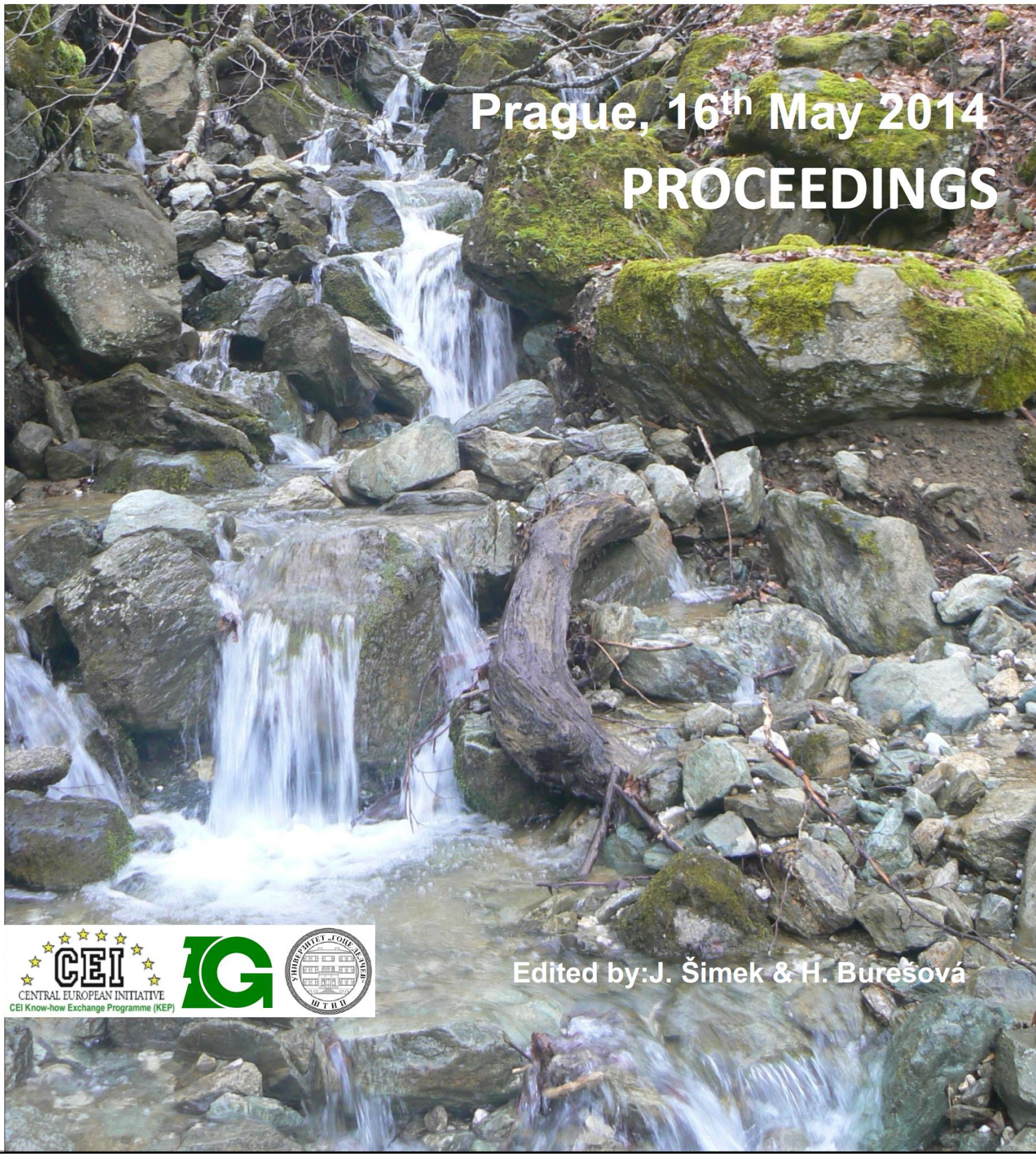
GIS-GEOINDUSTRY, s.r.o., Czech Republic CEI Know-how Exchange Programme

2nd INTERNATIONAL WORKSHOP

Environmental impact assessment of the Kozuf metallogenic district in southern Macedonia in relation to groundwater resources, surface waters, soils and socio-economic consequences (ENIGMA)



Prague, 16th May 2014

PROCEEDINGS

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2nd INTERNATIONAL WORKSHOP
ON THE PROJECT

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GEOCHEMISTRY OF THE ENDEMIC FLORA VIOLLA ALSHARICA AND THYMUS ALSHARENSIS OF THE ALSHAR SITE-MACEDONIA

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Abstract: This paper presents the specific geochemistry of the endemic flora Viollа Alsharica and Thymus Alsharensis which appears at the area of the location of Alsar (Republic of Macedonia). The locality Alsar is a unique mining findings where a number of minerals of thallium which in their basics are sulfates of thallium with arsenic, sculpture, iron and mercury. As a result of this mineralization of the entire space of a few square kilometers is enriched with arsenic, thallium, antimony and mercury, that is, there are increased concentrations of these elements in the soils and water. The conducted research on the geochemistry of the endemic flora shows that it is enriched with thallium and arsenic and that there is a strong geochemical correlation between the contents of arsenic and thallium in the soils of the endemic flora. This study was designed to assess total contents of 7 trace metals (Pb, Cu, Zn, Co, Ni, Tl, As, Pb and Mn in the soil and plant samples of two endemic species (Viollа Alsharica and Thymus alsharensis) collected from soils in part of the Alshar site. The content, transfer and accumulation of metals from soil to endemic species was evaluated in terms of Biological Concentration Factor (BCF),

Total metal content of Zn, Ni, Cu, Ba, Tl, As,Pb, Co and Mn in soils varied between of 70-465 mg/kg, 17-190 mg/kg, 6-68 mg/kg, 20-1100 mg/kg, 98-154 mg/kg, 443-2000 mg/kg, 10-48 mg/kg, 8-217 mg/kg, 22-6410 mg/kg, respectively.

In soil from Alshar site content of Tl (98-154 mg/kg) and As (443-2000 mg/kg), drastically exceeding the intervention value according to a New Dutch list.

Mn has the highest bio-accumulation factor in comparison with other metals in both endemic species. BCF for other trace elements decreases in the order Pb> Cu> Ba> Zn> TL,> Ni> Tl> How to Viollа Alsharica, and Cu> Zn> Ba> Ni> Pb> Tl> Co> As for Thymus alsharensis.

The BCF values of Viollа Alsharica, was highest for Pb (2,98), Tl (1.85) and Mn (10.94), while Thymus alsharensis has higher BCF values for Zn (2.93), Ba(2.80), Cu(5.68), Ni (2.51) and Co (0.73). Both endemic species have low bio-accumulation factor for As.Thumus alsharensis and Viola alsharica from Alshar site, had very high BCF values and could be useful for phytostabilization of soils contaminated with Cu, Zn, Tl, Ni and Pb.

INTRUDUCTION

Alshar area is located in the Vardar zone and it represents geotectonic unit of regional character which is Pelagonian zone between the west and the Serbo - Macedonian mass to the east. The area (mine site) is located near the Macedonian - Greek border, and is genetically linked to tertiary magmatic activity that occurred in this region in the late Miocene and early Pleistocene - Quaternary, ie in the range of 6.5-1.8 million years (Lipolt and Fuhrman, 1986, Kolios et all, 1988, Boev, 1988). As a result of this magmatic activity in this part of the mountain Kozuf there is a presence of large amounts of pyroclastic materials represented with tuffs, conglomerates, and lacustrine tuff ignimbrites, while the volcanic rocks of the intrusive stages of this magmatic activity are underrepresented. The magmatic activity is controlled by the structures which are ideally shaped and occurring in places where old

structures are cut Vardarski direction and youth structures in the direction of NE- SW extension (Jankovic and Jelenkovic, 1994). The volcanic rocks in this area are presented with andesite, and kvarclatites latiti while the west side is present and rioliti trahiti (battlefield 1988). The volcanic rocks are generally enriched in alkali elements and belong to the group of alkaline rocks in Macedonia (Karamata and others., 1994). Alshar mine site itself is located in a tectonic zone about 2 km long with the general direction of stretching NNW-SSE. The area appeared several ore bodies have variable sizes from a few meters up to 200-300 m in width and length. Morphological ore bodies have the appearance of Sochi, irregular veins or impregnation. The hydro-thermal changes are mainly presented with salification, argilitization in some parts adularization. The mine mineralization has been presented with Fe- sulfides , As-Tl minerals , Pb and Sb sulphates , cinabarit and in some places has appeared on samoroden sulfur and gold (Jankovic , 1960 , Ivanov , 1986) . The main ore bodies of thallium found in the northern part of the site in the locality and Red Doll mineralization mainly been presented with realgar, auripigment, lorandites and markasit.
Based on the research of the fluid inclusions it is determined that the mineralization in Alsar is formed in a relatively low temperature frame (100-250 ^{o}C) and salt of the dissolvent from 8-13 mass% NaCl equivalent and a high activity of the oxygen and fS2 (Beran et all, 1994).Based on the research of the sulphure isotopes (Frantz et all, 1994) it is determined that the sulphur has a volcanic origin, and based on the research of lead it is determined that the lead originates from the continental core. Jankovik and Jelenkovik (1994) determine the primary depth of mineralization of 250 to 400 meters. Due to the current research it is determined that in the Alsar founding there are around 4000 tons of thallium mine with content from 0.3% Tl, 2.5 %As, 1.5 %Sb (Jankovik and Jelenkovik, 1994). The age of the founding is determined based on the isotopic measures with the method Ar-Arof the mineral sanidin/ adularia, it is 4.22 +-0.07 MA (Troesh and Frantz, 1992).

WAY OF APPEARANCE OF THE ENDEMIC FLORA

Violla Alsharica and Thymus Alsharensis

The best places where this community is present is the nearby of the village Majden, from the right side of the Majdenska River, on the locations of Alsar and Crven Dol, as well as the left bank of the Majdanska River, below the Majdan village. The community is developed on a shallow eroded soil, but steep inclined sides of southwest, northeast and southeast exposition, on a sea altitude of 700 to 800 meters.

Within the community Violla Alsharica (see Fig. 1) the following types are included (Mateski, 2007); *Viola allchariensis, Viola frondosa, Achillea holosericea, Acinos arvensis* subsp. *villosus* var. *villosus, Acinos hungaricus, Aethionema saxatile, Allium ampeloprasum, Alyssum stribrnyi var. macedonicum, Anthemis tinctoria, Anthemis tinctoria* var. *pallida, Anthyllis vulneraria* subsp. *spruneri, Asperula aristata, Astragalus onobrychis* var. *Chlorocarpus, Asyneuma canescens* subsp. *cordifolium, Asyneuma limonifolium, Bromus riparius* (*B. fibrosus subsp.macedonicus*), *Calamagrostis epigejos, Campanula lingulata, Centaurea leucomala, Cerastium decalvans* var. *dollinieri, Cerastium petricola, Chamaecytisus triflorus* var. *albanicus, Crepis sancta, Dinathus pinifolium* subsp. *serbicus, Draba athoa, Erysimum cuspidatum, Festuca callieri* var. *mariovoensis, Festuca valesiaca, Galium purpureum, Helianthemum canum, Hypericum rumeliacum* var. *macedonicum, Jurinea consanguinea* var. *arachnoidea, Koeleria glaucovirens var. macedonica, Leontodon crispus* subsp. *Crispus, Linaria dalmatica* subsp. *macedonica, Linum hirsutum* subsp. *hirsutum, Minuartia glomerata* subsp. *macedonica, Minuartia verna* subsp. *verna, Myosotis sylvatica, Onobrychis degenii,*

Onobrychis pindicola, Onosma heterophyllum, Opoponax hispidus, Petrorhagia thessala var. *thessala, Phleum graecum, Poa bulbosa, Poa timoleontis, Potentilla sulphurea, Scabiosa webiana, Sedum acre, Silene bupleuroides* subsp. *staticifolia, Silene conica* subsp. *subconica, Silene italica, Silene otites, Stachys macedonica, Stipa epilosa, Teucrium polium, Thymus alsarensis, Thymus thracicus* var. *stribrnyi, Trinia glauca, Vicia pannonica* subsp. *pannonica.*

The community Viollа arsenica develops on the same location like the communityViolla alcharensis (Mateski, 2007), on soil rich with arsenic and antimony, but with a complete different ecological characteristics (mesofil depression with deep soil and slight inclination) attitude above sea level from 720 to 800 м. Within the community Viollа arsenica the following types are included: *Viola arsenica, Viola frondosa, Arrhenatherum elatius, Asyneuma canescens* subsp. *cordifolium, Bromus riparius* (*B. fibrosus* subsp.*macedonicus*), *Campanula sparsa* subsp. *Sphaerothrix, Campanula trachelium, Centaurea napulifera* subsp. *nyssana, Centaurea nervosa,Chamaecytisus triflorus* var. *albanicus, Cirsium ligulare, Dactylis glomerata, Dianthus giganteus* subsp. *haynaldianus, Digitalis ferruginea, Echinops bannaticus* var. *acutilobus, Erysimum cuspidatum, Euphorbia amygdaloides, Festuca callieri* var. *mariovoensis, Festuca valesiaca, Galium speciosum, Hesperis matronalis, Hesperis theophrasti, Knautia caroli-rechingeri, Knautia drymeja* subsp. *nympharum, Koeleria glaucovirens* var. *macedonica, Lathyrus pratensis, Linum hirsutum* subsp. *hirsutum, Linum nervosum, Muscari comosum, Myosotis sylvatica, Opoponax hispidus, Peucedanum carvifolium, Phleum graecum, Picris hieracioides, Poa angustifolia, Potentilla sulphurea, Pteridium aquilinum, Silene alba* subsp. *divaricata* var. *thessala, Solidago virgaurea, Thymus alsarensis (Fig.2),Thymus thracicus* var. *stribrnyi, Veronica teucrium* subsp. *crinita, Vicia pannonica* subsp. *Pannonica.*

Fig.1. Violla alsharica

Fig.2. Thymus alsharensis

Methodology of work

The aim of the investigation was to determine the correlation between individual micro elements present in the soils and those in the ashes of Viola alsharica and Thymus Alsharaensis. In this regard a number of samples were collected from the rocks in the site. Samples were collected in oblong grids in which the distances between profiles amounted to 100 meters, whereas the distance between the samples collected amounted to 50 meters. Samples were analyzed by the method of instrumental neutron activation in order to determine the contents of individual micro elements such as Sb, Se, W, Zn, Ba, Tl, As, Co, Cu, Mn, Ni and Pb.

Besides samples taken from the soils, samples of plants such as Viola Alsharica and Thymus Alsharensis were also collected for analysis. It is worthwhile to point out that samples taken from the plants were representative of the whole plant. The samples were dried at temperature of 105^{o} C until there was no loss in weight. Samples dried in this manner were heated at temperature of 700^{o} C for two hours and then determination of individual microelements (those of Sb, Se, W, Zn, Ba, Tl, As, Co, Cu, Mn, Ni and Pb) was performed by the ICP-AES method.

RESULTS AND DISCUSSION

Trace metals in soil from Alshar site

Descriptive statistics for the total content of Zn (70- 465 mg/kg), Ni (17-190 mg/kg), Cu (6-68 mg/kg), Ba (20-1100 mg/kg), Tl (98-154 mg/kg), As (443-2000 mg/kg), Pb (10-48 mg/kg), Co (8-217 mg/kg), Mn (22-6410 mg/kg) in the soils in part of the Alshar region, are presented in Table 1.

Table 1: Descriptive statistic for the microelements in the soils in part of the Alshar site(ICP-AES method, in mg/kg)

soil	*Zn mg/kg*	*Ba mg/kg*	*Tl mg/kg*	*As mg/kg*	*Co mg/kg*	*Cu mg/kg*	*Mn mg/kg*	*Ni mg/kg*	*Pb mg/kg*
Mean	211.4	602	113.9	1409.8	52.5	28.1	2183.3	69.8	29.5
SD	142.2	374.9	18.0	638.3	64.0	21.4	2506.8	57.1	11.5
Minimum	70	20	98	443	8	6	22	17	10
Maximum	465	1100	154	2000	217	68	6410	190	48

The average natural level of zinc in the earth's crust is 70 mg/kg (dry weight), ranging between 10 and 300 mg/kg (V. P. Singh,2005). The contents of Zn in no contamination soils reported for several European countries vary between 7 and 89 mg/kg (Kabata-Pendias, 2001). Elevated Zn content of natural source, at the range of 126–683 mg/kg (Davies et al., 2003).

In this work, the total Zn content in the soil from Alshar site, was variable within the sampling point (70 – 465 mg/kg, SD=142.2) (fig.2). In most of the sites of sampling, the content of zinc is higher than the natural background content (140 mg/kg),but not exceeding the intervention value (720 mg / kg) according new Dutch list (table 2).

Table 2. The Now Dutch list for soil remediation intervention values and natural background content soil for metals.

microelement	*Zn mg/kg*	*Ba mg/kg*	*Tl mg/kg*	*As mg/kg*	*Co mg/kg*	*Cu mg/kg*	*Mn mg/kg*	*Ni mg/kg*	*Pb mg/kg*
natural background content (NBC)	**140**	**120**	**1**	**29**	**9**	**36**	**/**	**35**	**85**
intervention values (IV)	**720**	**625**	**15**	**55**	**240**	**190**	**/**	**210**	**530**

The copper content of world soil ranges from 2 to 100 mg/kg with an average value of about 30mg/kg. Most of this is in unavailable mineral form. (Kabata Pendias, 2011) According to a New Dutch list, natural background content of copper in nature is 36mg/kg, while interventional value is 190mg/kg. In the sampling point of Alshar site, the total Cu, content ranged from 6 to 68mg/kg, and similar to zinc, (fig.2), in most of the studied sites of Cu content is higher than the natural background content (39mg/kg), but not exceeded soil remediation intervention values (160 mg/kg) according the New Dutch List. http://www.contaminatedland.co.uk/std-guid/dutch-l.htm (table 2).

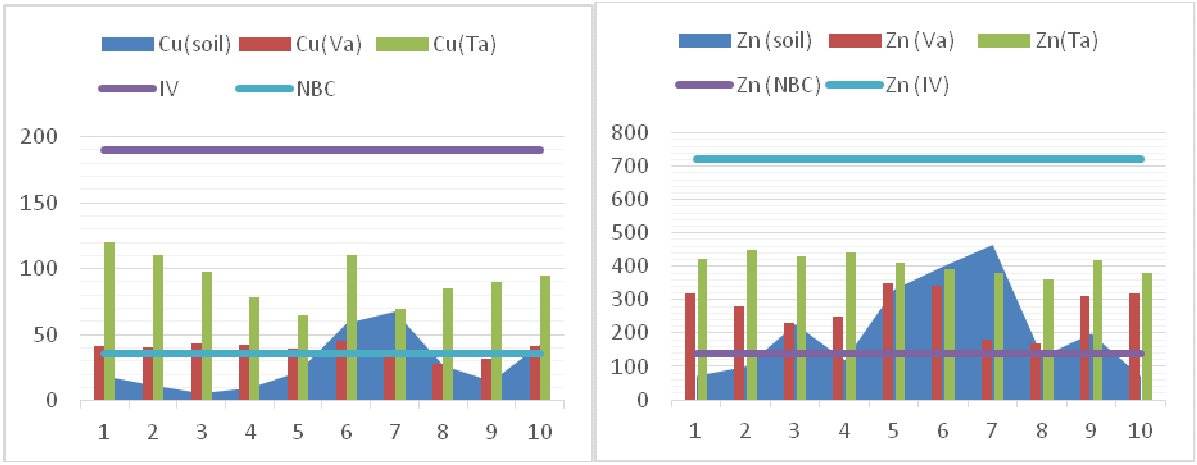


Figure 2. Cu and Zn content (mg/kg, dry weight) in soils and in ashes of Viola Alsharica (Va) and Thymus alsharensis (Ta), in part of the Alshar site, in comparison with natural background content (NBC) and intervention values (IV) according to a new Dutch list of metals in soil.

According to a New Dutch list, natural background content of Tl is 1.0mg/kg, while interventional value is 15 mg/kg, and for As natural background content is 29 mg/kg, and the interventional value is 55 mg/kg, appropriate.

The total content of Tl in uncontaminated soils from different countries is reported as follows (in mg/kg): <0.04–0.52 in Sweden (Eriksson, 2001); 0.01–0.41 in Poland (Łukaszewicz et al., 1996); 0.39–0.59 in Japan (Takeda et al., 2004).

While 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 (Curlik and Šefcik, 1999).

In soil from Alshar site content of Tl (98-154 mg/kg) and As (443-2000 mg/kg), drastically exceeding the intervention value according to a New Dutch list, in that arsenic (SD=638.3) shows some variation indifferent points of the study area, and thallium (SD=18.0) is almost constant in the investigation region (table 1).

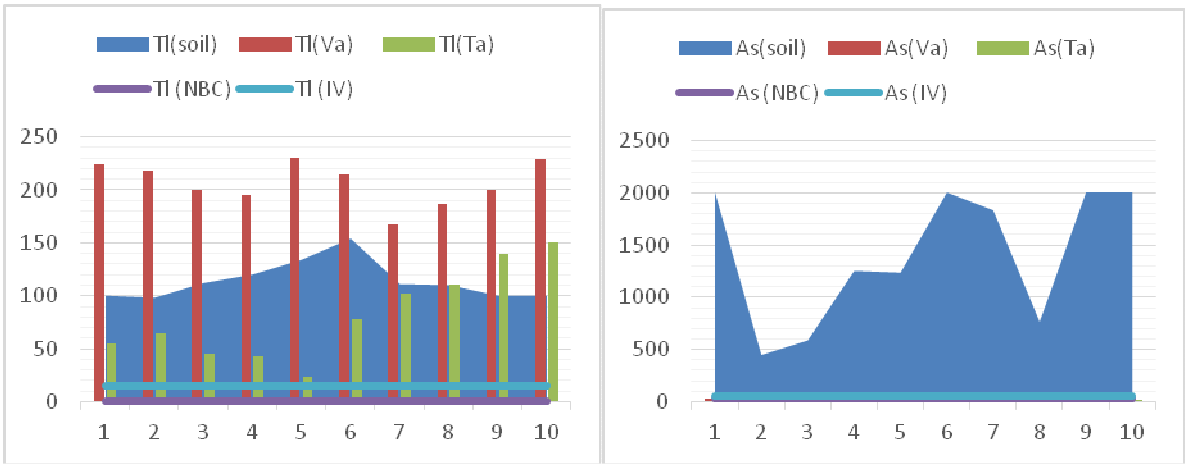


Figure 3. Tl and As content (mg/kg, dry weight) in soils and in ashes of Viola Alsharica (Va) and Thymus alsharensis (Ta), in part of the Alshar site, in comparison with natural background content (NBC) and intervention values (IV) according to a new Dutch list of metals in soil.

The reported Ba average range for soils on the world scale is from 362 to580 mg/kg (Kabata Pendias, 2011)**.** 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 to778 mg/kg (Eriksson, 2001).

In the soils of the area Alshar, the barium content varies from place to place (20-1100 mg/kg, SD = 374,9) (Figure 2), and in most of the sampling sites, the measured values exceeding the intervention value (600 mg/kg) in accordance with Dutch new List.

The Co content of soils is inherited mainly from parent materials. The worldwide mean value of Coin surface soils is calculated as 10 mg/kg (Kabata Pendias, 2011**).** Higher Co contents of surface soils are found in arid and semiarid regions, for example, Egyptian soils contain Co from 16.5 to 26.8 mg/kg (Nasseemand 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 (Eriksson, 2001). 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 cobalt in some parts of the area Alshar varies considerably from place to place (8-217 mg/kg, SD=64.0).

According to a New Dutch list, natural background content of Co is 9 mg/kg, while remediation interventional value is 240mg/kg. In the sampling point of Alshar site, the total Co content varies considerably from place to place (8-217 mg/kg, SD=64.0), but most of the area, moving around a natural background content with the exception of some places where it occurs significantly increase the content of cobalt (fig.4).

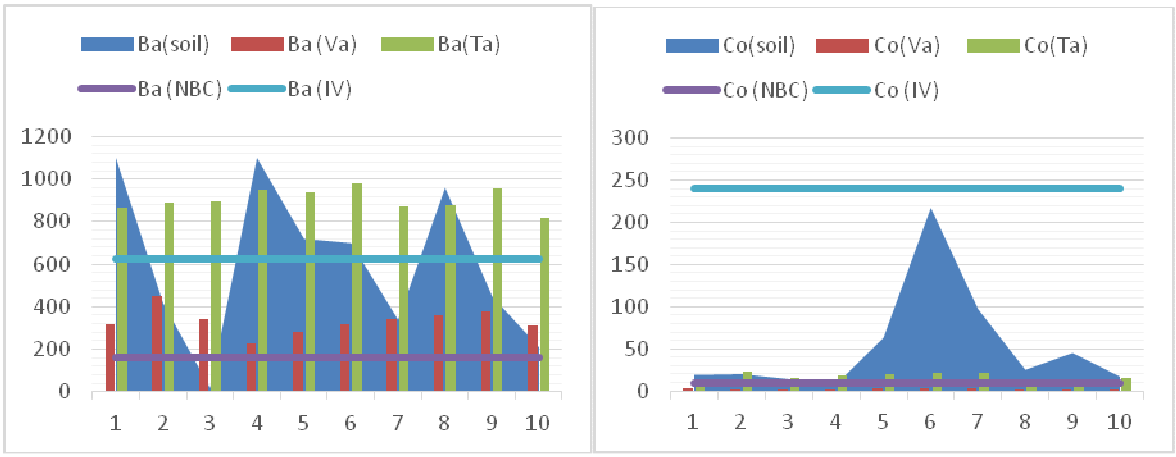


Figure 4. Ba and Co content (mg/kg, dry weight) in soils and in ashes of Viola Alsharica (Va) and Thymus alsharensis (Ta), in part of the Alshar site, in comparison with natural background content (NBC) and intervention values (IV) according to a new Dutch list of metals in soil.

Soils throughout the world contain Ni in the very broad range, however its mean concentrations, as reported for various countries are within the range 13–37 mg/kg. The highest Ni contents are given for uncontaminated soils of various countries, as follows (in mg/kg): (1) 119, Canada (Frank, R.,1976), (2) 450, China (Quiping, Z.1984.), and 3240, Italy (Bini, C.,1988).

The natural background content of Ni in soil according to the New Dutch list is 35mg/kg, and intervention value is 260 mg/kg. in the region of Alshar nickel content varies from 17 to 190, and most of the area around the natural background content with the exception of some places where, there are a significant increase in nickel content, but does not exceed the maximum permitted content according the new Dutch list.

Content of Pb in Alshar site ranges from 10 to 40 mg/kg (fig.5), and well below natural background content (85mg/kg) and intervention value (530mg/kg) according the now Dutch list.

Manganese is one of the most abundant among trace elements in the lithosphere, and common occurrence in rocks ranges from 350 to 2000 mg/kg and higher content are associated with mafic rocks (Kabata Pendias, 2011). The Mn total content in soil from Alshar site ranged from 22 to 6410 mg/kg.

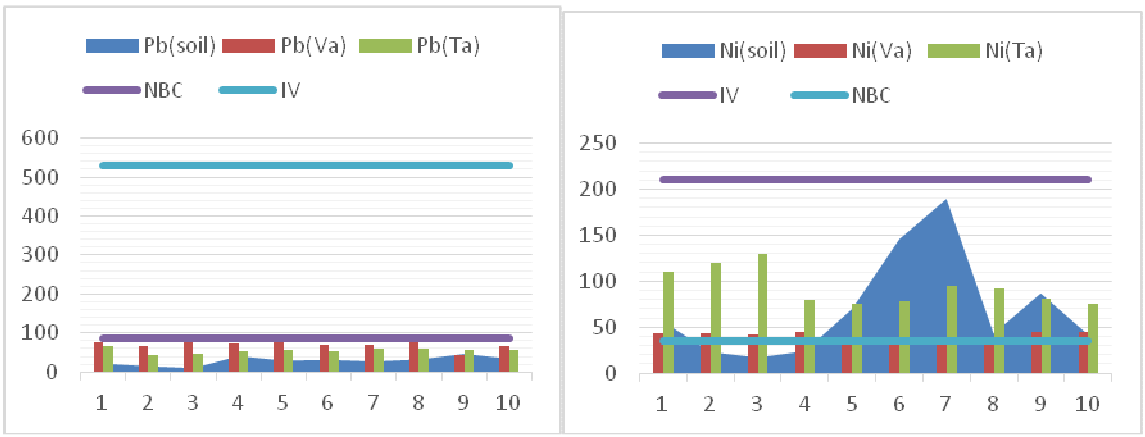


Figure 5. Pb and Ni content (mg/kg, dry weight) in soils and in ashes of Viola Alsharica (Va) and Thymus alsharensis (Ta), in part of the Alshar site, in comparison with natural background content (NBC) and intervention values (IV) according to a new Dutch list of metals in soil.

In Table 3 given the correlation between trace elements in the soil of some places in the Alshar site. Significant positive relationships have between Co, Ni, Mn, Cu and Zn. Tl have significant positive correlation only with Co (r=0.789) and Zn (r=0.645). Between As and Tl correlation is significantly low. As have positive relation with Cu, Ni and Pb.

Tab. 3. *Pearson correlation matrix for total metal contents* in the soils in part of the Alshar site.

	Zn	*Ba*	*Tl*	*As*	*Co*	*Cu*	*Mn*	*Ni*	*Pb*
Zn	1								
Ba	-0.229	1							
Tl	**0.645**	0.220	1						
As	0.228	0.113	0.124	1					
Co	**0.768**	0.002	**0.789**	0,427	1				
Cu	**0.648**	-0.140	0.373	**0.562**	**0.719**	1			
Mn	**0.606**	0.101	0.414	0.339	**0.744**	**0.749**	1		
Ni	**0.841**	-0.082	0.403	**0.594**	**0.777**	**0.855**	**0.777**	1	
Pb	0.004	0.346	0.116	**0.549**	0.126	0.160	0.257	0.223	1

Bio accumulation of microelement in endemic species Thymus alsharensis and Viola alsharica from Alshar site

Mean, minimum, maximum value and standard variation of Zn, Ba, Cu, Ni, Co, As, Ti and Pb in collected plant species are provided in Table 4.

Table 4. Mean, minimum, maximum value and standard variation of Zn, Ba, Cu, Ni, Co, As, Ti and Pb inendemic species Thymus alsharensis and Viola alsharica from Alshar site.

Viola Alsharica	*Zn mg/kg*	*Ba mg/kg*	*Tl mg/kg*	*As mg/kg*	*Co mg/kg*	*Cu mg/kg*	*Mn mg/kg*	*Ni mg/kg*	*Pb mg/kg*
Mean	275	333	206.5	33.9	2.16	38.6	1706	40.4	70.3
SD	64.9	58.7	20.3	5.1	0.5	5.3	148.8	4.8	10.3
Minimum	170	230	167	28	1.5	28	1400	33	45
Maximum	350	450	230	45	2.9	45	1900	45	81
Thymus alsharensis	*Zn mg/kg*	*Ba mg/kg*	*Tl mg/kg*	*As mg/kg*	*Co mg/kg*	*Cu mg/kg*	*Mn mg/kg*	*Ni mg/kg*	*Pb mg/kg*
Mean	408.4	904.5	81.1	11.8	16,8	92.1	1470	93.7	55.1
SD	29.6	49.7	42.9	5.5	4,7	18.1	182.9	19.8	6.2
Minimum	360	820	23	5	10	65	1200	75	45
Maximum	450	980	150	23	23	120	1800	130	65

In endemic plant Thymus alsharensis collected from the region of Alshar degree of bio accumulation of Zn ranges from 360 to 450 mg/kg, while endemic plant Viola alsharica, 170 to 340 mg/kg. In Viola Alsharica content of zinc is characterized by variability from one place to another. Zn contents in the ashes of Thymus alsharensis, did not differ significantly between sites of collection.

The Zn accumulation levels in this study are similar and sometimes higher, the other recorded in the literature for other plants. Mbila and Thompson (2004) evaluated the uptake of Zn by Osmorhiza longistylis and Sanicula marilandica plants present in mine spoils, reporting accumulation levels ranging from 114 to 159 mg/kg dry wt. in the roots and 42 to 55 mg/kg dry wt. in the shoots. In several wild species growing in polluted areas, such as Scolymus hispanicus, Lupinus angustifolius, Fumaria agraria, Solanum nigrum, Anagallis arvensis and Amaranthus blitoides, measured a wide range of accumulated zinc, which ranges from 76 to 930 mg/kg dry WT (Del Rio et al. (2002).

In this study, despite the clear influence of the soil contamination in the accumulation of Zn by Thymus alsharensis and Viola alsharica, the amounts were almost always below the Zn phytotoxic levels reported for plants – 500 to 1500 mg/ kg (Chaney (1989).

Ba is reported to be commonly present in plants, it is apparently not an essential component of plant tissue. Ba mean contents in most plants range from 2 to 13 mg/kg, with an exception of blueberries in which highly elevated Ba levels are reported, about 160 mg/kg **(Anke M., 2002)**. Much higher concentrations of Ba are also found in different trees and shrubs grown in area of arid climate. The highest contents of Ba are reported for Brazil nuts, depending on the location of growth, from about 3000 to 4000 mg/kg (Kabata-Pendias and Mukherjee, 2007).

Ba content in endemic species from Alshar site is relative higher, and ranges from 310-450 mg/kg, for Violla Alsharica, and from 820 to 980 mg/kg for Thymus alsharensis. In addition, it can be concluded that the Thymus alsharensis has higher affinity for zinc than other endemic species (fig. 5)

Some plant species have a great tolerance to increased concentrations of Cu and can accumulate extremely high amounts of this metal in their tissues (McGrath, 2007). Cu in ash of a variety of plant species, growing under widely ranging natural conditions, is reported to range from 5 to 1500 mg/kg. However, Cu contents of whole plant shoots do not often exceed 20 mg/kg, and thus values from 20 to 100 mg/kg are usually considered to indicate the threshold of excessive contents.

In this work, Cu content in endemic species collected from different site in the Alshar area ranges from 65 to 120 mg/kg and from 32 to 45 mg/kg in Thymus alsharensis and Violla alsharica respectively. This shows that Thymus alsharensis a higher affinity for copper than Violla alsharica (fig. 2, 5 and 6).

The Tl content of plants seems to be a function of Tl concentration in soil. Anthropogenic Tl in soils seems to be easily mobile and thus readily available to plants. Plants of the Brassicaceae family grown in soils contaminated by tailing dam waste accumulated up to 5 mg/kg; the highest values were 321 and 607, depending upon the species (Madejon et al., 2005). Several experiments have been conducted on the hyperaccumulator Iberis intermedia (Al-Najar et al., 2003). This plant may accumulated up to 4000 mg/kg when grown in a soil containing only 16 mg/kg.

The content of Thallium in endemic species from Alshar site, especially in Violla Alsharica (167-230 mg/kg) is very high, significantly higher than the content of Tl in soil, indicating a very high degree of bio-accumulation of thallium by these endemic species that grow only in this area of Macedonia (fig. 3, 5 and 6). Therefore it can be assumed that these plants can serve as hyperaccumulators for phytoremediation of soils with a high content of Thallium

Concentrations of As in plants grown in uncontaminated soils vary from 0.5 to 80 µg/kg. The uptake of As by plants depends on the As species and follows the order: Asorg > As^{5+}> As^{3+}.(Greger (2007). Total content of As in both endemic species, from 28 to 45 mg/kg in the ashes of Violla Alsharica, and from 5 to 23 in the ashes of Thymus alsharensis, suggested very low bio-accumulation index (Figure 3, 5 and 6).

As arsenic, and cobalt content of the endemic species collected from the region of Alshar has very low bio-accumulation (Figure C). In fact, the content of cobalt in Thymus alsharensis ranges from 10 to 23 mg/kg and the Violla Alsharica from 1,5 to 2,9 mg/kg.

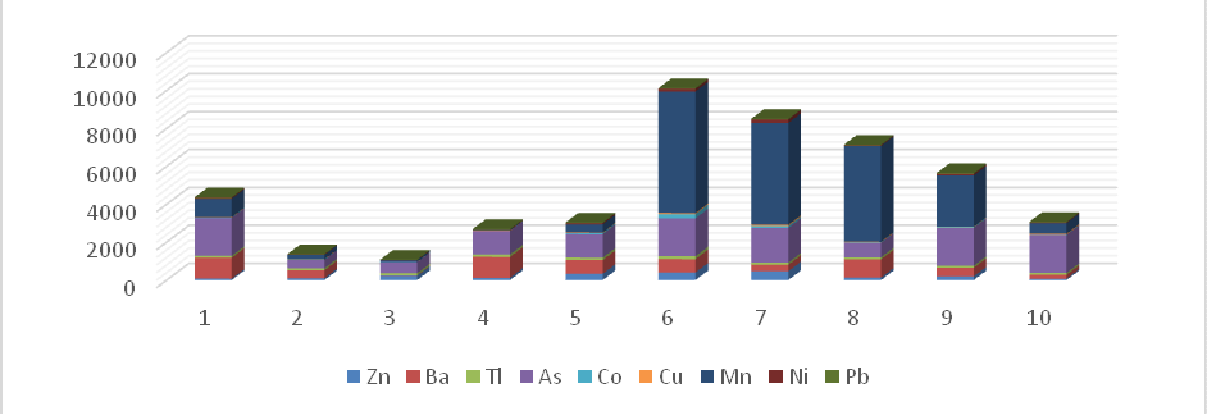


Figure 5. Sum of Zn, Ba, Tl, As, Co, Cu, Mn, Ni and Pb in soil

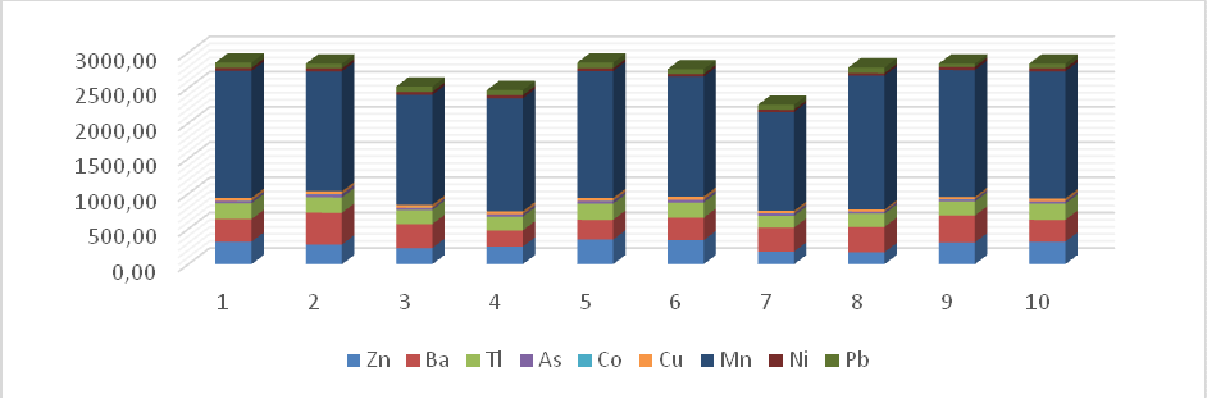


Figure 6. Sum of Zn, Ba, Tl, As, Co, Cu, Mn, Ni and Pb in ashes of Viola Alsharica

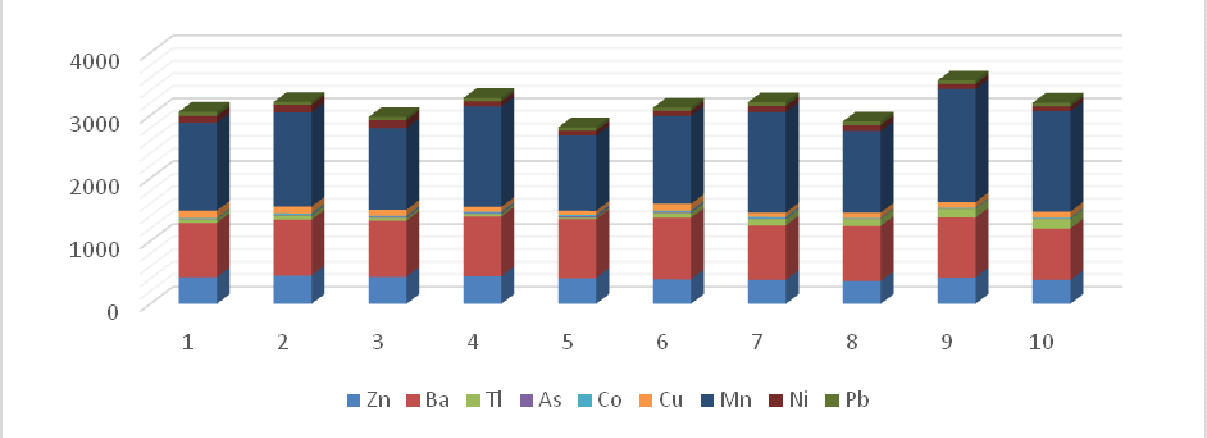


Figure 7. Sum of Zn, Ba, Tl, As, Co, Cu, Mn, Ni and Pb in ashes of Thymus alsharensis

Content of Mn was significantly higher in both soil and endemic species from Alshar site. In Thymus alsharensis range from 1200 to 1800 mg/kg, and in Violla Alsharica from 1400-1900 mg/kg. Mn is known to be rapidly taken up and translocated within plants as a result complex interactions between roots and microorganisms which can be affected the phytoavailability of

Mn (Marschner and Rengel, 2005) However, the high correlation between the content of manganese in soil and endemic species suggests that the Mn content of plants is not only an effect of plant characteristics, but also of the pool of available Mn, which is highly controlled by soil properties.

Nickel is the seventeenth element recognized as essential for plant growth and development (Liu 2001). The content of Ni in plants are dependent on many factors including plant species.Plants have two transport systems (Brown 2006): low affinity and high affinity. With the low-affinity transport system, plants can absorb Ni^{2+} ions at the low concentration of 4.4 µg/kg. With the high-affinity transport system, plants can take up 1.8 mg/kg of Ni.The Ni conent in plant leaves ranges from 0.05 to 5 mg/kg on a dry weight basis. The critical Ni concentration in plant tissues is about 1.0mg/kg. Nickel concentrations >10 mg/kg are generally considered to be toxic to sensitive species or cultivars (Brown 2006).

The Ni content in endemic species collected from Alshar area ranging from 33 to 45 mg/kg, and from 75 to 130 mg/kg respectively for Viola Alsharica and Thymus alsharensis (fig. 4). In fact, it is a high affinity of these endemic species to nickel, especially by Thymus alsharensis.

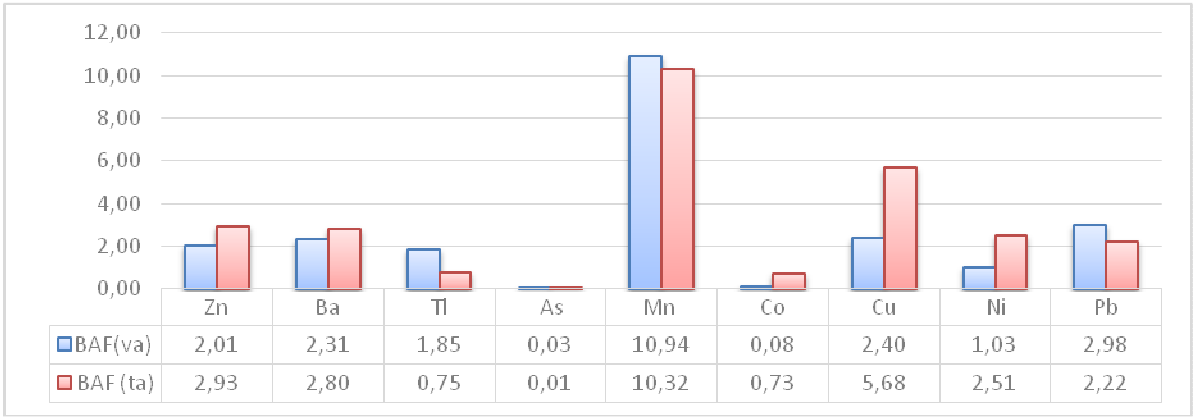
The great variation of Pb contents of plants is influenced by several environmental factors, such as the presence of geochemical anomalies, pollution, seasonal variation, and genotype ability to its accumulation (Alexander et al., 2006). According to previous studies, Pb content in plants growing in uncontaminated soils were 0.3–18.8 mg/kg for Pb, whereas the highest Pb content in plants growing in contaminated soils were 1506 mg/kg for Pb (Kabata-Pendias and Pendias, 1992). In this study, int wo endemic species collected from 10 locations at theAlshar site, the content of Pb in plant ashes are provided in Tables 2, 3 and 4. Total Pb content in the Viola Alsharica ranged from 45 to 81 mg/kg , and in Thymus alsharensis ranged from 45 to 65 mg/kg.

Bio-accumulation factor of trace metals

In order to assess the influence of soil properties on trace metal uptake by plants, the soil-to-plant transfer ratio were evaluated by calculating the biological absorption factor (BAF), i.e., the ratio of trace metal content in the plant (Me_{plant}) to either the total metal content in soils (Me_{tot}) according to (Chen et al, 2002).

$$BAF = \frac{Me_{plant}}{Me_{soil}} \quad (1)$$

The bio-accumulation factor (BCF) for Viola Alsharica and Thymus alsharensis, from the Alshar region are shown in Figure 7.



	Zn	Ba	Tl	As	Mn	Co	Cu	Ni	Pb
BAF(va)	2,01	2,31	1,85	0,03	10,94	0,08	2,40	1,03	2,98
BAF (ta)	2,93	2,80	0,75	0,01	10,32	0,73	5,68	2,51	2,22

Figure 7. Bio-accumulation factor (BAF) for Mn, Cu, Ba, Pb, As, Zn, TL and Ni for thumus alsharensis and viola alsharica.

Most of plant species, with the exception of Co and As, had BCF >1, although the content of most trace metals remained below 1000 mg/kg. In general, Mn has the highest bio-accumulation factor in comparison with other metals in both endemic species. BCF for other trace elements decreases in the order Pb> Cu> Ba> Zn> TL,> Ni> Tl>Co for Violla Alsharica, and Cu> Zn> Ba> Ni> Pb> Tl> Co> As for Thymus alsharensis (Table 7).
The BCF values of Violla Alsharica, was highest for Pb (2,98), Tl (1.85) and Mn (10.94), while Thymus alsharensis has higher BCF values for Zn (2.93), Ba (2.80), Cu (5.68), Ni (2.51) and Co (0.73).
Figure 8 shows the sum of BCF for Mn, Cu, Ba, Pb, As, Zn, TL and Ni in different places of sampling area, for Thymus alsharensis and Violla Alsharica. Total degree of bio accumulation of different places is highest for thumus alsharensis, and for both endemic species varies from place to place. The highest degree of variation indicates Manganese with BAF values of 0.27 to 72.73 in both endemic species.

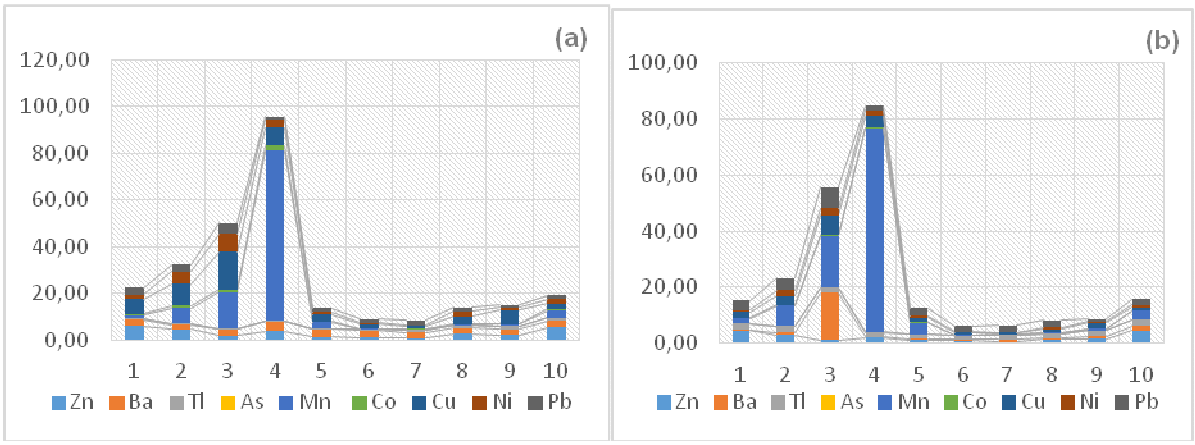


Figure 8. Sum of bio-accumulation factor (BAF) for Mn, Cu, Ba, Pb, As, Zn, TL and Ni,for thumus alsharensis (a) and viola alsharica(b)

Conclusion

Accumulation of selected metals varied among endemic species (thumus alsharensis and viola alsharica) and uptake of an element by a plant is primarily dependent on the plant species and the soil quality. So violla alsharica has greater affinity for Tl, As and Pb unlike thumus alsarensis which has a higher affinity for Zn, Ba, Co, Cu and Ni, but identical variation of sum of trace element from place to place suggests that these endemic species have almost identical absorption capacity.In general the results indicated that both endemic species (thumus alsharensis and viola alsharica) from Alshar site, were identified as hyperaccumulator because all species accumulated Pb, Cu, Zn, Ni, Co and As less than 1000 mg/kg (Macnair, M.R., 2002.). However, based on BCFs values this endemic species were identified which have the potential for phytostabilization and phytoextraction. Thumus alsharensis and Viola alsharica from Alshar site, had very high BCF values and could be useful for phytostabilization of soils contaminated with Cu, Zn, Tl, Ni and Pb.

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