.22	0.04	0.37	0.04
Mg, mg/kg	15900	754	1110	1115	1089	5700	1269	969	1239	18700	767	457	1356	1162
Mn, mg/kg	1397	24.1	12.2	32.7	27.9	1272	144	8.95	16.7	1000	18.7	2.37	21.3	9.89
Mo, mg/kg	11.2	12.8	2.37	0.47	0.70	11.7	0.55	0.31	0.42	<1	0.21	0.24	0.60	<0.20
Na, mg/kg	3300	83.7	22.3	19.9	17.9	3700	47.1	24.3	21.4	7600	67.9	20.7	34.4	37.5
Ni, mg/kg	71.0	2.89	24.9	8.84	10.0	40.1	1.80	1.35	3.50	415	8.11	0.91	7.78	7.46
P, %	0.15	0.04	0.15	0.12	0.22	0.20	0.12	0.24	0.19	0.23	0.09	0.10	0.19	0.28
Pb, mg/kg	85.1	0.65	1.01	0.68	0.50	110	1.40	0.52	1.02	113	0.54	0.51	1.17	0.99
Rb, mg/kg	7.25	11.1	14.3	10.5	15.0	7.80	19.1	25.7	15.1	10.5	2.93	2.67	6.47	5.72
S, %	0.18	0.19	0.11	0.17	0.17	0.24	0.10	0.12	0.18	0.15	0.10	0.07	0.19	0.18
Sr, mg/kg	250	11.6	3.71	2.88	1.96	214	12.8	3.91	2.88	569	11.1	7.88	21.9	10.9
V, mg/kg	82.6	1.17	<0.05	<0.05	<0.05	87.8	1.36	<0.05	<0.05	74.8	1.41	<0.05	0.87	<0.05
Zn, mg/kg	341	8.87	16.4	12.6	19.7	283	81.9	19.4	34.7	654	16.2	4.39	13.0	18.2

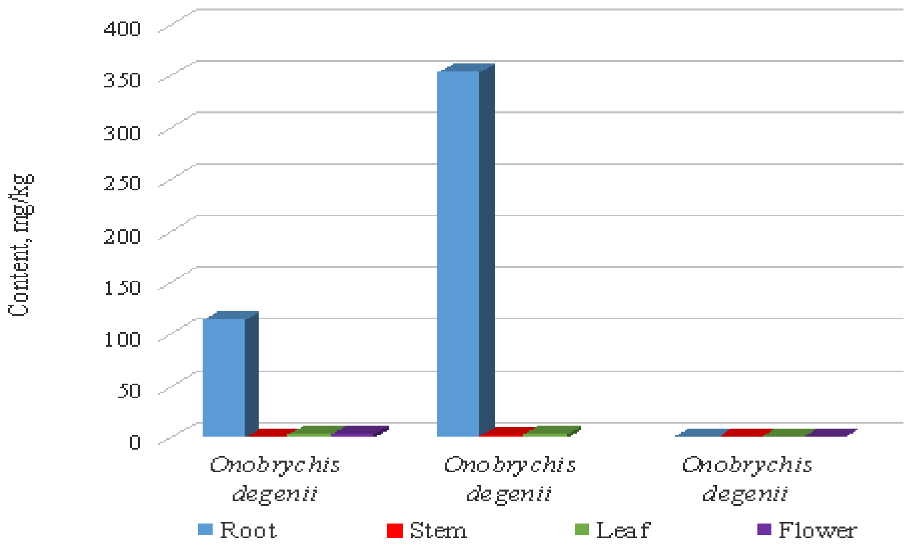

Fig. 6. Content of As in the various parts of the three endemic species: *Onobrychis degenii*, *Knautia caroli-rechingeri* and *Centaurea kavadarensis*

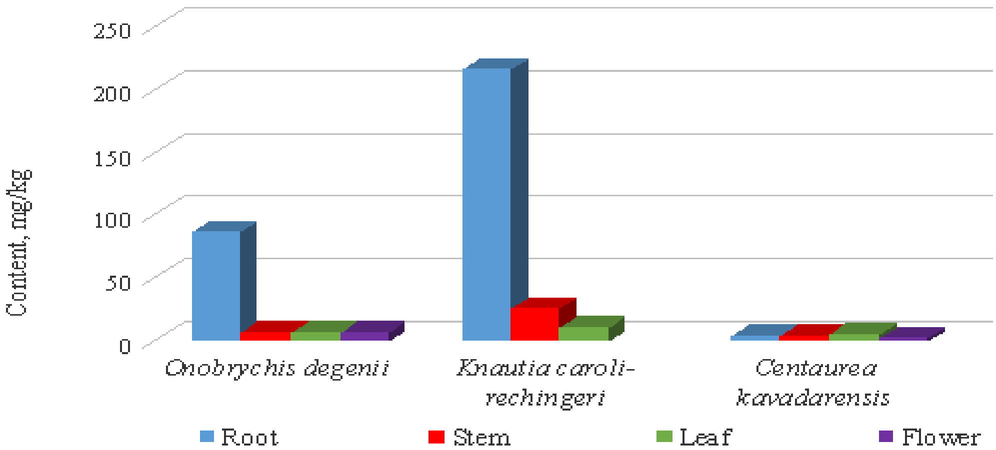

Fig. 7. Content of Tl in the various parts of the three endemic species: *Onobrychis degenii*, *Knautia caroli-rechingeri* and *Centaurea kavadarensis*

The extraction, mobility and accumulation of toxic elements from the soil depend on the solubility of those metals present in the soil, as well as on the ability of the plant to extract and accumulate elements of the soil. Until now, the accumulation of As, Sb and Tl was investigated, as well as the connection between the accumulation and mobility of these elements to the endemic plant species of Allchar – *Viola allchariensis* G. Beck, *Viola arsenica* G. Beck and *Viola macedonica* Boiss. & Heldr. (Bačeva et al., 2014b), as well as *Thymus alsarensis* Ronn. (Bačeva et al., 2016) which are present in the area of Allchar locality with the increased contents of As, Sb and Tl in the soils. In order to determine the degree of extraction and accumulation of toxic elements from the soil, for the new three endemic species (*Onobrychis degenii*, *Knautia caroli-rechingeri* and *Centaurea kavadarensis*) the same extraction solutions as described in the experimental section were used (Bačeva et al., 2014b; 2016).

Extracted amounts of As, Sb and Tl from the soil samples are summarized in Figures 8–10, which indicate that As and Tl were the most abundant in soil samples based on HCl extraction. From the same locations from which the endemic plant species were collected, soil samples were taken and the extractions with three extraction solutions were carried out on these soil samples: 0.1 M HCl; H_2O (at pH = 7) and DTPA-$CaCl_2$-TEA.

Different degrees of availability can be estimated depending on the extracting power of the reagent that is used. The most commonly used extraction media for testing the bioavailability of soil elements are the following (Pansu and Gautheyrou, 2006): extraction with water (provides information on the actual availability of elements from the soil solution), sequestering reagents as a DTPA-$CaCl_2$-TEA solution (pH = 7.3) of triethanolamine (0.1 mol l^{-1} TEA), $CaCl_2$ (0.01 mol l^{-1}) and diethylenetriaminepentaacetic acid (DTPA, 0.005 mol l^{-1}) according to the ISO 14870 method; and acid reagents (usually 0.1 mol l^{-1} HCl solution).

From Figure 8 it can be seen that the highest amount of As was extracted with an extraction solution of 0.1 M HCl. Thus, the extracted content of As from the soil where the plant *Knautia caroli-rechingeri* grows is 343 mg/kg or 6.96% of As in the soil. From here it can be concluded that the plant *Onobrychis degenii*, although it does not grow on a soil with high content of As, yet it has a lower ability to extract arsenic by 0.1 M HCl solution (0.85 mg/kg or 0.85% of the As in the soil). The extractability of As from the soil originated from the area where *Centaurea kavadarensis* was collected is also low (0.45% of the As from the soil).

Although the content of Sb in the soil is much lower that those for As and Tl, its extractability is noticeable for the soil samples from all three locations and for all of three extraction solutions (Figure 9). However, the extraction of Sb is the highest in 0.1 M HCl with the degree of extraction up to 1.19% for soil from the location of *C. kavadarensis*. It is also obvious that the extractability of Sb is higher for the same soil with the two other extraction solutions (Figure 9).

From Figure 10 it can be noticed that the extractions with 0.1 M HCl, H_2O and DTPA + TEA + $CaCl_2$ extraction solutions for Tl from the soil of the three endemic plants are similar. Thus, the extraction with 0.1 M HCl provides the highest thallium extraction. It has also been shown that the soils from *Centaurea kavadarensis* and *Knautia caroli-rechingeri* locations provide the highest Tl extractability with all the extraction solutions (Figure 10).

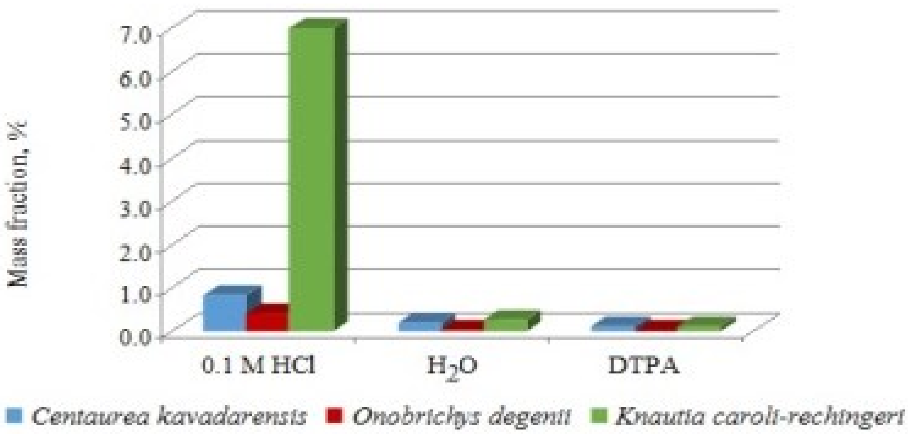

Fig. 8. Extraction of As in 0.1 M HCl, H_2O and DTPA+TEA+$CaCl_2$ extraction solutions

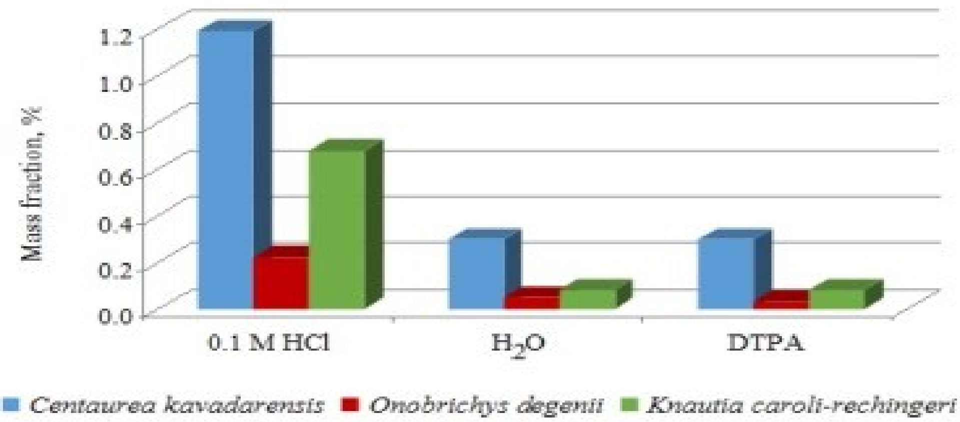

Fig. 9. Extraction of Sb in 0.1 M HCl, H_2O and DTPA+TEA+$CaCl_2$ extraction solutions

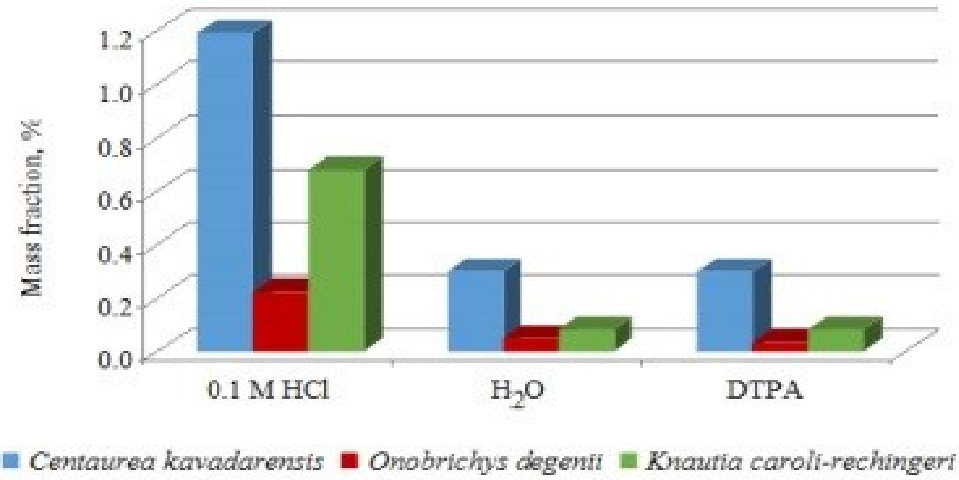

Fig. 10. Extraction of Tl in 0.1 M HCl, H_2O and DTPA+TEA+$CaCl_2$ extraction solutions

For the rest of the analyzed elements (Table 1) it can be concluded that there was no statistically significant correlation between their contents in all three investigated endemic species. Data show that the contents of Al, Ca, Fe, K and Mg in soil samples is significantly high (from 1.96 to 5.03% for Al, from 0.80 to 5.83% for Ca, from 2.67 to 3.06% for Fe, from 0.99 to 1.60% for K and from 0.57 to 1.87% for Mg). Relatively higher contents were found also for Ba, Mn, P and Zn. It can be also seen (Table 1) that the Allchar locality has relatively uniform amounts of major oxides such as CaO, Na_2O and K_2O that classifies these rocks as monzonites. Mineralization is associated with Triassic carbonates (dolomites and marbles), the Tertiary magmatic rocks and volcano-sedimentary sequence (tuffaceous dolomite) (Volkov et al., 2006; Jelenković and Boev, 2011; Boev and Jelenković, 2012). It refers to their contents in the endemic species and no big differences were observed for the contents of Al, Ba, Ca, Fe, K, Mg, Na, P and S in all of the investigated endemic species. From most of the analyzed trace elements the highest content was observed in the roots of the plants compared with their contents in the other parts of the plants (stem, leaf and flower).

Bioaccumulation is a process in which chemical substances are absorbed in the organism by all routes of exposure as occurs in the natural environment. Essential and non-essential elements naturally bioaccumulated using different pathways and the toxicity of metals were highly influenced by geochemical factors that can affect metal bioavailability. Assessment of the mobility and accumulation of As, Sb and Tl in endemic plants is of great importance for environmental monitoring.

Table 2 presents the results of the contents of As, Sb and Tl in soil and in all parts of *Centaurea kavadarensis, Onobrychis degenii* and *Knautia caroli-rechingeri*, and calculations for the bioaccumulation (BAF) and biotransfer factor (BTF). From these results it can be concluded that the bioaccumulation factor (transfer of the element from soil to all parts of the plant) is very low for As (0.023 in *Onobrychis degenii* and 0.073 in *Knautia caroli-rechingeri*, while for *Centaurea kavadarensis* it could not be calculated because the contents of As in the parts of the plants are below the detection limit), and for Sb. From the other side, BAF for thallium is very higher ranges from 0.322 in *Onobrychis degenii*, 0.618 in *Knautia caroli-rechingeri* and the highest values of 3.819 were found for *Centaurea kavadarensis*.

From the other side, biological transfer factor (BTF) which represents the ratio between the content of that element in the flower (or seeds) and in the root and again it is the highest for Tl (from 0.048 in *Knautia caroli-rechingeri* to 0.737 in *Centaurea kavadarensis*) which shows their capability to accumulate high amounts of Tl. Probably this behavior of thallium could be explain by the interpretation given by Madejon et al. (2007) that when Tl is present in soils it may be easily taken up by plants because it is generally present as thermodynamically stable Tl(I), an analogue to potassium or by the explanation that thallium(I) has a very low stability that is constant with both organic and inorganic ligands and therefore should be easily extractable from the leaves (Kaplan and Mattigod, 1998; Nriagu, 1998; Scheckel et al., 2004; Bačeva et al., 2014b).

Table 2

Results from the analysis of plant material and calculations for bioaccumulation and biotransfer factor

Sample	Root	Stem	Leaf	Flower	Sum	Soil	BAF[a]	BTF[b]
As, mg/kg								
Onobrychis degenii	114	0.58	4.03	3.49	122	5288	**0.023**	**0.031**
Knautia caroli-rechingeri	354	1.66	3.78	-	360	4932	**0.073**	**0.011**
Centaurea kavadarensis	<0.50	<0.50	<0.50	<0.50	–	102	–	–
Sb, mg/kg								
Onobrychis degenii	<0.50	<0.50	<0.50	<0.50	–	80.7	–	–
Knautia caroli-rechingeri	0.68	<0.50	<0.50	–	0.68	30.9	**0.022**	–
Centaurea kavadarensis	<0.50	<0.50	<0.50	<0.50	–	8.40	–	–
Tl, mg/kg								
Onobrychis degenii	86.7	6.34	6.83	6.19	106	330	**0.322**	**0.071**
Knautia caroli-rechingeri	216	26.3	10.4	–	253	409	**0.618**	**0.048**
Centaurea kavadarensis	4.31	4.13	5.38	3.18	17.0	4.45	**3.819**	**0.737**

[a]BAF – Bioaccumulative factor: the ratio between the total content of the element in all parts of the plant and the presence of that element in the soil

[b]BTF – Biological transfer factor: the ratio between the content of that element in the flower (or leaf) and the root

CONCLUSION

The goal of this investigation is to establish the intensity of bioaccumulation of various elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sr, Tl, V and Zn), with focus on As, Sb and Tl, in three endemic plant species from Allchar locality, *Onobrychis degenii* Dörfler, *Knautia caroli-rechingeri* Micev. and *Centaurea kavadarensis* Micev. It was found that the contents of As and Tl in soil samples that were collected from the locations where the plants grow were higher than the content of Sb. The results showed that the accumulation of As and Tl in the investigated endemic species is significantly high, and they are able to hyperaccumulate toxic elements in their roots and shoots. Accumulation of these elements in the studied endemic species may be used as a valuable tools for bioindication, while, from the other hand, the accumulation of these toxic elements in the plants causing high risk to the human and animal health. Such behavior of As, Sb and Tl was confirmed by the extraction tests with various solvents of soil samples collected from the same locations as the endemic plants. It was established that in all extraction solutions Tl was the most extractable element.

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Р е з и м е

БИОАКУМУЛАЦИЈА НА НЕКОИ ТОКСИЧНИ ЕЛЕМЕНТИ ОД ЕНДЕМСКИТЕ ВИДОВИ РАСТЕНИЈА *ONOBRYCHIS DEGENII* DÖRFLER, *KNAUTIA CAROLI-RECHINGERI* MICEV. И *CENTAUREA KAVADARENSIS* MICEV. ОД ЛОКАЛИТЕТОТ АЛШАР, РЕПУБЛИКА МАКЕДОНИЈА

Катерина Бачева Андоновска[1], Трајче Стафилов[2], Владо Матевски[1,3]

[1]*Истражувачки центар за животна средина и материјали, Македонска академија на науките и уметностите, 1000 Скопје, Република Македонија*
[2]*Институт за хемија, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“ во Скопје, п.фах 162, 1001 Скопје, Република Македонија*
[3]*Институт за биологија, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“ во Скопје, п.фах 162, 1001 Скопје, Република Македонија*
kbaceva@manu.edu.mk; trajcest@pmf.ukim.mk

Клучни зборови: акумулација; мобилност; антимон, арсен; талиум; ендемски растенија; Алшар; BAF; BTF

Рудникот Алшар, кој се наоѓа во јужниот дел на Република Македонија, е уникатен депозит познат во светот поради разновидноста на минералошкиот состав, особено поради високата содржина на арсен и талиум. Целта на ова истражување е да се утврди интензитетот на акумулација на различните елементи (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sr, Tl, V и Zn), со посебен осврт на As, Sb и Tl, во три ендемски видови растенија од локалитетот на Алшар, *Onobrychis degenii* Dörfler, *Knautia caroli-rechingeri* Micev. и *Centaurea kavadarensis* Micev. Земени се примероци од различни делови од овие растенија и соодветните почви, потоа подготвени, растворени и анализирани со примена на индуктивно спрегната плазма со атомска емисиона спектрометрија (ИСП-АЕС). Со оваа истражување е утврдено дека содржината на As во примероците од почвата, земени од локациите каде што растат растенијата, се движи од 102 mg/kg до 5288 mg/kg, додека содржината на Tl во примероците од почвата исто така е висока, од 4,45 mg/kg до 409 mg/kg. Во споредба со содржината на Sb и Tl во примероците од почвата, содржината на Sb беше ниска и се движи од 8,4 mg/kg до 80,7 mg/kg. Добиените резултати покажуваат дека акумулацијата на As, Sb и Tl во испитуваните ендемски видови растенија е значајна, што покажува дека тие имаат способност да ги акумулираат токсичните елементи во нивните корени и надземните делови. Акумулацијата на овие елементи во испитуваните ендемски видови овозможува тие да се користат како значајни биоиндикатори, додека, од друга страна, акумулацијата на овие токсични елементи во растенијата може да предизвика висок ризик за здравјето на луѓето и животните.