Accumulation of Heavy Metals in Some Plants Grown on Serpentine Soils of Mersin, Turkey

Nurcan Koleli
Mersin University, Faculty of Engineering, Department of Environmental Engineering, 33342 Mersin, Turkey
nkoleli@mersin.edu.tr

Aydeniz Demir
Mersin University, Faculty of Engineering, Department of Environmental Engineering, 33342 Mersin, Turkey
aydenizdemir@mersin.edu.tr

Muzeyyen Eke
Mersin University, Faculty of Engineering, Department of Environmental Engineering, 33342 Mersin, Turkey
muzeyyeneke@hotmail.com

Ozlem Cakmak
Cukurova University, Faculty of Agriculture, Department of Soil Science, 01330 Adana, Turkey
ocakmak@cu.edu.tr

Abstract: The purpose of this study was to determine hyperaccumulator species growing in Findikpinari-Mersin. The soils derived from ultrafamic rocks lead to unusual and sparse associations of flora that are tolerant to extreme environmental conditions such as high heavy metal contents. As the geological structure, Mersin-Findikpinari has rocks containing ultramafic and serpentine, but this site is one of the less studied areas. The 26 specimens of total 755 plants identified systematically from Mersin-Findikpınari in between in 1997-2002 were randomly selected and studied whether hyperaccumulator or not. Twenty six plants collected (members of 26 genera and 8 families) from different sampling locations were analyzed for their total As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn concentrations using an ICP-MS. A certified reference material (SRM 1573A, SRM 1547) was also analyzed to check the accuracy of the used extraction technique. In the present study, Mn content (548 mg kg-1) of Anthemis aciphylla Boiss. (Asteraceae) was higher than the critical Mn value (300-500 mg kg-1) and Ni content (115 mg kg-1) Crocus graveolens Boiss&Reute (Iridiceae) was higher than the critical Ni value (10-100 mg kg-1) but unfortunately none of the plants studied was hyperaccumulator.

Keywords: hyperaccumulator, Findikpinari-Mersin, serpentine, heavy metal

Introduction

Heavy metal contamination in soil is a global environmental and health safety issue in the world. Remediation of contaminated soils is essential for sustainable soil use. Conventional remediation technologies for soils contaminated with heavy metal cations are generally termed as ‘pump and treat’ and ‘dig and dump’ techniques (Chin, 2007). They can be divided into either *in situ* or *ex situ* remediation. The conventional technologies used for *in situ* and *ex situ* remediation are typically expensive and destructive (Prasad and Freitas, 1999). The environmental impact of such technologies can be very high. For example, soil washing methods may render the soil infertile or spread the contaminant, and excavation methods can produce high waste volumes. Additionally, these remediation methods are often limited to small areas and depend on accessibility to the contaminated site (Chin, 2007). The high cost and environmental concerns of conventional remediation technologies has fuelled the need for alternative remediation method. Phytoremediation is one of alternative remediation technologies (Chaney *et* al., 1997; Chin, 2007). Phytoremediation is defined as the use of green plants to remove pollutants from the environment or render them harmless (Raskin *et* al., 1997). The five classes of phytoremediation are outlined below. (i) *Rhizofiltration,* (ii) *Phytostabilisation*, (iii) *Phytodegradation,* (iv) *Phytovolatilisation,* (v) *Phytoextraction* (Chin, 2007). The phytoextraction and rhizofiltration technologies are the most useful branches for heavy metal removal from soil and water respectively. The goal of phytoextraction is to reduce heavy metal levels in the soil to acceptable levels within three to ten years (Huang and Cunningham *et* al., 1996). In order to achieve this goal, plants must be screened and selected for certain attributes. The ideal plant for phytoextraction would have: (i) a rapid growth rate, even under harsh conditions, (ii) a high shoot biomass (20 metric tons dry mass (DM) ha^{-1} yr^{-1}) (Huang *et* al., 1997), and (iii) a capacity to accumulate or tolerate high amounts of metals in shoots; in the case of Pb, 10,000 mg kg^{-1} (1% DM) (Brooks, 1998). There are three types of metal-tolerant plants which are classified according to their tolerance and accumulation response on soils contaminated with heavy metal cations: (i) *excluders* - restrict metal uptake into roots except at extreme metal concentrations (ii) *indicator plants* - metal level accumulated in the shoot is relative to metal levels in soil and (iii) *hyperaccumulators* – concentrate metals in shoots, regardless of soil metal concentrations (Greger, 1999; Ghosh and Singh, 2005). Metal hyperaccumulator plants comprise species that accumulate (in mg kg^{-1})>10000 (Mn or Zn), >1000 (Cu, Co, Cr, Ni, Pb) or >100 (Cd) in their shoots (Baker and Brooks, 1989; Wenzel and Jokwer, 1999). Initial phytoextraction research began with hyperaccumulator plants, such as *Thlapsi caerulesences* and *Alyssum bertoloni* (Keller *et* al., 2003). Whilst these plants are useful for studying metal tolerance and accumulation mechanisms, their slow growth rate and small biomass may limit their application in phytoremediation (Ebbs and Kochian, 1998). This is because the total amount of metals extracted (a measure of phytoremediation potential) is the product of biomass and tissue concentration (Kayser *et* al., 2000). Of the over 450 plant species which have been identified as hyperaccumulators, about 75% of their have been Ni hyperaccumulators (Clemens, 2001). These hyperaccumulator plants have attracted the interest of plant and soil scientist because of their role in the development of phytoremediation technologies for the treatment of heavy metal contaminated soils, sediments and water resources (Wenzel *et* al., 1999; Lombi *et* al., 2000). For instance, some varieties of *Thlaspi* and ecotype of *Silene vulgaris* have been found to be Cd accumulators; *Larrea tridendata*, a desert inhabitant shrub, accumulates Cu, several wild species of *Sutera* accumulate Cr, and other cultivated species accumulate Cd, Cr and Cu, maize and ambrosia accumulate Pb (Gardea Torresday *et* al., 2004). However, researchers all over the world are searching new plant species susceptible to be used in phytoremediation (Gardea Torresday *et* al., 2004). First, hyperaccumulators are usually specific for one particular metal (Baker and Brooks, 1989), and are adapted to precise climate and soil conditions. Furthermore, they cannot be managed as a conventional crop, have low biomass, and often a short life cycle. Therefore it seems more reasonable to search for non hyperaccumulator plants showing good features for phytoremediation and then transfer biotechnologically traits that make the modified plant even a more powerful tool than natural hyperaccumulators.

Over the last few years on heavy metal tolerance and accumulation studies, the genetic modification approach has gained significant momentum. The goal of genetic modification approach is to develop fast growing, high shoot biomass plants with the metal accumulation traits of natural small biomass hyperaccumulators: 'engineered phytoremediators' (Ow, 1996). The advantage of this technique is the relatively short space of time and selective targeting of genes for improvement. With genetic engineering, plants can be manipulated to accumulate, translocate and tolerate heavy metals, thus creating the ideal transgenic plant for environmental cleanup in the shortest possible time (Pilon-Smits, 2005; Bennett, 2003; Persans *et* al., 2001). For instance, genes can be isolated from metal hyperaccumulators and inserted into fast growing high biomass plant species (Persans *et* al., 2001). It has been suggested that especially phytoextraction would become commercially available if metal removal and tolerance properties of hyperaccumulator plants, such as *Thlapsi caerulescens* (Brown *et* al., 1995; Bennett, 2003) or *Pteris vittata* (Ma *et* al., 2001), could be transferred into fast growing, high biomass producing crop species. For example, most recently, Cd accumulation was enhanced when a metallothionein gene from *Silene vulgaris* L. was overexpressed in the high biomass *Nicotiana tabacum* L. (tobacco) (Gorinova *et* al., 2006).

Ultramafic rocks exposed to heavy tectonic activities usually contain high amounts of serpatine soils in the Earth's crust. Serpentine areas are generally characterized by high levels of heavy metals such as nickel, cobalt and chromium. The soils derived from ultrafamic rocks lead to unusual and sparse associations of flora that are tolerant of extreme environmental conditions such as high heavy metal contents. Serpentine soils, "hotspots" of metallophyte endemics are a rich source of toxic trace elements. There are serpentine soils derived from ultramafic rocks in various parts of the world. Serpentinized rocks are distributed all over the world viz., western north America; Newfoundland, Mount Albert in eastern Canada; Lizard peninsula, Wales and Scotland; north-east Cuba; Portugal; Italy; Balkan peninsula; Turkey; topical far east; Central Brazil; New Caledonia; south east Asia; Philippines; Japan; Zimbabwe; eastern Transvaal Loweveld of South Africa, New Zealand; greenstone belts of western Australia (Proctor and Woodell, 1975; Sequeira *et* al., 1991). Significant exposures of ultramafic rocks and soils are found in many parts of Turkey (Figure 1), although they are not such important features of the geology of the eastern and south-eastern provinces. Notable areas include the central part of the North-west (Kutahya and Balikesir provinces), the South-west between Antalya and Marmaris (Antalya and Mugla provinces), the Amanus Mountains (Hatay and Adana provinces), regions of the eastern Taurus (north and north-east of Mersin) and its extension into the Aladag massif (Nigde and Adana provinces), and numerous areas in a band running generally north-eastwards for several hundred kilometers from near Adana to near Erzincan (Figure 1). Other significant outcrops include several smaller areas near Ankara and in Canakkale province. Soils developed on serpentine rocks cover a large area in Fındıkpınarı (Mersin, Turkey) where there are a large number of mines (e.g., chromium). Little is known about heavy metal contents of the natural plants grown on Mersin-Findikpinari. Findikpinari is one of the plataeus used as a settlement place and has 1250 m altitude (Orcan *et* al., 2004). Research area is on the Bolkar Mountains which is an interesting place from the point of endemism (Orcan *et* al., 2004). The geological structure of the area is formed upper Crataceous ultramorphic and serpentine. Common soil formations distinguished in the area as follows: brown forest soils, reddish Mediterranean soils and brown calcareous soils (Orcan *et* al., 2004). Koleli *et* al., (2008) reported that the maximum concentrations of metals in 11 soil samples collected from Mersin-Findikpinari (as dry mass) were 909 mg kg^{-1} Cr, 3615 mg kg^{-1} Ni, 246 mg kg^{-1} Cu, 467 mg kg^{-1} Zn, 8.2 mg kg^{-1}Cd and 111 mg kg^{-1} Pb. Koleli *et* al., (2008) to determine hyperaccumulator species growing in serpentine soils in Findikpinari-Mersin, total 123 plant species (members of 23 genera and 15 families) from 5 different sampling locations were collected and analyzed for their total Cd, Cr, Cu, Ni, Pb, and Zn contents using an ICP-MS. The results indicate that four plants species, mainly *Thlaspi elegans* Boiss. and *Alyssum murale* Waldst.& Kit. contained Ni concentrations up to 15693 and 13591 mg kg^{-1} Ni dry matter, respectively. Similarly, *Anthemis cretica* L. and *Sanicula europaea* L. also contained Ni concentrations of 7741 and 4247 mg kg^{-1} DM, respectively. The collected 755 specimens (52 family, 149 genera and 327 species) in Mersin-Findikpinari were identified by Orcan et. al. (2004) in between 1997-2002. Orcan *et* al., (2004) reported that the largest family according to number of the species is *Fabaceae* and the largest genus is *Trifolium* in this area.

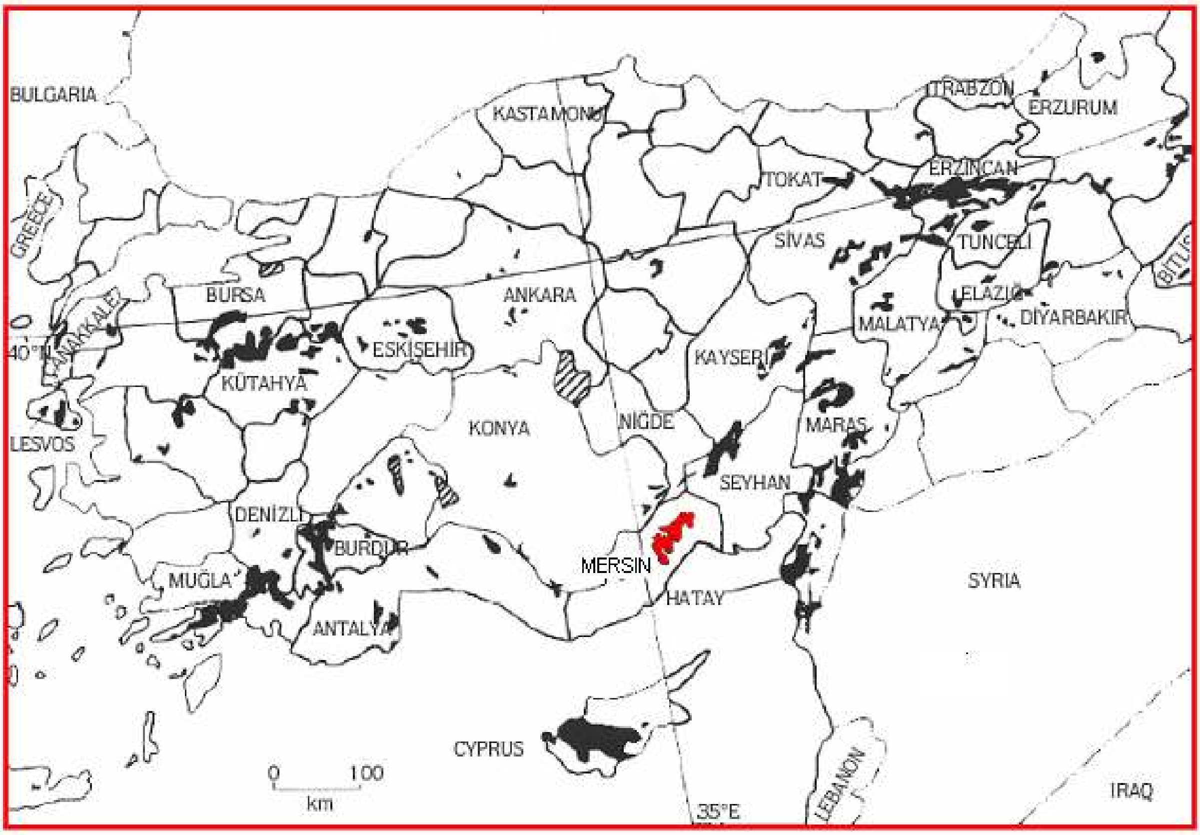

Figure 1: Map of Turkey showing areas of ultramafic geology (in black) and of Mersin-Findikpinari (in red) (from Reeves and Adiguzel, 2004)

The main objective of this study is to evaluate heavy metal accumulation ability of the different plantspecies grown on Mersin-Findikpinari. The 26 specimens from the 755 specimens collected and identified in between 1997-2002 by Orcan et. al. (2004) in Mersin-Findikpinari The plants were randomly selected to evaluate heavy metal accumulation capacity..

Material and Methods

The shoots of identified plants were oven-dried at 70 °C for dry matter amount determination. Dried shoot samples were ground and digested in 2 mL 30% H_2O_2 and 5 mL 65% HNO_3 in sealed vessels of a microwave (MarsXpress) apparatus. Each plant was replicated three times. Arsenic, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn concentrations were analyzed using an ICP-MS (Inductively Coupled Plasma-Mass Spectroscopy, Agillent 7500ce). Certified reference materials (*SRM 1573A, SRM 1547*) were also analyzed in order to check the accuracy of the extraction technique used in the study.

Family	Name of the plant	Plant no	Collection site	Altitude, m	Collection date
Asteraceae	*Conyza bonariensis* (L.) Cronquist	689	Purcu surroundings, under forest, rocky places	1350	14.06.1998
Asteraceae	*Crupina curipinastrum* (Moris) Vis.	699	Purcu surroundings, under forest, rocky places	1350	14.06.1998
Asteraceae	*Anthemis aciphylla* Boiss.var. *aciphylla*	85	Cayirbogazi surroundings, waste places, open forest, under forest	1300-1500	20.04.2002
Boraginaceae	*Alkanna aucherana* A.DC.	2	Akarca Guzlesi-Findikpinari, roadside, under forest and open forest	1150	14.03.2002
Caryophyllaceae	*Silene dichotoma* Ehrh. subsp. *dichotoma*	111	Akarca Guzlesi-Findikpinari, roadside, under forest and open forest	900-1150	11.05.2002
Iridaceae	*Crocus graveolens* Boiss. &Reuter	4	Akarca Guzlesi-Findikpinari, roadside, under forest and open forest	900-1150	14.03.2002
Lamiaceae	*Scutellaria salviifolia* Bentham	141	Akarca Guzlesi-Findikpinari, roadside, under forest and open forest	900-1150	11.05.2002
Lamiaceae	*Micromeria carica* P. H. Davis	744	Capurgedigi, surroundings, under forest	1200-1300	28.06.1998
Lamiaceae	*Prunella vulgaris* L.	740	Purcu surroundings, under forest, rocky places	1350	14.06.1998
Lamiaceae	*Lamium garganicum* L. subsp. *reniforme*(Montbret & Aucher ex Bentham) R. Mill	758	Capurgedigi surroundings, under forest and open forest	1200-1300	09.05.1998
Lamiaceae	*Marrubium astracanicum* Jacq.subsp.*astracanicum*	208	Devekoyagi surroundings, under forest and open forest	1800	27.06.2002
Lamiaceae	*Purunella orientalis* Bornm.	741	Findikpinari-Caglarca village, roadside	1300-1400	15.07.1998
Lamiaceae	*Prunella vulgaris* L.	739	Cayirbogazi surroundings, under forest, rocky places, waste places	1300-1500	31.05.1998
Lamiaceae	*Lamium crinitum* Montbret & Aucher ex Bentham.	747	Capurgedigi surroundings, under forest and open forest	1200-1300	09.05.1998
Lamiaceae	*Nepeta nuda* L. *subsp. nuda*	759	Capurgedigi surroundings, under forest and open forest	1200-1300	09.05.1998
Papaveraceae	*Fumaria kralikii* Jordan	72	Cayirbogazi surroundings, waste places, open forest, under forest	1300-1500	20.04.2002
Papaveraceae	*Corydalis solida* (L.) Swartz subsp. *tauricola* Cullen & Davis	775	Findikpinari, under forest	1300-1350	14.03.1999
Poaceae	*Briza humilis* Bieb.	712	Bozon Guzlesi-Findikpinari, roadside, stony, rocky places	1250	01.06.1997
Poaceae	*Poa speluncarum* Edmondson	720	North of the Findikpinari, under forest, rocky slopes	1300-1400	18.05.1997
Poaceae	*Aegilops neglecta* Req. ex Bertol.	722	Bozon Guzlesi-Findikpinari, roadside, stony, rocky places	1250	01.06.1997
Poaceae	*Festuca jeanpertii* (St.- Yves) F. Markgraf subsp. *jeanpertii.*	707	Bozon Guzlesi-Findikpinari, roadside, stony, rocky places	1250	01.06.1997
Poaceae	*Bramus diandrus* Roth	728	Bozon Guzlesi-Findikpinari, roadside, stony, rocky places	1250	01.06.1997
Poaceae	*Festuca pinifolia* (Hackel ex Boiss.) Bornm. var. *pinifolia*	708	Akarca Guzlesi-Findikpinari, roadside, under forest, stony places	900-1150	21.06.1997
Poaceae	*Cynosurus echinatus* L.	713	Bozon Guzlesi-Findikpinari, roadside, stony, rocky places	1250	01.06.1997
Ranunculaceae	*Ranunculus ficaria* L. subsp. *calthifolius* (Reichb.) Arc	5	Akarca Guzlesi-Findikpinari, roadside, under forest and open forest	900-1150	14.03.2002

Table 1: Family, genus, altitude, name of the collected site, and the collection date (from Orcan et. al., 2004)

Findings

Research area is on the Bolkar Mountains which is an interesting place from the point of endemism of Turkey. The collected 26 plants from different sampling locations have 26 genera and 8 families. Different 8 families were Asteraceae (3), Boraginaceae (1), Caryophyllaceae (1), Iridaceae (1), Lamiaceae (9), Papaveraceae (2), Poaceae (7) and Ranunculaceae (1). In the identified 755 plant, the largest family according to number of the species is *Fabaceae* and the largest genus is *Trifolium*. In the tested 26 plants, the largest family according to number of the species is Poaceae (7). Table 1 shows family, genus, altitude, name of the collected site, altitude and collection date of the tested plant samples.

Table 2 shows heavy metal concentrations in shoots of the investigated plant specimens. The highest As (6), Co (10), Cr (46), Mn (548), Se (4) concentrations were *Anthemis aciphylla* Boiss. (Asteraceae). Manganese concentration in *Anthemis aciphylla* Boiss. (Asteraceae) was higher than the critical concentration (300-500) in plants according to Kabata-Pendias and Pendias (1992). *Fumaria kralikii* (Papaveraceae) has higher metal content, except for Cd and Zn, than other plants and higher than normal concentration in plants according to Kabata-Pendias and Pendias (1992). The highest Ni concentration was 115 mg kg^{-1} DM for *Crocus graveolens* Boiss&Reute (Iridaceae) and this value was higher than the critical concentration (10-100) in plants according to Kabata-Pendias and Pendias (1992).

In the future, the identified 755 plants will be studied to evaluate heavy metal accumulation capacity because of the research area is an interesting place from the point of endemism and remediation of contaminated soils is essential for sustainable soil use. New selected metal hyperaccumulator plant may be genetically modify and remediate metal-contaminated soils. But metal hyperaccumulator plants after treatment evaluated as hazardous waste because of the higher concentration of the extracted metals. Therefore, further treatment of this biomass is environmentally necessary.

Family	Name of the plant	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Se	Zn
Asteraceae	*Conyza bonariensis* (L.) Cronquist	**3**	1	**6**	**24**	**29**	**335**	**40**	15	**3**	137
Asteraceae	*Crupina curipinastrum* (Moris) Vis.	1	1	<bd	1	11	47	3	15	2	179
Asteraceae	*Anthemis aciphylla* Boiss.var. ***aciphylla***	**6**	1	**10**	**46**	**29**	***548***	**67**	16	**4**	84
Boraginaceae	*Alkanna aucherana* A.DC.	1	**3**	1	8	18	88	**35**	**28**	1	79
Caryophyllaceae	*Silene dichotoma* Ehrh. subsp. ***dichotoma***	2	1	**2**	11	16	**235**	**13**	9	2	65
Iridaceae	*Crocus graveolens* Boiss. &Reuter	1	1	**8**	**31**	**21**	**189**	***115***	6	1	104
Lamiaceae	*Scutellaria salviifolia* Bentham	9. <BD	<bd	**4**	**16**	20	67	**79**	13	1	112
Lamiaceae	*Micromeria carica* P. H. Davis	<bd	<bd	<bd	<bd	2	3	1	2	<bd	6
Lamiaceae	*Prunella vulgaris* L.	1	1	<bd	3	**21**	**126**	5	7	1	79
Lamiaceae	*Lamium garganicum* L. subsp. ***reniforme***(Montbret & Aucher ex Bentham) R. Mill	1	1	<bd	4	11	97	**6**	**22**	1	52
Lamiaceae	*Marrubium astracanicum* Jacq.subsp.***astracanicum***	1	1	<bd	7	17	96	**24**	20	1	79
Lamiaceae	*Purunella orientalis* Bornm.	1	<bd	1	3	16	80	**9**	3	1	100
Lamiaceae	*Prunella vulgaris* L.	1	2	1	5	15	**101**	**7**	17	1	207
Lamiaceae	*Lamium crinitum* Montbret & Aucher ex Bentham.	<bd	<bd	<bd	2	12	76	**24**	8	1	58
Lamiaceae	*Nepeta nuda* L. *subsp.* ***nuda***	1	1	<bd	3	12	**158**	**6**	20	1	165
Papaveraceae	*Fumaria kralikii* Jordan	**6**	1	**6**	**26**	**31**	**247**	**30**	**35**	**3**	144
Papaveraceae	*Corydalis solida* (L.) Swartz subsp. ***tauricola*** Cullen & Davis	<bd	1	<bd	3	17	**107**	9	7	1	175
Poaceae	*Briza humilis* Bieb.	1	<bd	<bd	7	11	76	**12**	6	<bd	68
Poaceae	*Poa speluncarum* Edmondson	<bd	<bd	<bd	2	11	67	4	9	1	106
Poaceae	*Aegilops neglecta* Req. ex Bertol.	<bd	1	<bd	4	6	49	**6**	4	1	46
Poaceae	*Festuca jeanpertii*(St.- Yves) F. Markgraf subsp. ***jeanpertii.***	<bd	<bd	<bd	4	18	49	**35**	14	1	60
Poaceae	*Bramus diandrus* Roth	<bd	<bd	<bd	3	10	83	**6**	18	1	45
Poaceae	*Festuca pinifolia* (Hackel ex Boiss.) Bornm. var. ***pinifolia***	1	1	**2**	9	16	**133**	**16**	19	1	68
Poaceae	*Cynosurus echinatus* L.	<bd	1	<bd	2	9	48	**9**	3	1	76
Ranunculaceae	*Ranunculus ficaria* L. subsp. ***calthifolius*** (Reichb.) Arc	1	<bd	**3**	**19**	**24**	**137**	**60**	9	1	92
Asteraceae	*Conyza bonariensis* (L.) Cronquist	<bd	1	<bd	2	18	34	3	6	1	80
	The highest value in plants	**6**	**3**	**10**	**46**	**31**	***548***	***115***	**35**	**4**	207
	The lowest value in plants	1	1	1	1	2	3	1	2	1	6
	Common concentrations in plants*	0.02-7	0.1-2.4	0.02-1	0.03-14	5-20	20-100	0.02-5	0.2-20	0.001-2	1-400
	The critical concentration in the plants*	5-20	5-30	15-50	5-30	20-100	300-500	10-100	30-300	5-30	100-400
	Hyperaccumulation threshold value	1000	100	1000	1000	1000	10000	1000	1000	1000	10000

Table 2: Heavy metal concentrations of the tested plants (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn), mg kg^{-1} DM *Kabata-Pendias (1992)

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