Acta Bot. Croat. 66 (2), 177–195, 2007

CODEN: ABCRA 25
ISSN 0365–0588

Flora of heavy metal-rich soils in NW Iran and some potential hyper-accumulator and accumulator species

ROGHIEH HAJIBOLAND*, MIR H. MANAFI

Plant Science Department, University of Tabriz, 51666 Tabriz, Iran

In the northwestern part of Iran (Azerbaijan province), there are numerous, active mining areas with heavy metal-rich soils. The plant vegetation growing on these soils could represent a specific flora having potential hyperaccumulators, accumulators and excluder species. In this work, soils rich in Ni, Zn, Cu and Mn and plants were identified during April to September in 2002–2003. Plant species belonging to 39 families were collected and the heavy metals in the above-ground parts of specimens were analyzed. For the majority of species, though metal concentrations were orders of magnitude higher than plants growing on soils without mineralization, the concentrations were below the threshold defining hyper-accumulation. The Ni, Zn, Cu and Mn excluder species that grow on metal rich soils but restrict metal absorption and/or transport into shoots were also identified. These species are suitable for the rehabilitation and stabilization of contaminated lands around mining areas as well as the remediation of agricultural soils affected by wastewaters of metal smelters in mining sites of NW Iran.

Key words: flora, accumulator species, heavy metal, Iran

Introduction

The presence of high levels of metals in soils exerts a pressure on plant species leading to the selection of a specific flora. Plants growing on metal-loaded soils respond by exclusion, indication or accumulation of metals (BAKER 1981). A number of plant species endemic to metalliferous soils accumulate metals to extraordinarily high levels (> 1%) as compared to the normal concentrations in plants (SHALLARI et al. 1998).

A hyperaccumulator is defined as a plant with a concentration of heavy metal in its above ground parts, 10–500 times higher than in usual plants (SHEN and LIU 1998).

The existence of metal-accumulating plants has attracted the attention of plant scientists for many years. These plants are strictly confined in their distribution to soils enriched in heavy metals, either naturally through mineralization or as a result of the extraction and smelting of matalliferous resources (VAZQUEZ et al. 1992). Approximately 45 families containing metal-accumulating species and more than 400 hyperaccumulator species have been reported. In addition to taxonomic applications, some hyperaccumulator species are used as geobotanic indicators in mineral exploration (BROOKS 1998a).

* Corresponding author, e-mail: ehsan@tabrizu.ac.ir

Ni-rich soils mainly belong to the serpentine type. Serpentine soils are considerably different from normal soils, being very rich in Ni and containing high amounts of chromium, cobalt, iron and magnesium (BROOKS 1987). Serpentine floras are the most extreme examples of changes occurring in plant vegetation because of the substrate, so that geological boundaries are readily observable due to differentiation in the flora (BROOKS 1998a). It was suggested that nickel is the element responsible for serpentine vegetation. The total number of Ni hyper accumulator plants exceeded 400 (ROBINSON et al. 1997). The Ni hyperaccumulation threshold level has been set at 5000 (BROOKS 1983) or 1000 $\mu g\ g^{-1}$ dry weight (BAKER et al. 2000; POLLARD et al. 2002).

Plant communities growing over Zn deposits have a certain similarity with serpentine flora. In a recent report on the Zn hyperaccumulators, 16 hyperaccumulators were introduced which accumulated up to 1% Zn in dry weight (BROOKS et al. 1995). The hyperaccumulation threshold level for Zn has been set at 10,000 $\mu g\ g^{-1}$ dry weight (BROOKS 1983).

There are only few regions in the world where a true copper flora exists (BROOKS 1998a). The presence of 50 species endemic to copper/cobalt deposits was reported (BROOKS et al. 1995) and 24 copper hyperaccumulators were identified (BROOKS 1998a). The hyperaccumulation threshold level for Cu has been set at 5000 (BROOKS 1983) or 1000 $\mu g\ g^{-1}$ dry weight (BAKER et al. 2000; POLLARD et al. 2002).

In contrast to Ni, Zn and Cu, the occurrence and geobotany of Mn hyperaccumulators has not been fully studied. This is because very little is known about the uptake of Mn by plants and it is not a common pollutant. However, 11 Mn hyperaccumulators from three families were reported (BROOKS 1998a) and the Mn hyperaccumulation threshold level has been set at 10,000 $\mu g\ g^{-1}$ dry weight (BROOKS 1983).

Hyperaccumulator plants represent a potential for the remediation of soils polluted by heavy metals (BAKER et al. 2000). Hence, it is necessary to obtain more information about the flora existing on metal-enriched soils in order to determine their potential for the management of polluted soils and particularly for metal extraction (SHALLARI et al. 1998).

New hyperaccumulators will be identified by more studies on metal-enriched environments. There are abundant metal resources in Azerbaijan province, NW Iran, and the province is characterized by a high density of mines and metal smelters. These metal resources include Cr-Ni, Pb-Zn, Cu and Mn. From the geological point of view, Azerbaijan is the most complex part of the Iran Plateau and is characterized by numerous sites of metal and non-metal element-rich deposits (CALAGARI, 2003). Deposition of metals took place during various metallogenic periods and heavy metal rich soils were formed by the destruction, movement and sedimentation of these deposits.

This work was undertaken to acquire information about the flora of heavy metal-rich sites in Azerbaijan province with a special emphasis on metal accumulation by plants.

Materials and methods

Studies were undertaken during two successive growing seasons including April– September 2002 and 2003. Azerbaijan is located in the NW Iran (Fig. 1), with an average altitude of 1500 m, an annual precipitation of 257.5 mm and annual average temperature of

12.3 °C. The mean temperature in summer is 26.4 °C and in winter is -1.3 °C. This province can be classified bioclimatically as Mediterranean.

Metal rich sites were described in this work according to recent research on the geology of NW Iran (CALAGARI 2003, KHALATBARI-JAFARI et al. 2005, MOAZZEN and MODJARRAD 2005).

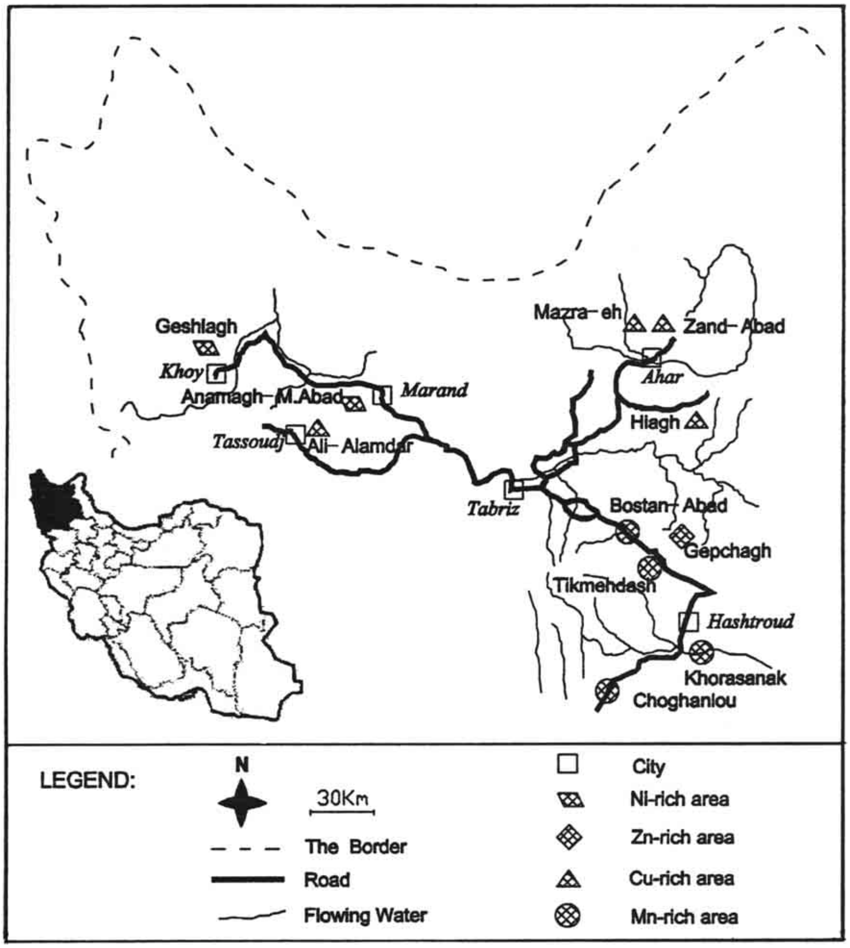

Fig. 1. Map of NW Iran indicating the studied sites.

Soil samples were taken from the plant sampling location of the upper horizon at a depth of approximately 15 cm. Samples were air dried and sieved through a 2-mm screen. All plant species grown at each site were collected during two time intervals including April–May and August–September in two years (2002–2003). For each species 5–6 samples were taken at each site and time; one of them was prepared for taxonomic identification and the others were used for elemental analysis. The identification of species was realized with the Flora Iranica (RECHINGER 2002) and Flora of Turkey (DAVIS 1988).

Soil pH and EC were determined in the saturated soil paste with a pH and EC-meter. Soil texture was determined using the hydrometer method (GEE and BAUDER 1986) and organic carbon was analyzed using the method of WALKELY and BLACK (1934). The concentration of heavy metals in a water-soluble and NH_4-acetate (AAc) extractable fraction in soil (2h, 5 m L g^{-1} soil) was determined according to the method described by JIANG et al. (2004).

For analysis of plant specimens, the leaves of each plant were separated and washed with double-distilled water, blotted on filter paper and dried at 70 °C for 2 days for determination of dry weight. The dry weight (DW) of samples varied between 0.5 and 2.0 g. Four replicates consisting of leaves of four plants were analyzed for each plant species and heavy metal. For determination of Zn, Cu and Mn, samples were ashed in a muffle furnace at 550°C for 8 h and for Ni, samples were digested with 1:1 perchloric acid/nitric acid mixture (1:8 W/W) on a heating plate (200°C) for 5 h. Thereafter, samples were suspended in 2 ml 10% HCl and made up to volume (25 mL) with double-distilled water. Metal concentrations were determined by atomic absorption spectroscopy (Shimadzu, AA 6500) using 232, 213.9, 324.8 and 279.5 nm for Ni, Zn, Cu and Mn determination respectively provided by metal-specific lamps. Reference standard solutions (Merck) were used after proper dilution for calibration of the instrument. All collected plant specimens were analyzed, but only those belonging to species with the highest and lowest accumulation are reported.

Investigated area

Ni-rich sites

Geshlagh: This site is located near the city of Khoy, 190 km from Tabriz (45°38' E, 38°22' N) at an elevation of 1750 m. The site covers an area of 500 m^2. Annual average temperature is 13 °C with a precipitation of 294 mm and real transpiration of 283 mm. This site can be classified bioclimatologically as temperate semi-humid with dry and warm summers. The main geological units in this site include metamorphic (amphibolite and greenschist), mafic, ultramafic and serpentine rocks. The majority of ultramafic rocks are lherzolite. These rocks are suitable substrates for the accumulation of heavy metals such as Cr and Ni. The total reservoir of Ni is 10,000 tons.

Anamagh and Mahboob-Abad: This site is located in north of Mishoudagh, near the city of Marand, 65 km south of Tabriz (45°38' E, 38°22' N) at an elevation of 1850 m. The site covers an area of 2 km^2. Annual average temperature is 11 °C, annual precipitation is 426 mm, and real transpiration is 370 mm. This site can be classified as temperate humid with dry and warm summers. Mafic rocks such as diabasic and gabbroic rocks are present in this area.

Zn-rich sites

Gebchagh: This site is located 75 km from Tabriz (46°58' E, 37°43' N), at an elevation of 1960 m. The area covered by the site is 1000 m^2. Annual average temperature is 8 °C, annual precipitation is 268 mm and real transpiration is 250 mm. This site can be classified as cold and humid with dry and cold summers. Sulphur-silicon mineralization took place in this area and silica bands low in Fe and rich in Zn, Cd, Pb and Mn could be found. The total reservoir of Zn is 50,000 tons.

Cu-rich sites

Copper mines are dominant in the mining in Azerbaijan, for the total reservoirs of Cu mines in this province come to 160 million tons.

Hiagh: Located east of the city of Hiagh, 120 km from Tabriz (45°15' E, 37°12' N) at an elevation of 2450 m. The area covered by the site is 3 km^2. This site localized near the intrusive mass of quartz monzonite. A porphyry type of Cu mineralization took place and at marginal areas there are numerous bands of Cu, Pb and Zn.

Zand Abad and Mazra-eh: Both of these areas are located north west of the city of Ahar, 120 km from Tabriz (47°5' E, 38°37' N) at an elevation of 2010 m. The area covered is 500 km^2. This site is situated at the marginal area of a granitoid mass in Shivar-Dagh. A skarn type of mineralization (Fe-Cu skarn) took place and the majority of Cu deposits are localized in exo-skarn.

Annual average temperature for these two sites is 10 °C, annual precipitation is 314 mm and real transpiration is 283 mm. Both of these sites can be classified as temperate semi-humid with dry and cold summers.

Ali Alamdar: This site is located northeast of Tassoudj, 75 km from Tabriz (45°30' E, 38°37' N), at an elevation of 2300 m. with an annual average temperature of 9 °C, annual precipitation of 323 mm and real transpiration of 294 mm and the area covered is 200 km^2. This site can be classified as temperate humid with dry and cold summers. The mineral deposits are lens-shaped to band form. Barite commonly occurs in Cu and Pb veins in limestones.

Mn-rich sites

Manganese mines are the second dominant mines in Azerbaijan, and the total reservoirs of Mn are 400,000 tons in this province. The annual average temperature of these sites is 11.8 °C, annual precipitation is 323 mm, and real transpiration is 294 mm.

Tikmeh-Dash and Bostan Abad: Both of these sites are situated near Tabriz (50 km) at an elevation of 1895 m (47°56' N, 37°44' N). The area covered is 20,000 m^2. This site can be classified as temperate humid with dry and cold summers. The Mn skarn have a thickness of 2 km, trace amounts of Cu mineralization could be found. In Bostan Abad, Mn, Zn and Pb mineralization also took place.

Khorasanak: This site is located near the city of Hashtroud, 115 km from Tabriz (47°10' E, 37°20' N) at an elevation of 1850 m. The area covered is 500 km^2. Manganese-rich bands are localized within the gypsum deposits.

Choghanlou: This site is located near the city of Hashtroud, 150 km from Tabriz (46°50' E, 37°43' N) at an elevation of 2200 m. The area covered is 10,000 m^2. Mn is associated with Fe (Hematite) and sedimentary rocks (Travertine).

Results

During two growing seasons, 254 specimens were collected from 11 sites and determined. These species belong to 39 families. The family Asteraceae was the most abundant plant familygrowing on heavy metal-rich soils, Cupressaceae, Fumariaceae, Onagraceae, Paronochiaceae, Polygalaceae, Ranunculaceae, Thymelaceae and Urticaceae, each having only one species among collected specimens, were the least frequent plant families occurringin these sites (Tab. 1).

Tab. 1. Flora of Ni, Zn, Cu and Mn-rich soils in Northwest of Iran. **1** – Geshlagh (Khoy), **2** – Anamagh and Mahboob Abad, **3** – Gepchagh, **4** – Hiagh, **5** – Zand-Abad, **6** – Mazra-eh, **7** – Ali-Alamdar, **8** – Tikmedash, **9** – Bostan-Abad, **10** – Khorasanak, **11** – Choghanlou.

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Apiaceae											
Daucus orientalis L.		+									
Eryngium caeruleum Bieb.		+		+	+		+				+
Ferula sp.					+						
Peucedanum sp.		+					+				
Scandix iberica Bieb.	+										
Zozima absinthifolia Vent.		+									
Asteraceae											
Achillea sp.						+	+		+		
Achillea millefollium L.	+		+								
Achillea santoliona L.					+					+	
Achillea vermicularis Trin.				+							+
Anthemis tinctoria L.		+									
Cartamus oxycantha M.B.										+	
Centaurea virgata Lam.	+		+	+	+	+		+	+	+	+
Chrysanthemum sp.			+					+			
Cichorium intybus L.		+	+	+	+				+		
Circium sp.	+	+	+	+	+	+			+		
Cirsium lanceotatum L.								+			
Cousinia sp.				+						+	
Cousinia calocephala Jaub.								+			
Crepis sp.					+						
Crepis rigida Waldst. Kit.							+				
Echinops sp.					+						
Echinops persicum Stev.	+										
Echinops pungens Trautv.		+									+
Helychrisum argenteum L.	+										
Helychrisum armenium D.C.			+								
Helychrisum pallasii Sprengel.		+	+		+	+					
Lactuca sp.											+
Onopordon sp.								+	+		
Scabiosa sp.								+	+		
Scabiosa argentea L.				+							

Tab. 1. – continued

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Scariola sp.	+					+					+
Scariola orientalis Boiss.				+							
Scariola leucoclada Rech. Tulsl.							+		+		
Senecio sp.	+					+					
Senecio vulgaris L.							+				
Tanacetum sp.		+		+	+		+				
Tanacetum kotschyi Bois.										+	
Tanacetum myriophyllum Willd.										+	
Asteraceae											
Taraxacum microcephaloides V. S.						+					
Thevenotia sp.								+			
Tussilago farfara L.		+			+						
Xeranthemum squarrosa Boiss.			+								
Xeranthemum sp.					+						
Brassicaceae											
Alyssum sp.				+					+		
Alyssum dasycarpus Steph.			+								
Alyssum alpestre L.											+
Alyssum campestre L.											+
Alyssum desertorum Stapf.			+							+	+
Alyssum lanigerum DC.	+										
Alyssum Murale Walast.								+		+	
Alyssum strigosum Schrad.								+			
Alyssum szowitsianum Fish.							+				
Erysimum sp.	+					+				+	+
Goldbachia laevigata L.											+
Lepidium sp.									+		
Malcolmia Africana L.											+
Nonea persica Boiss.						+					
lEuclidium syriacum L.											±
Borraginaceae											
Alkanna sp.									+		
Alkanna orientalis L.			+			+					
Moltkia caerula Willd.										+	
Myosotis sp.											+
Myosotis sparsiflora Mikan.										+	
Nonea persica Boiss.										+	
Onosma microcarpus DC.	+										
Onosma pachypodum Boiss.	+										

Tab. 1. – continued

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Onosma bulbotrichum DC.	+										
Caryophyllaceae											
Acanthophyllum gracile Bge.	+										
Cevastium sp.		+									
Dianthus sp.	+			+		+					
Dianthus ta					+						
Herniaria sp.								+			
Holosteum sp.											+
Silene sp.		+						+			+
Silene aucheriana Boiss.					+						
Chenopodiaceae											
Chenopodium sp.		+			+		+				
Convulvulaceae						+					
Convulvulus sp.								+			
Cupressaceae											
Juniperus communis Rech.		+									
Cyperaceae											
Carex sp.			+								
Crassulaceae											
Sedum sp.						+					
Dipsaceae											
Cephalaria sp.								+			
Scabiosa argentea L.			+								
Equisetaceae											
Equisetum ravens L.		+			+						
Euphorbiaceae											
Euphorbia sp.						+					
Euphorbia gerardiana Jacq.		+	+	+	+		+	+		+	+
Fabaceae											
Astragalus sp.	+		+	+	+	+	+	+	+	+	+
Astragalus hohenackeri Boiss.										+	
Hedysarum ibericum M. Bieb.											+
Lotus corniculatus L.			+					+			
Medicago sativa L.			+	+				+			
Melilotus officinalis L.		+			+						
Fumariaceae											
Fumaria vaillantii Loise.	+										+
Geraniaceae											
Geranium tuberosum L.						+					

Tab. 1. – continued

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Hypericaceae											
Hypericum sp.							+				
Hypericum perforatum L.		+	+		+						
Iridaceae											
Gladiolus atroviolaceus Boiss.										+	
Iris acutiloba C.A. Mey.										+	
Lamiaceae											
Ajuga sp.					+						
Calamintha sp.		+			+						
Marrubium pariviflorum Fish Mey	+		+				+				
Menthea longifolia (L.) Hudson		+			+	+					
Nepeta sp.		+			+	+					
Phlomis caucasica Rech. Fil	+			+			+	+			
Phlomis pungens Willd			+								
Salvia sp.	+		+					+			+
Stachys sp.							+				
Stachys inflata Bentham.						+					
Stachys lavandulifolia Boiss.	+		+	+						+	
Teucrium sp.			+					+			
Lamiaceae											
Thymus sp.									+		
Thymus kotschyanus Boiss.	+		+		+		+	+			
Ziziphora clinopodioides Lam.						+					
Liliaceae											
Allium sp.						+					
Muscari sp.						+					
Ornithogalum sp.											+
Linaceae											
Linum perenne L.										+	
Onagraceae											
Epilobium minutiflorum He.		+			+						
Papaveraceae											
Glaucium corniculatum L.	+									+	
Hypecoum pendulum L.											+
Papaver sp.		+		+		+	+				
Papaver dubium L.					+						
Paronochiaceae											
Herniaria sp.	+										

Tab. 1. – continued

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Plantaginaceae											
Plantago lanceolata L.			+								
Plumbaginaceae											
Acantholimon sp.			+								
Acantholimon hohenackeri (Jaub Spach) Boiss	+				+			+			
Poaceae											
Aira sp.	+										
Agropyrom sp.		+	+		+		+	+			
Agropyrum repens L	+										
Agropyrum orientale L.										+	
Agropyrum cristatum (Schreb) Gaertn.	+										
Agrostisa sp.								+			
Avena fatua L.				+							
Boissiera pumilio (Trin.) E. Hack								+			
Bromus sp.					+	+					
Bromus macrostachys Desf.			+								
Bromus tectorum L.	+						+	+			
Bromus tomentellus Boiss.	+										
Cynodon dactylon (L) Pers.								+			
Cynosorus echinatus L.			+								
Dactylis glomerata L.	+	+		+	+	+		+			
Eragrostis sp.			+								
Eremopoa arundinaceae L. Rosh.			+								
Poaceae											
Eremopyrum sp.	+										
Festuca sp.		+			+		+				
Melica cupani Guss.		+			+						
Poa bulbosa L.			+		+		+				+
Poa pratensis L.		+									
Poa trivialis L.								+			
Secale montanum Guss.		+									
Stipa barbata Desf.			+		+			+	+	+	+
Polygalaceae											
Polygala sp.									+		
Polygonaceae											
Atraphaxis spinosa L.				+							

Tab. 1. – continued

Heavy metal-enriched soils	**Ni**		**Zn**	**Cu**				**Mn**			
Site code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Rumex sp.				+							
Polygonum sp.						+					
Polygonum alpestre C.A. Mey							+				
Polygonum maritimum L.								+			
Ranunculaceae											
Adonis aestivalis L.											+
Ceratocephalus falcatus L.											+
Ficaria kochii Ledeb.						+					
Rosaceae											
Poterium sanguisorba L.			+		+						
Rosa canina L.					+	+					
Rubiaceae											
Asperula orientalis Boiss. Et Hohen.											+
Galium leiophyllum Boiss.					+						
Salsolaceae											
Ceratocarpus arenarius L.							+				
Noaea mucronata Forsk.	+	+							+		+
Scrophulariaceae											
Scrophularia sp.		+			+						
Verbascum speciosum Schrad.	+	+		+	+	+	+	+			+
Veronica angallis L.		+	+		+						
Veronica multifida Bed. Linn											+
Thymelaeceae											
Daphne mucronata Royle.		+									
Urticaceae											
Urtica dioica L.		+									
Violaceae											
Viola sp.	+										

Concentration of Ni, Zn, Cu and Mn in the soils

The concentration of heavy metals in both water soluble- and AAc-fractions was much higher in samples collected from the outer surface of mine rocks (for Ni and Zn) or from the vicinity of mine (Cu and Mn), than those collected a distance of 5–10 m from it. The amount of labile metal fractions (AAc-fraction) was 14% to 600-times higher than readily absorbable fractions (water soluble heavy metals) depending on heavy metal species and soil type (Tab. 2).

Tab. 2. Description of sites and soil characteristics in soils rich in Ni, Zn, Cu and Mn from Northwest of Iran. **1** – Geshlagh (Khoy), **2** – Anamagh and Mahboob Abad, **3** – Gepchagh, **4** – Hiagh, **5** – Zand-Abad, **6** – Mazra-eh, **7** – Ali-Alamdar, **8** – Tikmedash, **9** – Bostan-Abad, **10** – Khorasanak, **11** – Choghanlou.

Site code	Description	pH	EC ($d\ Sm^{-1}$)	Soil Texture			%OC	Water soluble ($\mu g\ Kg^{-1}$)	NH_4 Ac-extractable ($\mu g\ Kg^{-1}$)
				% Sand	% Silt	% Clay			
	Ni-rich soils								
1	On mine rock	7.9	0.51	40	48	12	1.17	648±29	258±41
1	With plant cover	7.7	0.46	50	36	14	1.50	359±31	524±21
2	On mine rock	7.5	0.42	82	8	10	1.89	275±12	321±18
2	With plant cover	7.6	0.52	68	14	18	1.91	58±9	228±17
	Zn-rich soils								
3	On mine rock	7.5	0.40	62	19	20	0.76	21±4	157±12
3	Near mine with plant cover	7.7	0.49	40	28	32	0.85	8±2	129±2
3	Around mine with plant cover	7.5	0.42	50	30	20	0.75	6±1	105±9
	Cu-rich soils								
4	With plant cover	7.6	0.96	80	16	4	2.1	8.70±0.9	46.3±8.0
5	Near mine with plant cover	8.1	0.54	52	30	18	0.58	29.5±4.8	345±7.2
5	Around mine with plant cover	7.2	0.58	36	32	32	2.98	9.93±0.8	56.6±0.8
6	Near mine with plant cover	7.0	0.62	34	34	32	3.21	30.5±5.8	18881±1020
6	Around mine with plant cover	7.3	0.59	33	34	33	3.81	69.3±5.4	1276±184
7	Near mine with plant cover	8.0	4.10	74	22	4	0.89	7.42±0.1	70.7±14.1
7	Around mine with plant cover	8.3	1.65	44	22	34	1.4	11.6±1.2	18.8±3.8
	Mn-rich soils								
8	Near mine with plant cover	8.0	0.44	46	28	26	2.04	0.86±0.01	6.14±1.01
9	Near mine with plant cover	8.1	0.46	32	34	34	1.02	6.28±1.11	10.12±0.11
10	Near mine with plant cover	7.7	3.46	34	30	36	0.17	0.98±0.12	4.35±0.10
10	Around mine with plant cover	7.8	1.61	16	36	48	0.23	1.20±0.79	5.21±0.11
10	Around mine with plant cover	7.6	2.57	16	38	46	0.31	1.76±0.65	5.17±0.09
11	Near mine with plant cover	8.0	0.63	42	32	26	1.80	2.24±0.83	1.65±0.41
11	Around mine with plant cover	8.0	0.54	38	38	24	1.20	1.90±0.11	3.79±0.10
11	Around mine with plant cover	8.2	0.59	41	34	25	1.56	1.10±0.12	2.53±0.08

The highest Ni concentration in water soluble fraction was found in soil samples collected directly from mine rock without plant cover (648 μg kg^{-1}), while in contrast, the highest AAc-extractable amount from the same area (Geshlagh) was observed in samples collected from soil covered by plants (524 μg kg^{-1}). Zinc concentration in AAc-Ac fraction of soil with plant cover at Gebchagh (129 μg kg^{-1}) was 16 times higher than that in a water soluble fraction (8 μg kg^{-1}), while the difference between these two fractions was lower for soil without plant cover e.g. only 7 times higher than the water soluble fraction. AAc-extractable copper concentrations in the soils of Hiagh and Alialamdar were 18 to <100 μg kg^{-1}. Corresponding amounts for Zandabad and Mazraeh were significantly higher; the highest AAc-extractable Cu (18 mg kg^{-1}) was found in soil samples from Mazraeh collected from the immediate vicinity of the mine but with plant cover. The average manganese concentration in the soil was 4.78 with the highest value observed in samples from Khorasank and Bostan-Abad with up to 10 μg kg^{-1} extractable manganese. The site Choughanlou was low in Mn, e.g. 1.65 μg kg^{-1} in soil in the immediate vicinity of the mine (Tab. 2).

Concentration of Ni, Zn, Cu and Mn in plants

As mentioned above, different sites rich in given heavy metals have different metal availabilities for plants. Accordingly, concentration of heavy metals in plant specimens reflected mainly the concentration of the available metals in soils. For a given site, accumulator plants were found along with plants with a low content of metal (Tab. 3).

Ni concentration in plants varied from 3 to 186 μg g^{-1} DW and was dependent on species and collection site. The Ni concentration of plants collected at the Anamagh site, was low, ranging from a few μg g^{-1} DW to <50 mg μg g^{-1} DW. On Geshlagh soils, however, the range of variation of Ni concentration in plants was considerable. The maximum concentration of Ni was observed in the leaves of *Senecio vulgaris* and *Acanthophyllum gracile* (89–186 μg g^{-1} DW) collected at Geshlagh site. Low values for *Onosma pachypodum* (Geshlagh), *Tussilago farfara* and *Euphorbia gerardiana* (Anamagh) were recorded (average 20 μg g^{-1} DW).

In some plant species, including *Lotus corniculatus*, *Helycrisum pallasii* and *Chrysanthemum* sp., the Zn concentration of leaves exceeded 1000 μg g^{-1} DW and the average concentrations for other specimens were in the range of 200–800 μg g^{-1} DW. For a few species Zn concentration was lower than 50 μg g^{-1} DW, e.g. *Alyssum desertorum*, *Cirsium* sp. and *Cynosorus echinatus*.

Copper concentrations of some species collected from the three studied Cu-rich sites were in the range of 1000–16000 μg g^{-1} DW. The highest Cu concentration was observed in *Phlomis caucasica* (Lamiaceae) collected at Ali-Alamdar. A large number of species collected from all four Cu-rich sites have a Cu concentration of about 50–100 μg g^{-1} DW, followed by the species that had accumulated either lower than 10 or higher than 900 μg g^{-1} DW Cu in leaves.

A wide range of Mn concentration was observed in plants collected from the four studied Mn rich sites. Plants mainly accumulated 50–300 μg Mn in g^{-1} DW. Species that accumulate 700–2000 μg g^{-1} DW were not abundant, e.g. *Asperula orientale* (1821 μg g^{-1} DW), *Ornithogalum sp*. (1236 μg g^{-1} DW), *Astragalus* sp. (1180 μg g^{-1} DW) and *Lactuca* sp. (948 μg g^{-1}DW). Manganese concentration of *Bromus tectorum*, *Cousinia calocephala* and *Goldbachia laevigata* was as low as 3 μg g^{-1} DW.

Tab. 3. Amounts of Ni, Zn, Cu and Mn (µg g^{-1}DW) in leaves of plant species collected from metal rich soils of Northwest of Iran. Amounts are mean±SD (n=4). Sites: 1. Geshlagh (Khoy) 2. Anamagh & Mahboob Abad 3. Gepchagh 4. Hiagh 5. Zand-Abad 6. Mazra-eh 7. Ali-Alamdar 8. Tikmedash 9. Bostan-Abad 10. Khorasanak 11. Choghanlou.

Heavy metal	Site code	Plant species with the highest concentration	Concentration (µg g^{-1}DW)	Site code	Plant species with the lowest concentration	Concentration (µg g^{-1}DW)
Nickel	1	*Senecio vulgaris*	186±27	2	*Euphorbia gerardiana* Jacg.	34±5
	1	*Acanthophyllum Gracile* Bge	175±13	2	*Tussilago farfara* L.	22±3
	1	*Fumaria vaillantii* Loise	95±12	1	*Alyssum lanigerum*	14±2
	1	*Stachys lavandulifolia* Boiss.	89±21	1	*Onosma pachypodum* Boiss	3±2
Zinc	3	*Lotus corniculatus* L.	1279±54	3	*Alyssum desertorum* Stapt	25±5
	3	*Helychrisum pallasii*	1183±38	3	*Cirsium sp*	16±14
	3	*Chrysanthemum sp.*	1139±41	3	*Cynosorus echinatus* L.	17±6
Copper	7	*Phlomis caucasia* Rech. Fil	16624±858	5	*Tanacetum sp.*	31±11
	5,6	*Nepeta sp.*	2038±257	5	*Silene aucheriana*	28±9
	7	*Stachys inflata* Bentham.	1625±43	7	*Eryngium caerukeum* Bieb.	27±8
	5,6,7	*Verbascum speciosum* Schrad.	1156±28	7	*Poa bulbosa* L.	20±11
	6	*Alkanna orientalis* L.	968±56	5,6,7	*Achillea sp.*	19±5
Manganese	11	*Asperula orientale*	1821±65	8	*Bromus tectorum* L.	6±5
	11	*Ornithogalum sp.*	1236±101	8	*Cousinia calocephala* Jaub.	4±4
	10,11	*Astragalus sp.*	1180±74	11	*Goldbachia laevigata* L.	3±2
	11	*Lactuca sp.*	948±98			

Discussion

The floristic composition of each region is dependent on climate, physical geography as well as the chemical properties of soil. Therefore, it is not surprising that none of the reported elements of the flora of heavy metal-rich soils in the world was found in this work. The flora of Iran belongs mainly to the flora of the Orient, which includes the phyto-geographical region termed Irano-Touranian. The main characteristics of the Irano-Touranian region is the extreme diversity of the physical geography, low rainfall, extremely wide oscillations of temperature, the characteristic flora and vegetation, the enormous development of certain systematic groups, a high total number of species and a considerable number of endemic species and finally, the pronounced specialization of systematic groups above species. This is the explanation of the importance of any floristic study in general and that of heavy metals rich-soils in particular, also for other mining areas in the country.

Comparison of water soluble and AAc-extractable fractions, particularly for soil samples collected from the immediate vicinity of plant cover showed that vegetation could have an important role in mobilization and increasing the availability of heavy metals for plants. This was obvious particularly for Ni and Cu rich soils. Changes such as the release of organic chelators to the rhizosphere have been reported to increase the availability of Ni in accumulating species (WENZEL et al. 2003).

The water soluble and AAc-extractable Ni content in soils varied between 58 to 258 µg kg^{-1} and 228 to 648 µg kg^{-1} respectively. The amounts of the water soluble fraction were rather high compared with values reported by WENZEL et al. (2003) from serpentine soils in Austria (120 µg kg^{-1}), but the amount of Ni in AAc-fraction was much lower than the labile Ni concentration of that site (7720 µg kg^{-1}). Considering the effect of plants in the elevation of heavy metal availability, this difference could be the result of different vegetation density in the soils of these two sites. The samples from Geschlagh had higher concentrations of available Ni (648 µg kg^{-1}) than samples from Anamagh and Mahboob Abad (329 µg kg^{-1}). In Gechlagh, soil was derived from gabbros and ultrabasic rocks rich in Fe, Ni and Cr and the total Ni reservoir is 10,000 tons.

The Zn content of soil samples collected from the only Zn rich site in NW Iran, Gebchagh, varied from 6 to 21 µg kg^{-1} and 105 to 157 µg kg^{-1} for water soluble and exchangeable Zn respectively. This amount was much lower than metal values reported by ERNST et al. (2000) for water soluble Zn of a polymetallic soil from Belgium (10–130 mg kg^{-1}).

The site of Mazrah-eh appeared to be the site richest in Cu. Copper was present at high levels in the soil of this site and reached 70 µg kg^{-1} and 18,880 µg kg^{-1} in water soluble and exchangeable (AAc extractable) fractions respectively. Copper concentration in the water soluble fraction in one Cu-rich soil in central Europe was reported to be 5400 µg kg^{-1}(ERNST et al. 2000) and in AAc extractable fraction of a soil from Cu-mining area in South China was reported to be 2850 µg kg^{-1} (JIANG et al. 2004). Considering a factor of 1000 difference between water soluble and total Cu (ERNST et al. 2000, WENZEL et al. 2003), total Cu in soil of Mazrah-eh could be estimated in a range of 8–70 mg kg^{-1}. The total Cu concentration of soil in a copper/cobalt deposit in Zaïre was estimated from 0.28 to 25 mg kg^{-1} (BROOKS 1998b).

Concentration of Ni in leaves of *Senecio vulgaris* and *Acanthophyllum gracile* (89–186 µg g^{-1} DW) collected at Geshlagh site were 5–10 times higher than Ni concentrations of normal plants grown in mineralized soil (20 µg g^{-1} DW) (BROOKS 1983). Nickel hyperaccumulators indicate ultramafic substrates and they are associated with the presence of nickel, chromium and cobalt (SHALLARI et al. 1998). Most of the Ni hyperaccumulators have been found on serpentine soils, high in Ni, Cr, Mg and Fe, but low in Ca, P and other plant nutrients. Approximately 316 taxa containing 0.1–3% Ni have been identified worldwide as Ni-hyperaccumulators (BROOKS 1998a).

Zinc concentration of leaves in *Lotus corniculatus*, *Helycrisum pallasii* and *Chrysanthemum* sp. (1139–1279 µg g^{-1} DW) and the average concentrations in other specimens (200–800 µg g^{-1} DW), were 2–10 times higher than Zn concentrations of normal plants grown in mineralized soil (100 µg g^{-1} DW) (BROOKS 1983). In the studied Zn rich site sulfur-silica mineralization took place and Zn is found in association with Cd and Pb. A true Zn flora was found in western Germany and eastern Belgium where the soils are rich in zinc but do not contain high levels of copper or lead (BROOKS 1998a).

Approximately 69% of specimens collected from all four Cu-rich sites, have a Cu concentration of about 50–100 µg g^{-1} DW, followed by species accumulating 200–600 µgg^{-1}DW (17%), species with more than 900 µg g^{-1} DW (9%) and species with a Cu concentration of 20–30 µgg^{-1} DW (5%). The latter amounts were found in *Bromus tectorum*, *Cousinia calocephala* and *Goldbachia laevigata,* which could be classified as copper excluder species (BROOKS 1983).

If a hyperaccumulator is considered to be a plant containing concentrations of heavy metals in the above-ground part 10–500 times more than usual in the same plant (SHEN and LIU 1998), the Ni concentration of 2 samples (*Senecio vulgaris* and *Acanthophyllum Gracile*) satisfied this standard value, as did the Zn concentration of 3 samples (*Lotus corniculatus, Helychrisum pallasii* and *Chrysanthemum* sp.) and Cu concentrations of 5 samples (*Phlomis caucasica*, *Nepeta* sp., *Stachys inflata, Verbascum speciosum* and *Alkanna orientalis*). But compared with the absolute concentrations of hyperaccumulators (Ni>5000, Zn>10000, Cu>5000 and Mn>10,000 µg g^{-1} shoot DW) (BROOKS 1983), only *Phlomis caucasica* satisfied this standard value and could be considered a Cu hyperaccumulator species. However, according to BAKER (et al. 2000) and POLLARD (et al. 2002) and considering the hyperaccumulation threshold of Cu at 1000 µg g^{-1} shoot DW, *Phlomis caucascia*, *Nepeta* sp*., Stachys inflata, Verbascum speciosum* and *Alkanna orientalis* could be identified as new potential hyperaccumulators. Further investigations are necessary to prove the hyperaccumulation trait of these species under laboratory conditions.

The highest Mn concentration found in four species (*Asperula orientale*, *Ornithogalum* sp. *Lactuca sp. Astragalus* sp.) was 948–1821 µg g^{-1} DW, much lower than the absolute standard value for hyperaccumulation (10,000 µg g^{-1} DW) and in the range of Mn concentration in normal plants in mineralized soils (1000 µg g^{-1} DW) (BROOKS 1983) and similar to the critical toxicity concentration of crop species such as sweet potato (1380 µg g^{-1} DW) and sunflower (5300 µg g^{-1} DW) (MARSCHNER 1995). In a few plant species including *Bromus tectorum*, *Cousinia calocephala* and *Goldbachia laevigata*, Mn concentration was even lower than the background concentration in normal plants (400 µg g^{-1} DW). These species could be considered strong Mn excluders.

There are very few examples of plants that hyperaccumulate copper. All of these are from the copper/cobalt deposits of southeastern Zaïre. BROOKS et al. (1995) reported the presence of 24 species growing on copper deposits in Zaïre, from these, 6 species accumulate Cu up to 5000 µg g^{-1} DW, however, none of them are found in the flora of Iran. In a herbarium survey, an extraordinary accumulation of copper by *Aeollanthus biformifolius* was observed (BROOKS et al. 1978). This species is confined to copper-rich substrates and contains up to 1.37% copper on a dry weight basis.

Plant species with lower uptake potential of heavy metals such as *Euphorbia gerardiana*, *Tussilago farfara*, *Alyssum lanigerum* and *Onosma pachypodum* (for Ni), *Alyssum desertorum*, *Cirsium* sp. and *Cynosorus echinatus* (for Zn), *Tanacetum* sp., *Silene aucheriana*, *Eryngium caerukeum*, *Poa bulbosa* and *Achillea* sp. (for Cu) and *Bromus tectorum*, *Cousinia calocephala* and *Goldbachia laevigata* (for Mn) could be considered potential pioneer species for the restoration of vegetation and the rehabilitation of metal-contaminated soils with minor dangers of metal toxicity for animals and humans. It was reported that grasses are suitable for the rehabilitation of metal contaminated soils, in terms of high tolerance to heavy metals, biomass production and coverage (WONG 2003).

The genus *Astragalus* is one of the most important elements of Iran's flora in general and in NW Iran in particular. The accumulating potential of plants from this genus has previously been reported only for selenium. Hyperaccumulators of selenium (>1000 µg g^{-1} DW) in at least 13 species of *Astragalus* were reported by BROOKS (1998a). In this work it was shown that some species of this genus have a high accumulating potential for Mn (1180 µg g^{-1} DW). The Cu accumulation capacity of this plant is also high (519 µg g^{-1} DW). However, because of difficulties in the taxonomy of this genus, an exact identification of specimens collected from Cu and Mn rich sites was not possible and should be identified in further investigations.

Numerous species from *Alyssum* were reported to be Ni hyperaccumulators (BROOKS 1998a). The genus *Alyssum* has the greatest number of individual hyperaccumulators of nickel with up to 48 species of nickel plants. The distribution of hyperaccumulating *Alyssum* species is unusual and they are confined to ultramafic substrates from Portugal in the west to the Iraq/Turkey/Iran border areas in the east. Anatolia is the site of their maximum multiplicity and diversity (BROOKS 1998a). However, in this work the only species from the *Alyssum* genus (*Alyssum lanigerum*) collected at Ni rich sites was identified as a typical Ni excluder plant. The concentrations of Zn in leaves of two collected *Alyssum* species from a Zn rich area e.g. *A. desertorum* and *A. dasycarpus* were 25 and 839 µg g^{-1} DW respectively and the concentration of Cu in *A. szowitsianum* collected from Cu rich sites was only 26 µg g^{-1} DW. Five out of 7 *Alyssum* species collected in this work belonged to Mn rich sites and the range of Mn accumulation in leaves was from 17 µg g^{-1} DW (*A. desertorum*), to 578 µg g^{-1} DW (*A. campestre*). Therefore, none of the *Alyssum* species collected in this work could be considered hyperaccumulator or accumulator species. It is known that species of one genus and ecotypes of a given species can be different in metal uptake and accumulation capacity. For example, the differences between various species of *Thlaspi* in accumulation capacity were attributed to the differences in their uptake systems (LASAT et al. 1996).

This is the first report on the flora of heavy metal-rich sites in NW Iran and the concentration of metals in above-ground parts of plants. Information on natural flora on these soils is needed for selecting suitable species for the remediation of contaminated soils as well as the rehabilitation of highly contaminated soils by some pioneer species capable of tolerating high concentrations of heavy metals without taking up toxicity-inducing concentrations for animals and human.

Acknowledgment

Authors thank Dr. M. Moayyed, University of Tabriz, Geology Department for introducing metal rich sites. This work was supported financially by the Research Council, University of Tabriz.

References

BAKER, A. J. M., 1981: Accumulators and excluders- strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654.

BAKER, A. J. M., MCGRATH, S. P., REEVES, R. D., SMITH, J. A. C., 2000: Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: TERRY, N., BANUELOS, G. (eds.), Phytoremediation of contaminated soil and water, 85–107. Lewis Publishers, Boca Raton, FL.

BROOKS, R. R., MORRISON, R. S., REEVES, R. D., MALAISSE, F., 1978: Copper and cobalt in African species of *Aeolanthus* Mart. (Plectranthinae, Labiatae). Plant Soil 50, 503–507.

BROOKS, R. R., 1983: Biological methods of prospecting for minerals. Wiley, New York.

BROOKS, R. R., 1987: Serpentine and its vegetation – a multidisciplinary approach. Dioscorides Press, Portland, OR,

BROOKS, R. R., DUN, C. E., HALL, G. E. M., 1995: Biological systems in mineral exploration and processing. Ellis Horwood, Hemel Hempstead.

BROOKS, R. R., 1998a: Geobotany and hyperaccumulators. In: BROOKS, R. R. (ed.), Plants that hyperaccumulate heavy metals, 55–94. CAB International, USA.

BROOKS, R. R., 1998b: Biogeochemistry and hyperaccumulators. In: BROOKS, R. R. (ed.), Plants that hyperaccumulate heavy metals, 95–118. CAB International, USA.

CALAGARI, A. A. 2003: Concentration variations of major and minor elements across various alteration zones in porphyry copper deposit at Sungun, East Azarbaidjan, Iran. J. Sci. I.R. Iran 14, 27–36.

DAVIS, P. H., (ed.) 1965–1988: Flora of Turkey and the east Aegean islands. Edinburgh, Edinburgh University Press.

ERNST, W. H. O., NELISSENN, H. J. M., TEN BOOKUM, W. M., 2000: Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. Environ. Exp. Bot. 43, 55–71.

GEE, G. W., BAUDER, J. W., 1986: Particle-size analysis. In: KLUTE, A. (ed.), Methods of soil analysis. Part 1, 2nd Ed. American Society of Agronomy, Madison, WI.

JIANG, L. Y., YANG, X. E., SHI, W. Y., YE, Z. Q., HE, Z. L., 2004: Copper uptake and tolerance in two contrasting ecotypes of *Elshotzia argyi*. J. Plant Nutr. 27, 2067–2083.

KHALATBARI-JAFARI, M., JUTEAU, T., BELLON, H., WHITECHURCH, H., COTTON, J, EMAMI, H. 2005: New geological, geochronological and geochemical investigations on the Khoy ophiolites and related formations, NW Iran. J. Asian Earth Sci. 23, 507–535.

LASAT, M. M., BAKER, A. J. M., KOCHIAN, L. V., 1996: Physiological characterization of roots Zn^{2+} absorption and translocation to shoot in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. Plant Physiol. 112, 1715–1722.

MARSCHNER, H., 1995: Mineral nutrition of higher plants. Academic Press, London.

MOAZZEN, M., MODJARRAD, M. 2005: Contact metamorphism and CSD studies in the Shivar aureole, NW Iran. Geol. J. 40, 499–517.

POLLARD, A. J., POWEL, K. D., HARPER, F. A., SMITH, A. C., 2002: The genetic basis of metal hyperaccumulation in plants. Crit. Rev. Plant Sci. 21, 539–566.

RECHINGER, H. K., (ed.) 1965–2002: Flora Iranica. Granz-Austria: Akademishe Druck, Verlagsanstalt.

ROBINSON, B. H., CHIARUCCI, A., BROOKS, R. R., PETIT, D., KIRKMAN, J. H., GREGG, P. E. H., De DOMINICIS, V., 1997: The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. J. Geochem. Explor. 59, 75–86.

SHALLARI, S., SCHWARTZ, C., HASKO, A., MOREL, J. L., 1998: Heavy metals in soils and plants of serpentine and industrial sites of Albania. Sci. Total Environ. 209, 133–142.

SHEN, Z. G., LIU, Y. L., 1998: Progress in the study on the plants that hyperaccumulate heavy metals. Plant Physiol. Commun. 34, 133–139.

VAZQUEZ, M. D., BARCELO, J., POSHENRIEDER, C. H., MADIGO, J., HATTON, P., BAKER, A. J. M., COPE, G.H., 1992: Localization of Zn and Cd in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can heperaccumulate both metals. J Plant Physiol. 140, 350–355.

WALKELY, A., BLACK, I. A. 1934: An examination of the Degtjareff methods for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.

WENZEL, W. W., BUNKOWSKI, M., PUSCHENREITER, M. HORAK, O., 2003: Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ. Pollut. 123, 131–138.

WONG, M. H., 2003: Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775–780.