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ORIGINAL ARTICLE

Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential

J. Nouri · N. Khorasani · B. Lorestani · M. Karami · A. H. Hassani · N. Yousefi

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Abstract Contamination of heavy metals represents one of the most pressing threats to water and soil resources, as well as human health. Phytoremediation can be potentially used to remediate metal contaminated sites. In this study, concentrations of copper, zinc, iron, and magnesium accumulated by native plant species were determined in field conditions of Hame Kasi iron and copper mine in the central part of Iran in Hamadan province. The results showed that metal accumulation by plants differed among species and tissue bodies. Species grown in substrata with elevated metals contained significantly higher metals in plants. Metals accumulated by plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance exists widely amongst them. The mentioned species could accumulate relatively higher metal concentrations far above the toxic concentration in the plant shoots. With high translocation factor, metal concentration ratio of plant shoots to roots indicates internal detoxification metal tolerance mechanism; thus, they have potential for phytoextraction. The factors affecting metal accumulation by plant species including metal concentrations, pH, electrical conductivity, and nutrient status in substrata were measured. Mostly, concentrations of zinc and copper in both aboveground and underground tissues of the plants were significantly, positively related to their total in substrata, while iron, zinc, and copper were negatively correlated to soil phosphorus.

Keywords Heavy metal accumulation · Phytoremediation · Polluted soils · Translocation factor

Introduction

Heavy metals make a significant contribution to environment as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations (Welch 1995; Samarghandi et al. 2007). All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants (Page et al. 1982). Phytoremediation that uses the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues appears very promising for the removal of pollutants from the environment (Gurbisu and Alkorta 2003). Since most of plant roots are located in the soil, they can play an important role in metal removal via filtration, adsorption and cation exchange, and through plant-induced chemical changes in the rhizosphere (Dunbabin and Bowmer 1992; Wright and Otte 1999). There is evidence that plants can accumulate heavy metals in their tissues such as *Sebera acuminate* and *Thlaspi caerulescens* (Cunningham and Ow 1996), *Arabidopsis thaliana* (Delhaize 1996), *Typha latifolia*, and *Phragmites australis* (Ye et al. 2001). *T. latifolia* and *P. australis* have been successfully used for phytoremediation of Pb/Zn mine (Ye et al. 1997a, b). Metal accumulation by plants is affected by many factors. In

J. Nouri · B. Lorestani (✉) · A. H. Hassani
Graduate School of the Environment and Energy,
Science and Research Branch,
Islamic Azad University, Tehran, Iran
e-mail: lorestani_B@iauh.ac.ir; nourijafar@gmail.com

N. Khorasani · M. Karami
Department of Fishery and Environment,
University College of Agriculture and Natural Resources,
University of Tehran, Tehran, Iran

N. Yousefi
College of Basic Science, Islamic Azad University,
Hamadan, Iran

general, variations in plant species, the growth stage of the plants and element characteristics control absorption, accumulation and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots (Guilizzoni 1991). As a result, metal removal by vegetation can be greatly enhanced by the judicious selection of plant species. The knowledge about the abilities of different plant species or tissues to absorb and transport metals under different conditions will provide insight into choosing appropriate plants for phytoremediation of the polluted regions. In Iran, there are numerous metal mines and the process of metal mining has caused severe heavy metal pollution. Our preliminary investigation at metal mine found that many plants could thrive in heavily metal-contaminated soils. For this reason, the concentrations of Fe, Zn, Cu, and Mn were examined in 12 wild plant species grown in surrounding area of Hame Kasi mine located in Hamadan, Iran. The primary objectives were as follows:

(a) To determine the ability of different wild plant species grown in metal-contaminated site to accumulate and tolerate Iron, zinc, copper and manganese in their tissues; (b) to explore the difference of metal accumulation by the same plant when growing on metal-contaminated and "unpolluted" sites; (c) to find the factors influencing the metal accumulation by plants; and (d) to discuss the potential use of plants for metal removal in polluted area. The above information then could be referred for selection of appropriate species in constructed soil treatment systems to exploit the removal potential of heavy metals by plants combined with other chemical or biological processes.

Materials and methods

Site characterization

The plant and soil samples used in this study were collected from a known metal-contaminated site located in a square (N 34°57′16″ and E 48°8′26″; N 34°56′14″ and E 48°8′22″; N 34°55′58″ and E 48°11′34″ and N 34°54′53″ and E 48°10′25″) in the surrounding area of Hame Kasi mine of northwest Hamadan province in Iran. The site has occupied approximately 10,000 m^2 and is covered mainly by grasses. Human activities such as mining have contributed to elevated metal concentrations in this site. Contamination of heavy metals was mainly concentrated in the top 20 cm at the site (Fig. 1).

Plant sampling and analysis

Twelve plant species were collected in the surrounding area of Hame Kasi mine (Hamadan, Iran) from January to June, 2007. The voucher specimens were deposited at Bu-Ali Sina University Herbarium (ICN 10998) and were labeled as follows: Iran, prov. Hamadan, 35 km from Hamadan to kordestan, Alt. 2,386 m. The studied species consisted of 11 genera and 6 families (Table 1) of which six species belonged to Asteraceae, forming the most dominant component in metal-polluted sites. Plant samples were thoroughly washed with running tap water and rinsed with deionized water to remove any soil particles attached to the plant surfaces. The aboveground and underground tissues were then separated and oven dried (70°C) to constant weight. The dried tissues were weighed and ground into powder for metal concentration analysis. Metal contents (Fe, Zn, Cu and Mn) of the plant samples were extracted by acid digestion followed by measurement of total concentrations of all elements of interest using atomic absorption spectrophotometer (GBC Avanta, Australia; Ward et al. 1975).

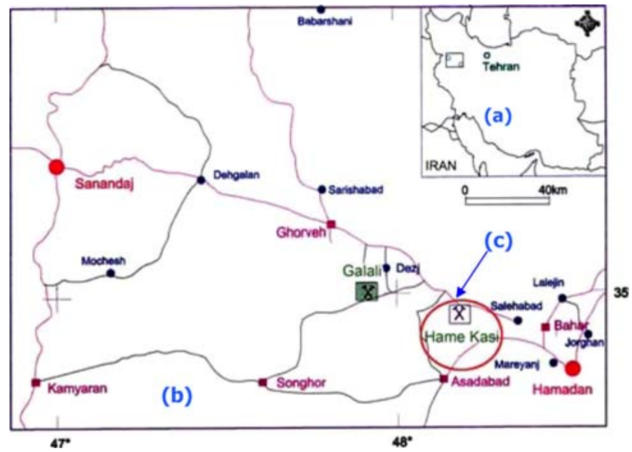

Fig. 1 Location of the study area; map of Iran (**a**), map of Hamadan province and (**b**) map of Hame Kasi mine (**c**)

Table 1 Species composition in surrounding area of Hame Kasi mine

Species no.	Plant species	Family
1	*Centaurea virgata* Lam.	Asteraceae
2	*Astragalus verus* L.	Fabaceae
3	*Chenopodium botrys* L.	Chenopodiaceae
4	*Stipa barbata* Desf.	Poaceae
5	*Ziziphora clinopodioides* Lam.	Lamiaceae
6	*Cousinia bijarensis* Rech. F.	Asteraceae
7	*Cirsium congestum* Fisch. and C. A.	Asteraceae
8	*Scariola orientalis* (Boiss.) Sojak.	Asteraceae
9	*Cousinia* sp.	Asteraceae
10	*Chondrila juncea* L.	Asteraceae
11	*Melica jacquemontii* Dence. Ex Jacqem.	Poaceae
12	*Verbascum speciosum* Schard.	Schorophulariaceae

Besides the above species located in close proximity to metal mines, *Chenopodium botrys*, *Astragalus verus*, *Scariola orientalis* and *Centaurea virgata* were also collected from an "unpolluted" site, located 15 km away from the polluted region under controlling conditions. The species, grown in surrounding area of mine, are listed in Table 1.

Soil sampling and analysis

Soils were sampled from the same sites and location points, as well as the plants. The top 20 cm soil from between the plant roots was collected, air-dried for 2 weeks, and then sieved through a 2-mm mesh. Samples were then analyzed for total metals (Fe, Zn, Cu and Mn), pH, electrical conductivity (EC), and total P. The pH and EC values (solid:distilled water = 1:5) of soil samples were measured by a pH meter and an EC meter, respectively (Thomas 1996; Rhoades 1996). Total phosphorus was measured using the method of Olsen (Rowell 1994). Total metal contents were extracted by acid digestion (Sposito 1989). Metal contents were measured by atomic absorption spectrophotometer (GBC Avanta, Australia) for Fe, Zn, Cu, Mn.

Calculation of TF

The ability of plants to tolerate and accumulate heavy metals is useful for phytoremediation purpose measured using translocation factor (TF) which is defined as the ratio of metal concentration in the shoots to the roots ($[\text{Metal}]_{\text{Shoot}}/[\text{Metal}]_{\text{Root}}$) to show metal translocation properties from roots to shoots (Kabata-Pendias and Pendias 1984).

Results

Characteristics of soils in the surrounding area of Hame Kasi mine

As shown in Table 2, soils in the surrounding area of mine were slightly alkaline with an average pH of approximately 7.8. The pH conditions were suitable for plant growth. The average of EC of the soil was 527 µs/m. The average of concentration of total P in the soil collected from the surrounding area of mine was 84.5 µg/g. In addition, heavy metal contents in the soil were extremely high and varied greatly.

Table 2 Soil characteristics of the surrounding area of Hame Kasi Mine (mean ± SE)

Characteristics	(*N* = 12)
EC (µs/m)	527 ± 39.5
pH	7.8 ± 0.09
P (mg/kg)	84.5 ± 3.3
Metals (µg/g)	
Fe	32,466.4 ± 1,565.1
Mn	459.5 ± 23.8
Zn	1,955.5 ± 137
Cu	488.4 ± 31.5

Metal accumulation and translocation by plants in the surrounding area of mine

Heavy metal (Fe, Zn, Cu and Mn) concentrations in plant tissues collected from the polluted area (shoots and roots, separately) are shown in Fig. 2. The data show that the metal contents in the plant tissues differed among species at the polluted site indicating their different capacities for metal uptake. *C. botrys* accumulated Cu (183 and 150 µg/g) and Mn (177.3 and 1,288 µg/g) in its root and shoot which were higher than other species while *V. speciosum* attained an average concentration of 15,343 µg/g Fe in the aboveground tissues and 9,226.3 µg/g in underground tissues, which was much higher than the other species. *S. orientalis* accumulated significantly higher Zn in its underground (1,208.3 µg/g) tissues than the other species and *S. barbata*, accumulated significantly higher Zn (329.3 µg/g) in its aboveground tissues than the other species at the polluted site.

Translocation factor, the ratio of shoot to root metals, indicates internal metal transportation. The data presented indicate that metals accumulated by the proposed plant species were largely retained in roots, as shown by general TF values (<1; Table 3). Exceptions occurred in *S. barbata*, *C. bijarensis*, *S. orientalis*, *M. jacquemontii* and *V. speciosum* for Fe (TF = 1.2, 1.06, 1.18, 1.26 and 1.66), *C. congestum* and *Cousinia* sp. for Mn (TF = 1.40 and 1.09) *S. barbata*, *C. bijarensis*, *C. juncea* and, *M. jacquemontii* for Zn (TF = 1.04, 1.01, 1.36 and 1.18) and *C. bijarensis* and *Cousinia* sp. for Cu (TF = 1.10 and 1.44). The TF values obviously varied among the plant species grown in the surrounding area of mine. For example, TF values for Fe in *V. speciosum* and *C. botrys* were 1.67 and 0.45, respectively, although both grew at the same site.

Metal accumulation by *A. verus*, *C. botrys*, *S. orientalis* and *C. virgata* in polluted and unpolluted sites

Species of *A. verus*, *C. botrys*, *S. orientalis* and *C. virgata* were collected from "unpolluted" sites which were located 15 km away from the polluted site. Although the same plant species were grown, pH, EC values, total P, and metal contents in their substrata were remarkably different (Figs. 2, 3).

In general, higher metal concentrations were found in plants from contaminated sites rather than the control.

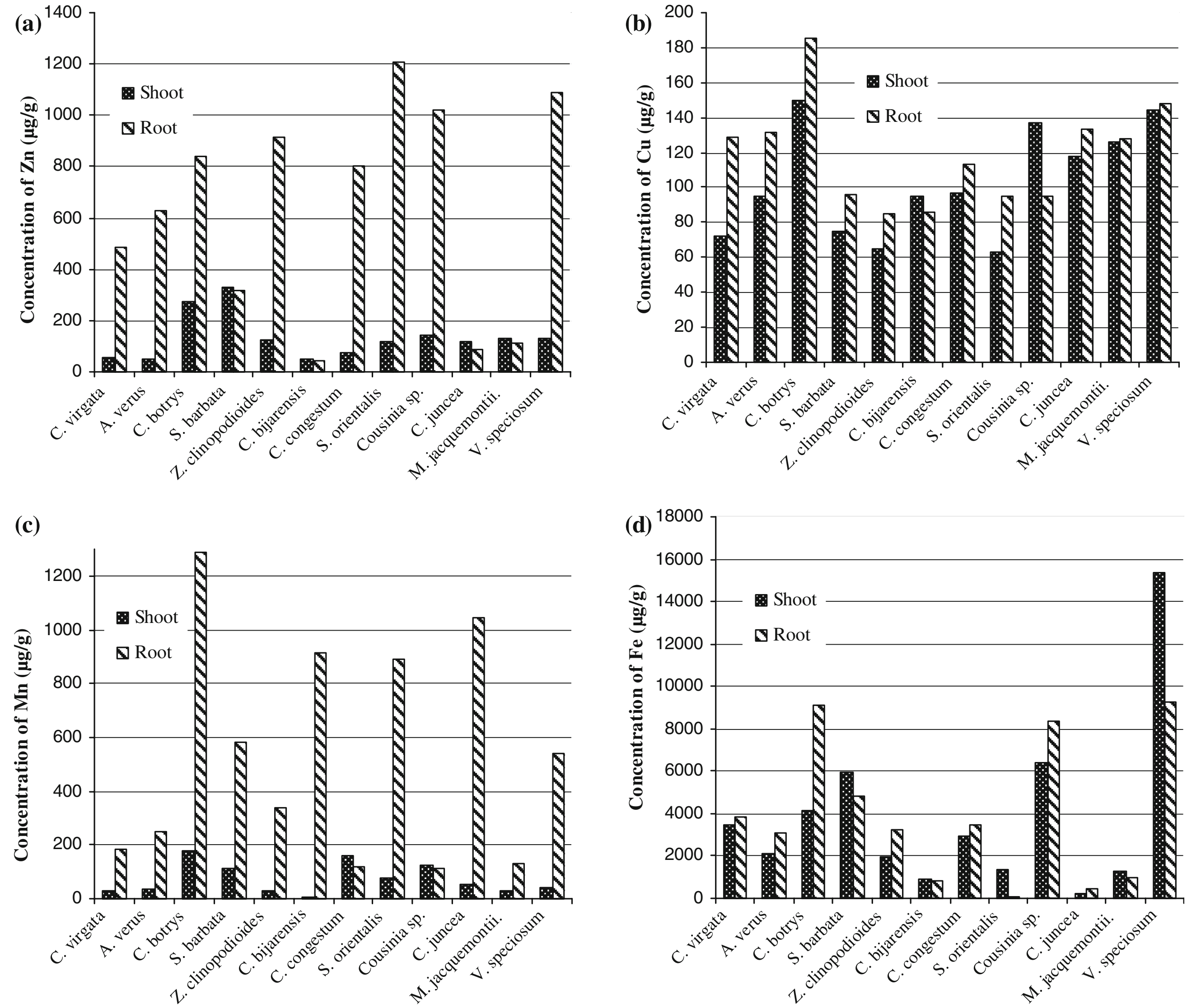

Fig. 2 Metal concentrations of Zn (**a**), Cu (**b**), Mn (**c**) and Fe (**d**) in shoots and roots of plants collected from surrounding study area of the mine

However, this was not always the case, especially in the aboveground tissues. For example, *A. verus* and *C. virgata* did not accumulate significantly higher Mn in their aboveground tissues than the control species, although Mn concentrations in their substrata were substantially high. The difference in metal uptake between species reflected the status of their associated soils which means that metal uptake could be affected by several factors that will be discussed later. Metal concentrations in species followed the order of Fe > Zn > Cu > Mn except for *C. botrys*, *Z. Clinopodioides*, *Cousinia* sp. and *M. jacquemontii*, the order of which was Fe > Zn > Cu > Mn. Meanwhile, *S. orientalis* and *S. barbata* followed the order of Fe > Mn > Cu > Zn resulting from elevated Cu in the sediment.

Discussion and conclusion

Metal accumulation and tolerance in plant species

The present study shows that some plants can colonize a wide range of metal concentrations in the soils (e.g. *C. botrys*, 256 µg/g Cu and 179 µg/g Mn; *V. speciosum* 15,343 µg/g Fe and *S. barbata* 329.3 µg/g Zn). According to Kabata-Pendias and Pendias (1984), 60–125 µg/g Cu, 70–400 µg/g Zn, 120–21,000 µg/g Fe and 300–500 µg/g Mn based on total fractions in soil would be considered toxic to plants. The metal (Fe, Zn, Cu and Mn) contents in the surrounding area of mine greatly exceed these ranges (Fig. 3); therefore, the 12 plant species grown in these contaminated sites have exhibited high metal tolerance.

Table 3 Translocation factors ($TF = [Metal]_{Shoot}/[Metal]_{Root}$) in plant species grown in surrounding study area of Hame Kasi mine

Species no.	Species	TF			
		Fe	Mn	Zn	Cu
1	*C. virgata*	0.91	0.17	0.12	0.56
2	*A. verus*	0.69	0.15	0.08	0.72
3	*C. botrys*	0.45	0.14	0.33	0.82
4	*S. barbata*	1.23	0.19	1.04	0.78
5	*Z. clinopodioides*	0.61	0.08	0.14	0.76
6	*C. bijarensis*	1.07	0.007	1.01	1.10
7	*C. congestum*	0.83	1.40	0.09	0.86
8	*S. orientalis*	1.18	0.08	0.09	0.66
9	*Cousinia* sp.	0.76	1.09	0.14	1.44
10	*C. juncea*	0.57	0.05	1.36	0.88
11	*M. jacquemontii*	1.26	0.23	1.18	0.98
12	*V. speciosum*	1.67	0.07	0.12	0.97

Plant species differ widely in their ability to accumulate heavy metals. Figure 2 shows that the root tissues accumulate higher concentrations of metals than shoots, which indicated greater plant availability of the substrate metals, as well as interior limited mobility of the plant. This is consistent with previous observations (Outridge and Noller 1991; Fitzgerald et al. 2003). Outridge and Noller (1991) reported that the concentrations of heavy metals in the root tissues of freshwater macrophytes from polluted areas were usually found to contain higher concentrations of most metals compared to the aboveground parts. Fitzgerald et al. (2003) observed that monocotyledonous species contained higher concentrations of Pb in the roots compared to shoots. In comparison with the ranges of metal concentrations in the soils and in the underground tissues, the concentrations of Fe, Zn, Cu and Mn in shoots were maintained at low levels (Fig. 2). The results presented here suggest that this metal-tolerating strategy is widely evolved and exists in plant species when they grow in metal-contaminated areas. The elevated metal concentrations in the underground tissues and low translocation to the aboveground tissues in the 12 species examined might also suggest that they are capable of rather well-balanced uptake and translocation of metals under heavily metal-polluted conditions. Gries and Garbe (1989) indicated that a well-balanced and largely independent ion budget is typical of many grass-like species and is supposed to partly create their wide ecological amplitude. The level of iron concentration toxic to plants is above 500 µg/g (István and Benton 1997). The results indicated that plants grown in Fe-contaminated areas usually contained higher concentrations than this threshold (Fig. 4). Zinc is an essential element to all plants the mean concentration of which in normal plants (aboveground tissues) is 66 µg/g (Outridge and Noller 1991) and the toxic level is above 230 µg/g

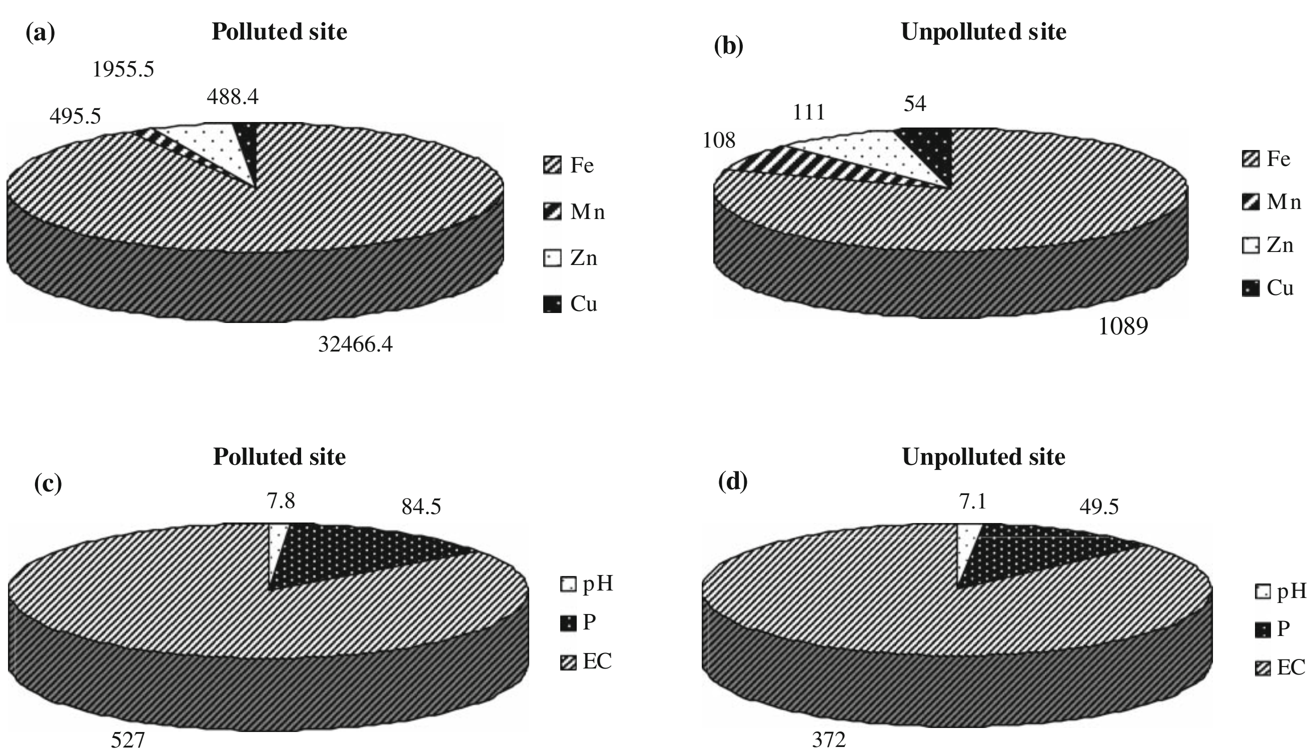

Fig. 3 Comparative of some soil characteristics and metal concentrations in the surrounding area of the study area of mine as a polluted area (**a**, **b**) and out of the area as an unpolluted area (**c**, **d**)

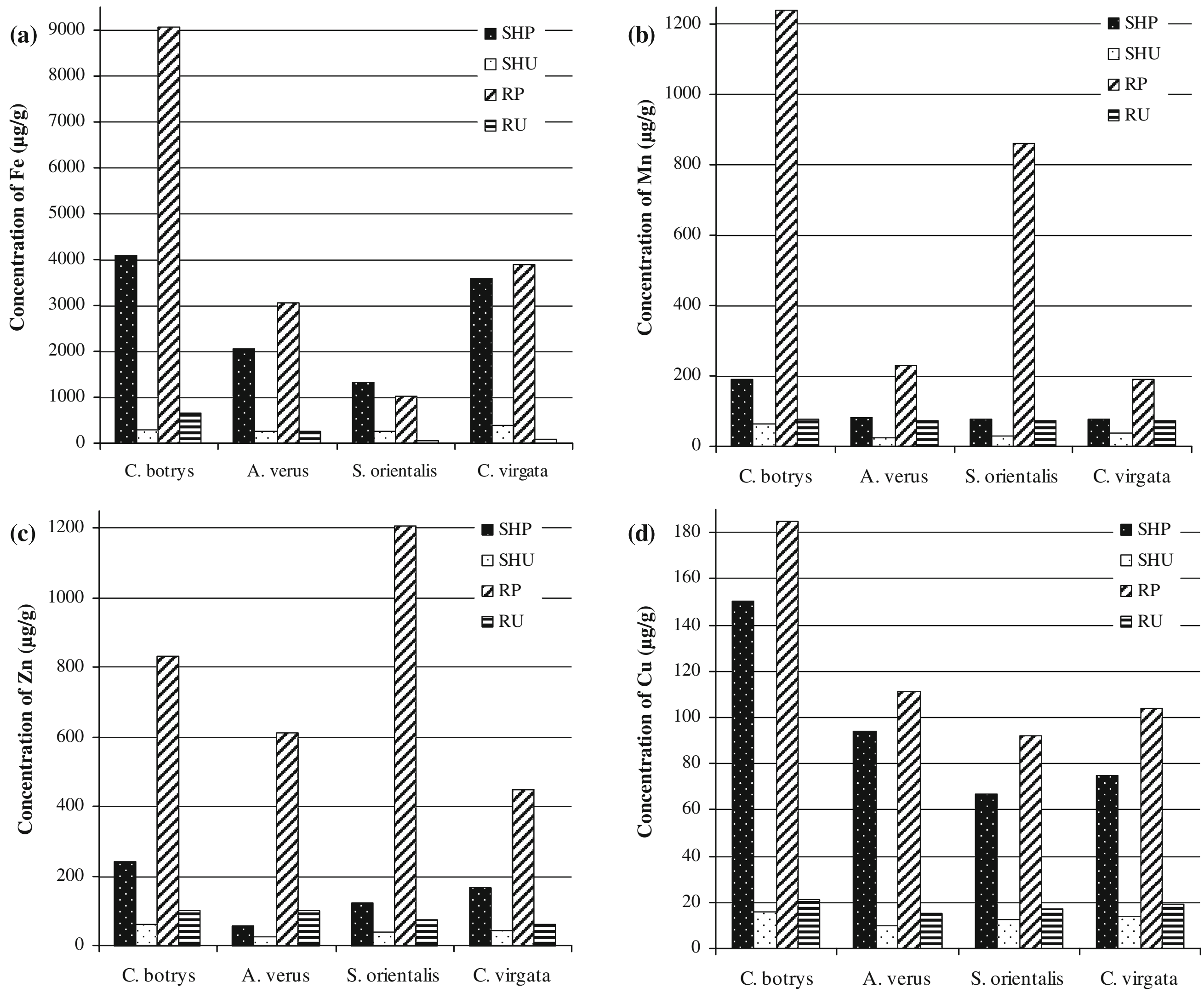

Fig. 4 Metal concentrations of Fe (**a**), Mn (**b**), Zn (**c**) and Cu (**d**) in shoots and roots of *A. verus*, *C. botrys*, *S. orientalis* and *C. virgata* grown in the polluted and unpolluted site. *SHP* plant shoot in polluted sites, *SHU* plant shoot in unpolluted site, *RP* plant root in polluted site, *RU* plant root in unpolluted site

(Borkert et al. 1998; Long et al. 2003). The ranges of Zn in plant species presented here were generally higher than the levels reported for other emergent vegetation. The highest Zn (329.3 μg/g) and (1,208.3 μg/g) in the aboveground and underground tissues were found in *S. barbata* and *S. orientalis*, respectively (Fig. 2a). Copper is also essential to plant growth, but will cause toxic effects when shoots or leaves accumulate Cu levels exceeding 20 μg/g Cu (Borkert et al. 1998). The majority of Cu values in this study were high. The highest Cu concentration in the aboveground of *C. botrys* was 185 μg/g (Fig. 2b). Manganese in normal plants is between 200 and 300 μg/g and toxic level to plants is between 300 and 500 μg/g (István and Benton 1997). The data for Mn in plants obtained in the present study were comparable to this value and showed large variance among species and tissues, except in the *C. congestum*. The metal concentration higher than toxic level in some species indicates that internal metal detoxification tolerance mechanisms might exist in these plant species, in addition to their exclusion strategies (Taylor and Crowder 1983a, b).

Relationship between plants and substrata

Plants grown in metal-enriched substrata take up metal ions in varying degrees. This uptake is largely influenced by the bioavailability of the metals which is in turn determined by both external (soil-associated) and internal (plant-associated) factors. In this study, Pearson correlation coefficients was calculated between metal concentrations in the aboveground and underground tissues and soil factors for determination of relationship between plants and soil factors. For this purpose of 12 collected plant species, the species of *Cousinia* sp., *C. congestum*, *C. juncea* and *V. speciosum* that had the highest TF for Cu, Mn, Zn and Fe elements were selected (Table 4). Most previous studies have shown only poor correlations between metal uptake by plants and soil concentrations (Keller et al. 1998; Greger 1999). Keller et al. (1998) considered that the uptake of metals, both by roots and leaves, was not linearly correlated to the external metal concentration increase. In this study total Cu and Zn in soil had positive relationship with any part of *Cousinia* sp. and *V. speciosum*, respectively, and also there was a similar trend between total Fe and underground of *C. juncea*. Other soil factors such as pH, EC, and soil nutrient also affected the metal uptake by plant species. Although soil pH indicates a positive effect on Cu and Zn concentrations in any part of *V. speciosum* and root of *C. juncea*, respectively, negative relationships have been observed with Fe contents in roots of *C. juncea* and *Cousinia* sp. and there was a similar trend between pH and Mn contents of any part of *C. congestum*. This relationship could be interpreted in terms of the chemical specification of the metals in the soil and competition between metal ions and protons at the plant–soil–water interface. Phosphorus had significant negative relationships with Fe contents in any part of *C. juncea* and *Cousinia* sp. with Zn and Cu in any part of *C. congestum* and *Cousinia* sp., respectively. P had a similar negative effect on Cu uptake by shoot of *C. congestum*. High P levels in soil may decrease Zn, Fe and Cu availability and uptake by plants due to chemical reactions in the rhizosphere where P has a strong tendency to absorb metals (Kabata-Pendias and Pendias 1984). EC is another factor which affects the bioavailability of metals to plant. In collected plants, there was positive correlation between EC values and Zn contents in any part of *Cousinia* sp. and *C. congestum*. Similar trend was observed between EC and Fe contents in underground tissues of *C. congestum*, *V. speciosum* and any part of *C. juncea*. EC had negative effect on Mn uptake in any part of *Cousinia* sp. and root of *C. juncea*. The lack of correlation in some cases only implies that, in isolation, these factors are not dominant in determining metal uptake by the plant. Indeed, it may be the variation and interaction of all these factors, plus forms of heavy metals (Nouri et al. 2001) and organic content (Wright and Otte 1999).

Table 4 Pearson correlation coefficients between metal concentrations in the above-ground and underground tissues and soil factors

	Soil factors		EC	Total
	pH	P		
Aboveground tissues				
Cousinia sp.				
Zn	NS	NS	0.978*	NS
Fe	NS	−0.992**	NS	NS
Cu	NS	−0.933*	NS	0.974*
Mn	NS	NS	−0.980*	NS
Cirsium congestum				
Zn	NS	−0.954*	0.986*	NS
Fe	NS	NS	NS	NS
Cu	NS	−0.952*	NS	NS
Mn	−0.985*	NS	NS	NS
Chondrila juncea				
Zn	NS	NS	NS	NS
Fe	NS	0.993**	0.960*	NS
Cu	NS	NS	NS	NS
Mn	NS	NS	NS	NS
Verbascum speciosum				
Zn	NS	NS	NS	0.956*
Fe	NS	NS	NS	NS
Cu	0.976*	NS	NS	NS
Mn	NS	NS	NS	NS
Underground tissues				
Cousinia sp.				
Zn	NS	NS	0.999**	NS
Fe	−0.989*	−0.976*	NS	NS
Cu	NS	−0.998**	NS	0.966*
Mn	NS	NS	−0.998**	NS
Cirsium congestum				
Zn	NS	−0.967*	0.980*	NS
Fe	−0.972*	NS	0.982*	NS
Cu	NS	NS	NS	NS
Mn	−0.990*	NS	NS	NS
Chondrila juncea				
Zn	0.975*	NS	NS	NS
Fe	NS	−0.990*	0.955*	0.984*
Cu	NS	NS	NS	NS
Mn	NS	NS	0.980*	NS
Verbascum speciosum				
Zn	NS	NS	NS	0.962*
Fe	NS	NS	0.990*	NS
Cu	0.996*	NS	0.982*	NS
Mn	NS	NS	NS	NS

NS no significant correlation

* Correlation is significant at the 0.05 levels; ** correlation is significant at the 0.01 levels

The potential use of plant species in phytoremediation

Phytoremediation is defined as the use of green plants to remove, contain, or render environmental contaminants harmless (Cuningham and Berti 1993). Phytoremediation of heavy metals can be divided into three groups:

phytoextraction—metal accumulating plants are established on contaminated soil and later harvested in order to remove metals from the soil; rhizofiltration—roots of metal accumulating plants absorb metals from polluted effluents and are later harvested to diminish the metals in the effluent and phytoestabilization—metals are established in the substrate (Salt et al. 1995). One reason for using plants for remediation concerns the relatively low cost and maintenance requirements (Cuningham and Berti 1993). Phytoremediation has been used in mined soil restoration since these soils are sources of air and water pollution by means of phytoestabilization and phytoextraction techniques to stabilize toxic mine spoil and remove toxic metals from the spoils, respectively (Wong 2003). According to the different capacities of metal uptake, species able to accumulate relatively high metal concentrations in the aboveground tissues could be good candidates for phytoextraction. According to the present results as shown in Table 3, species of *S. barbata*, *M. jacquemontii* and *C. bijarensis* with TF values greater than one (TF $>$ 1) would be good choices for extracting Fe and Zn; *S. orientalis* and *V. speciosum* for extracting Fe; *Cousinia* sp. for extracting Mn and Cu; *C. bijarensis* for extracting Cu; *C. juncea* for extracting Zn and *C. congestum* for extracting Mn from Fe/Zn/Mn or Cu-contaminated soils, respectively. In the view of toxicology, harvesting plants could be a desirable property, as metals would not pass into the food chain via herbivores and thus avoid potential risk to the environment. Among other plant species, which have strong ability to reduce metal translocation from roots to shoots, harvesting plants will not be an effective source of metal removal in a metal-polluted system and among the above-mentioned phytoremediation techniques; phytostabilization might be the most appropriate to remediate mine tailings and other metal-contaminated lands. Furthermore, the plant species examined in this study grew very well and propagated quickly in substrata with low nutrient conditions which would be a great advantage in the revegetation of mine tailings as cost would be reduced without fertilizer.

This study was conducted to screen plants growing on a contaminated site to determine their potential for metal accumulation. The result shows that most of the 12 plant species can colonize heavy metal-polluted areas, absorb a wide range of soil metals (Fe, Zn, Cu and Mn), not be affected by excessive metal contents and may possess metal resistance capabilities or higher tolerance than more sensitive species. As metal concentrations in shoots are usually maintained at low levels, metal tolerance in wild plants may mainly depend on their metal excluding ability. However, the metal concentrations higher than toxic level in some species indicates that internal detoxification metal tolerance mechanisms might also exist; therefore, their utility for phytoremediation is possible. Results within species revealed the factors influencing metal uptake in field. This implies that by controlling soil nutrients and other factors, metal removal by plants would be different and could be utilized for both phytostabilization and phytoextraction. It also suggests that full consideration of plant–soil interactions should be taken into account when choosing plant species and suitable conditions for developing bioremediation methods such as phytoremediation.

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