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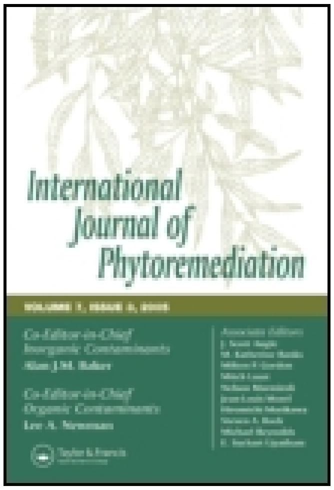

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Wild Flora of Mine Tailings: Perspectives for Use in Phytoremediation of Potentially Toxic Elements in a Semi-Arid Region in Mexico

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Wild Flora of Mine Tailings: Perspectives for Use in Phytoremediation of Potentially Toxic Elements in a Semi-Arid Region in Mexico

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The aim of this research was to identify wild plant species applicable for remediation of mine tailings in arid soils. Plants growing on two mine tailings were identified and evaluated for their potential use in phytoremediation based on the concentration of potentially toxic elements (PTEs) in roots and shoots, bioconcentration (BCF) and translocation factors (TF). Total, water-soluble and DTPA-extractable concentrations of Pb, Cd, Zn, Cu, Co and Ni in rhizospheric and bulk soil were determined. Twelve species can grow on mine tailings, accumulate PTEs concentrations above the commonly accepted phytotoxicity levels, and are suitable for establishing a vegetation cover on barren mine tailings in the Zimapan region. Pteridium sp. is suitable for Zn and Cd phytostabilization. Aster gymnocephalus is a potential phytoextractor for Zn, Cd, Pb and Cu; Gnaphalium sp. for Cu and Crotalaria pumila for Zn. The species play different roles according to the specific conditions where they are growing at one site behaving as a PTEs accumulator and at another as a stabilizer. For this reason and due to the lack of a unified approach for calculation and interpretation of bioaccumulation factors, only considering BCF and TF may be not practical in all cases.

Keywords: phytoextraction, phytoaccumulation, phytostabilization

Introduction

For hundreds of years mining has been an important economic activity in Mexico; during the last half century the production of mining wastes has strongly increased. This represents risks for the environment and the human population, mainly because of dispersion of potentially toxic elements (PTEs) (Armienta, Rodríguez and Cruz 1997). One way to avoid or reduce PTEs dispersion and to remediate contaminated sites is by the establishment of a vegetation cover (Peuke and Rennenberg 2005). Establishment of well-developed vegetation decreases contaminants' leaching and prevents the dispersal of contaminants through wind and water erosion from formerly bare or sparsely vegetated sites (Vangronsveld *et al.* 1995, 1996, 2009). The development of a stable and self-perpetuating vegetation cover can progressively reduce the soil labile PTEs pool leading to an attenuation of the impacts of PTEs on the contaminated site and to adjacent ecosystems (Mendez and Maier 2008). The most important limitations for the establishment and development of a plant cover on mine tailings not only are the high concentration of PTEs, which are toxic for most plant species, but also the generally low contents of organic matter and nutrients as well as the low water retention capacity (Vangronsveld *et al.* 2009). Consequently, only a few species are adapted to these extreme edaphic conditions; such species, however, could be suitable for phytoremediation purposes (Barrutia *et al.* 2011; Mendez and Maier 2008). PTEs phytoremediation has two main approaches: to remove (phytoextraction) or reduce the bioavailability of PTEs (phytostabilization) (Arthur *et al.* 2005). For all phytoremediation approaches it is desirable to use native species, since these plants proved to be adapted to the specific soil and climatological conditions (Barrutia *et al.* 2011) and will not become invasive. Therefore, the objective of the present study was to identify wild plant species suitable for phytoremediation and establishment of a plant cover on metalliferous mine tailings at Zimapan, Mexico.

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Materials and Methods

Sites of Collection

The study area is located in the municipality of Zimapan, Hidalgo State, a semi-arid region in the central part of Mexico. The temperature ranges 12–20°C and the annual precipitation is approximately 700 mm (INEGI 2009).

During the ore extraction procedure waste residues are discharged outside the mining site, and as a result, uncovered mine tailings are widely spread across the municipality. Due to their accessibility, two mine tailings sites, Santa Maria and San Francisco, were selected for the present study. Santa Maria tailing (SM) is located close to a populated area (20°44'8.89" N and 99°23'56.07"; approximately 11000 m^2). Here the residues are unoxidized and have a grey colour. The main minerals present are pyrite, galena, calcite and quartz (Moreno-Tovar *et al.* 2009). The San Francisco (SF) mine area (18000 m^2) is located between 20°49'32.5" N and 99°22'20.1"W. The residues are unoxidized (grey ones) and oxidized which have a red-orange colour. In the oxidized residues the most common minerals are ferric sulphates and iron oxides (Moreno-Tovar *et al.* 2009). Although only SM is close to habitation ($<$20 m), both mine tailings areas are uncovered, favourizing wind and water erosion; it further is common to find cattle searching for grazing or water. Therefore, these tailings are a significant environmental and public health concern (Moreno-Tovar *et al.* 2012 ; Armienta *et al.* 1997).

Plant and Tailings Sampling

At both sites 'plant covered islands' composed of several pioneer plant species were found, which were collected for identification. Bulk (non-rhizospheric) substrate samples from the tailings comprised five to eleven subsamples taken randomly from the heaps. According to Mexican regulation NOM-147-SEMARNAT/SSA1-2004, the rhizospheric substrate samples were taken from residues surrounding the roots of each plant species collected at a depth of 0–30 cm; three subsamples were combined to a composite sample for each plant species. All samples were air-dried and passed through a 2 mm sieve prior to PTEs extraction.

Plants Analysis

Shoot samples were first rinsed with tap water, washed with P-free detergent, rinsed with distilled water, washed with diluted HCl 10% (15 min) and finally rinsed three times with deionized water (15 min). Root samples were treated according to the same protocol, but doubling the time for rinsing with tap water and diluted HCl. Root and shoot samples were dried at 65°C for 72 h, then ground in a stainless steel mill. Three replicates of dry ground shoot and root samples were acid-digested using 4 mL H_2SO_4:$HClO_4$ (4:1 v/v) after the prior addition of 1 mL H_2O_2 to 0.5 g of sample. All samples were allowed to pre-digest for 24 h and then heated (220°C) until the solution had a clear appearance. Each digest solution was filtered (Whatman 42) and diluted with deionized water. Concentrations of Zn, Cd, Pb, Ni, Cu and Co were determined by flame atomic absorption spectrometry (Perkin Elmer 3100). For control of the digestion procedure, blanks were included in triplicate. Internal reference standards were also incorporated. Certified standard stock solutions (1000 mg L^{-1}) were used for calibrating the instrument used for sample analyses. The quality of the analytical methods was controlled considering detection limit and variation coefficient. The detection limit was calculated according to Wels and Sperling (2007).

Analysis of Tailings Substrates

For determination of total concentration of PTEs, substrate samples (both bulk and rhizospheric) were digested with HNO_3 using the USEPA 3050 method (EPA 1992). Extractable PTEs were analyzed by diethylene triamine pentaacetic acid-triethanolamine-calcium chloride (DTPA-extractable) procedure in an extraction ratio 1:4 (w:v) substrate:solution (Lindsay and Norvell 1978). Water-soluble elements were analyzed after extraction for 16 h using 1:2.5 (w:v) substrate:deionized water (Shuman 1985). For quality control of the procedures, blanks for both extractions were included in triplicate. pH, electric conductivity, and available P were determined as mentioned by Ruiz-Olivares *et al.* (2013). Particle distribution was determined by Bouyoucos method. Organic matter was analyzed by Walkley and Black procedure (Nelson and Sommers 1982).

The bioavalability index (BI) was calculated based on the quotient of DTPA-extractable and total concentrations of PTE in the rhizospheric substrate (Chen, Shian, and Qian 1996). The quotients were multiplied by 100 to express the index in percentages. We use the quotient as an estimation of the plant-available fraction of PTEs in the rhizosphere. However, we prefer the term 'extractable' instead of 'available'.

$$BI\,(\%) = \frac{DTPA - extractable\ concentration\ of\ PTE\ in\ rizosphere}{Total\ concentration\ of\ PTE\ in\ rhizosphere} \times 100$$

Bioaccumulation Factors

Two kinds of bioconcentration factors (BCF) were calculated in order to express the relationship between PTEs concentrations in shoot plants and in the substrate (Peuke and Rennenberg 2005), specifically in rhizosphere.

$$BCFT = \frac{PTE\ concentration\ in\ shoot}{Total\ concentration\ of\ PTE\ in\ rhizosphere}.$$

$$BCFD = \frac{PTE\ concentration\ in\ shoot}{DTPA - extractable\ concentration\ of\ PTE\ in\ rhizosphere}.$$

The translocation factor (TF), defined as the quotient of metal concentration in the shoots to the roots, is an estimation of the plant's capacity to translocate a PTEs from roots to the shoots (Yoon *et al.* 2006). A TF was calculated for each PTE.

Results

Identified Plant Species

On the mine tailing heaps in total 12 plant species were identified (Table 1). Four species occurred at both tailings: *Dalea bicolor* Humb. & Bonpl. Ex Willd., *Viguiera dentata* (Cav.) Spreng., *Brickellia veronicifolia* (Kunth) A. Gray and *Dichondra argentea* Willd. Species collected only from the SF tailings were *Cuphea lanceolata* Aiton, *Ruta graveolens* L., *Pteridium* sp. and *Juniperus* sp. Further, *Aster gymnocephalus* A. Gray*, Crotalaria pumila* Ortega*, Gnaphalium* sp. and *Flaveria trinervia* were only found on the SM.

Under normal conditions *B. veronicifolia*, *D. bicolor* and *R. graveolens* are shrubs with abundant foliage and commonly reach a size of 90 cm or higher (Rzedowsky and Rzedowsky 1985). At both sites, *B. veronicifolia* and *D. bicolor* were between 80 and 100 cm height, with abundant foliage. But, at the SF tailing, *R. graveolens* grew up to 20 cm. *Dichondra argentea* is a creeping species, which stems can grow up to 80 cm (Rzedowsky and Rzedowsky 1985), but the plants found were between 10 and 50 cm. It is commonly found in grasslands, xerophytic scrubs and dry sunny places (Sánchez-Sánchez 1979). *Viguiera dentata* is a perennial herb that grows up to 2.5 m (Rzedowsky and Rzedowsky 1985), but on the mine tailings it reached an average of 1.3 m. At the SM, *V. dentata* was found together with *A. gymnocephalus* and *Gnaphalium* sp. *Juniperus* sp. and *Pteridium* sp. grew in clusters on SF (Figure S1).

At SM, *C. pumila* was the most abundant plant; 40 specimens were together forming a patch (Figure S1), they had an average height of 20 cm and produced abundant seeds. *Aster gymnocephalus* plants were about 20 cm high but under favorable conditions it can reach up to 50 cm (Rzedowsky and Rzedowsky 1985). This species is common on eroded soils.

Characterization of Bulk and Rhizospheric Substrates of the Tailings

The average pH of non-oxidized SF bulk substrate was 7.8; but, in oxidized conditions was very acidic (1.7). However, for all rhizospheric samples the pHs were close to 7 (Table 1). EC of non-oxidized tailing varied 1.6–4.1 dS m^{-1}, in oxidized increased up to 5 dS cm^{-1}. Organic matter content varied 8.2 to 80.5 g kg^{-1}, the predominant particles were sand (410–830 g kg^{-1}), followed by lime (140–340 g kg^{-1}) and clay (30–210 g kg^{-1}). P extracted was very low (15.6–23.7 mg kg^{-1}).

On SM, the pH of the bulk substrate was 7.6, which was higher than those in the rhizospheres (5.8 to 6.8). EC values of rhizospheric samples were below 4 dS cm^{-1}. Organic matter content ranged from 1.1 to 8.2 g kg^{-1}. Sand content varied from 390 to 570 g kg^{-1}; lime 240–340 g kg^{-1} and clay 90–270 g kg^{-1}. pH and EC in rhizosphere should not be limiting factors for plant development, but phosphorus do, with 1.8– 8.7 mg kg^{-1}.

At both sites, Cd and Pb total concentrations in the bulk substrates were higher than the Mexican reference limit for industrial soils (NOM-147-SEMARNAT/SSA1 2004). While, only in oxidized bulk substrate from SF total Ni concentration exceed such regulation.

In general, total concentrations of PTEs in rhizospheric substrates were lower than those in non-rhizospheric bulk substrates (Table 1). Total Zn, Cd, Pb and Co concentrations in rhizospheres were similar in the two tailings, but the range was greater in those from SF than in SM. The opposite was observed for Cu and Ni.

The difference between total concentrations in rhizospheric and bulk soil has different possible explanations. Waste has been mixed with other material during tailing manipulation and construction of the heap. Since chemical and mineralogical characteristics of the residues are variable, it is possible to observe pH changes in discrete points in mine tailings, because of the difference in solubility of the various minerals. Due to residue management, it is possible to find specific points where the chemical conditions are not so stressful for plants, allowing them set up and progressive colonization. Along time plants might remove PTEs from rhizospheric soil.

DTPA-extractable Cu concentrations were similar for the two tailings (Table 1). Zn and Pb DTPA-extractable concentrations in rhizospheric substrates from the SF site were higher than the concentrations in non-vegetated tailings. The rhizosphere of *Juniperus* sp. contained approximately 8× more Zn in comparison to the bulk substrate. At SM, the DTPA-extractable Zn concentrations in rhizospheric substrates were higher than those in bulk substrates, except for *C. pumila* and *D. argentea* rhizospheres. Concentrations of Co in rhizospheric substrates were 2–7 times higher than in the bulk substrate. The DTPA-extractable concentrations of Cd in the bulk substrates were higher than in rhizospheric samples.

Root exudates release by plants may modify the pH (Blossfeld *et al.* 2010; Bravin *et al.* 2009), increasing the minerals dissolution (Houben and Sonnet 2012). The rhizospheric microorganisms (bacteria and mycorrhizal fungi) could contribute to this effect (Bravin *et al.* 2009); most of collected plants were associated with different arbuscular mycorrhizal fungal species (data not shown), which could have variation on the rhizosphere.

The highest concentrations of water soluble PTEs were observed for Zn in the rhizospheres of *Juniperus* sp. (3 mg kg^{-1}), *B. veronicifolia* (2 mg kg^{-1}), and *D. bicolor* (2 mg kg^{-1}). In general, the water soluble concentrations of PTEs were higher in bulk substrate than in rhizospheres (Table S1).

Zinc was the element with the highest BI; *F. trinervia* had 35%, followed by *D. argentea* (20%) from SF. Except for *C. pumila*, all BI-Cu were around 1 and 2%. The rhizosphere of *B. veronicifolia* from the SF site had the highest BI for Cd (9%), Ni (34%) and Co (3%). In the case of Pb, 5% was the highest BI observed in *Juniperus* sp., *A. gymnocephalus* and *Gnaphalium* sp. (Table S2).

PTEs Concentrations in Plant Tissues

The shoot concentrations of Zn and Cu (Figures 1a and 1d) were variables among species. *Cuphea lanceolata,* from SF, and *Gnaphalium* sp. from SM had the highest Zn concentrations in shoots. This last species also had the highest Cu concentration. Excepting *R. graveolens, D. bicolor, V. dentata* and *D. argentea* from SM, the rest of plants exceeded phytotoxicity level (PL)

Table 1. Potentially toxic elements concentrations (mg kg^{-1}) in two mine tailings in Zimapan, Mexico

Tailing/ rhizospheric tailing	pH	EC^a dS cm^{-1}	Zn		Cd		Pb		Cu		Ni		Co	
			Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
San Francisco non oxidized	7.8 ± 1.3	3.4 ± 1.5	4745 ± 1613	283 ± 357	157 ± 310	5 ± 3	1923 ± 943	183 ± 174	1045 ± 490	69 ± 57	60 ± 22	1.3 ± 1.1	52 ± 17	1.5 ± 1..3
San Francisco oxidized	1.7 ± 0.3	5.0 ± 0.5	5550 ± 67	139 ± 13	1190 ± 81	21 ± 2	5777 ± 60	8 ± 2	847 ± 29	6 ± 1	4550 ± 66	17 ± 1	1710 ± 65	2 ± 0.3
Rhizosphere of:														
Pteridium sp.	6.4 ± 0.4	2.8 ± 0.1	2631 ± 59	316 ± 19	60 ± 7	2 ± 0.4	4890 ± 44	26 ± 4	357 ± 11	6 ± 2	513 ± 25	5 ± 1	409 ± 21	2 ± 0.2
Juniperus sp.	5.8 ± 0.2	1.8 ± 0.1	4432 ± 74	1168 ± 23	127 ± 6	7 ± 0.3	3521 ± 62	184 ± 10	635 ± 45	4 ± 0.3	655 ± 26	7 ± 1	428 ± 51	1 ± 0.4
Cuphea lanceolata	6.5 ± 0.2	1.8 ± 0.1	2063 ± 65	184 ± 33	30 ± 5	2 ± 0.3	1598 ± 63	33 ± 5	188 ± 36	3 ± 0.4	795 ± 12	19 ± 3	231 ± 48	2 ± 0.1
Dichondra argentea	6.6 ± 0.2	2.4 ± 0.2	4703 ± 40	948 ± 19	80 ± 6	4 ± 0.1	2461 ± 46	60 ± 3	517 ± 30	3 ± 1	33 ± 3	9 ± 2	420 ± 41	1 ± 0.1
Brickellia veronicifolia	6.4 ± 0.2	1.9 ± 0.1	4642 ± 64	754 ± 20	72 ± 13	6 ± 1	2777 ± 54	121 ± 16	276 ± 51	6 ± 1	33 ± 3	11 ± 1	250 ± 37	6 ± 0.4
Ruta graveolens	6.8 ± 0.1	1.8 ± 0.1	4011 ± 60	513 ± 36	54 ± 9	2 ± 0.1	1889 ± 56	40 ± 2	315 ± 39	3 ± 1	57 ± 6	9 ± 0.4	374 ± 45	1 ± 0.2
Dalea bicolor	6.7 ± 0.1	1.7 ± 0.2	5368 ± 71	754 ± 20	105 ± 14	8 ± 2	1117 ± 61	32 ± 4	430 ± 11	4 ± 1	463 ± 14	20 ± 2	315 ± 33	2 ± 0.4
Viguiera dentata	6.8 ± 0.1	1.6 ± 0.2	2649 ± 47	250 ± 17	55 ± 10	2 ± 0.1	1959 ± 78	24 ± 7	178 ± 37	4 ± 0.4	495 ± 19	14 ± 1	182 ± 27	2 ± 0.4
Range	5.8–6.8	1.6–2.8	2063–5368	184–1168	30–127	2–8	4890–1117	24–184	178–635	3–6	33–795	5–20	182–428	1–6
Santa Maria	7.6 ± 0.4	0.7 ± 0.2.	4546 ± 58	65 ± 9	120 ± 58	9 ± 2	4183 ± 67	188 ± 21	1764 ± 35	5 ± 1	1112 ± 60	6 ± 0.4	972 ± 58	0.4 ± 0.1
Rhizosphere of:														
Aster gymnocephalus	6.4 ± 0.2	0.5 ± 0.1	4396 ± 48	182 ± 20	51 ± 8	1 ± 0.3	802 ± 43	43 ± 3	703 ± 24	4 ± 0.2	322 ± 11	5 ± 1	571 ± 29	1 ± 0.1
Gnaphalium sp.	5.8 ± 0.3	0.5 ± 0.1	4068 ± 41	164 ± 3	43 ± 6	1 ± 0.2	2080 ± 62	104 ± 10	1324 ± 25	2 ± 0.3	790 ± 24	6 ± 1	475 ± 36	2 ± 0.3
Viguiera dentata	6.5 ± 0.1	0.4 ± 0.1	4081 ± 34	173 ± 18	48 ± 8	1 ± 0.1	1493 ± 38	26 ± 3	527 ± 13	4 ± 0.2	687 ± 28	3 ± 1	444 ± 44	1 ± 0.2
Dalea bicolor	6.6 ± 0.1	0.4 ± 0.1	3799 ± 41	77 ± 14	40 ± 4	1 ± 0.3	2158 ± 62	20 ± 2	1154 ± 17	3 ± 1	230 ± 13	3 ± 1	381 ± 27	3 ± 0.1
Crotalaria pumila	6.4 ± 0.3	0.4 ± 0.1	326 ± 36	8 ± 1	24 ± 4	1 ± 0.2	1194 ± 63	8 ± 0.4	25 ± 7	7 ± 1	904 ± 29	4 ± 1	213 ± 43	3 ± 0.3
Brickellia veronicifolia	6.8 ± 0.3	0.4 ± 0.1	4381 ± 32	174 ± 17	60 ± 7	1 ± 0.3	1970 ± 61	34 ± 1	812 ± 43	9 ± 0.2	721 ± 8	6 ± 2	65 ± 15	2 ± 0.4
Flaveria trinervia	6.7 ± 0.2	0.3 ± 0.1	303 ± 26	107 ± 15	32 ± 7	1 ± 0.1	872 ± 13	12 ± 2	576 ± 11	1 ± 0.3	579 ± 8	3 ± 1	331 ± 21	1 ± 0.2
Dichondra argentea	6.8 ± 0.2	0.3 ± 0.1	275 ± 17	4 ± 1	33 ± 4	1 ± 0.1	932 ± 15	8 ± 0.3	618 ± 22	2 ± 0.2	443 ± 9	2 ± 1	60 ± 12	1 ± 0.1
Range	5.8–6.8	0.3–0.5	275–4396	4–182	24–60	1	802–2158	8–104	25–1154	1–9	230–904	2–6	60–571	1–3
TC-A/R/C					37		400				1600			
TC-I					450		800				20,000			

All the values are mean of three replicates ± standard deviation; n = 3.^{a} electrical conductivity: TC-A/R/C: Mexican reference for total concentrations in agricultural/residential/commercial soil (NOM-147-SEMARNAT/SSA1, 2004); TC-I: Mexican reference for total concentrations industrial soil (NOM-147-SEMARNAT/SSA1, 2004).

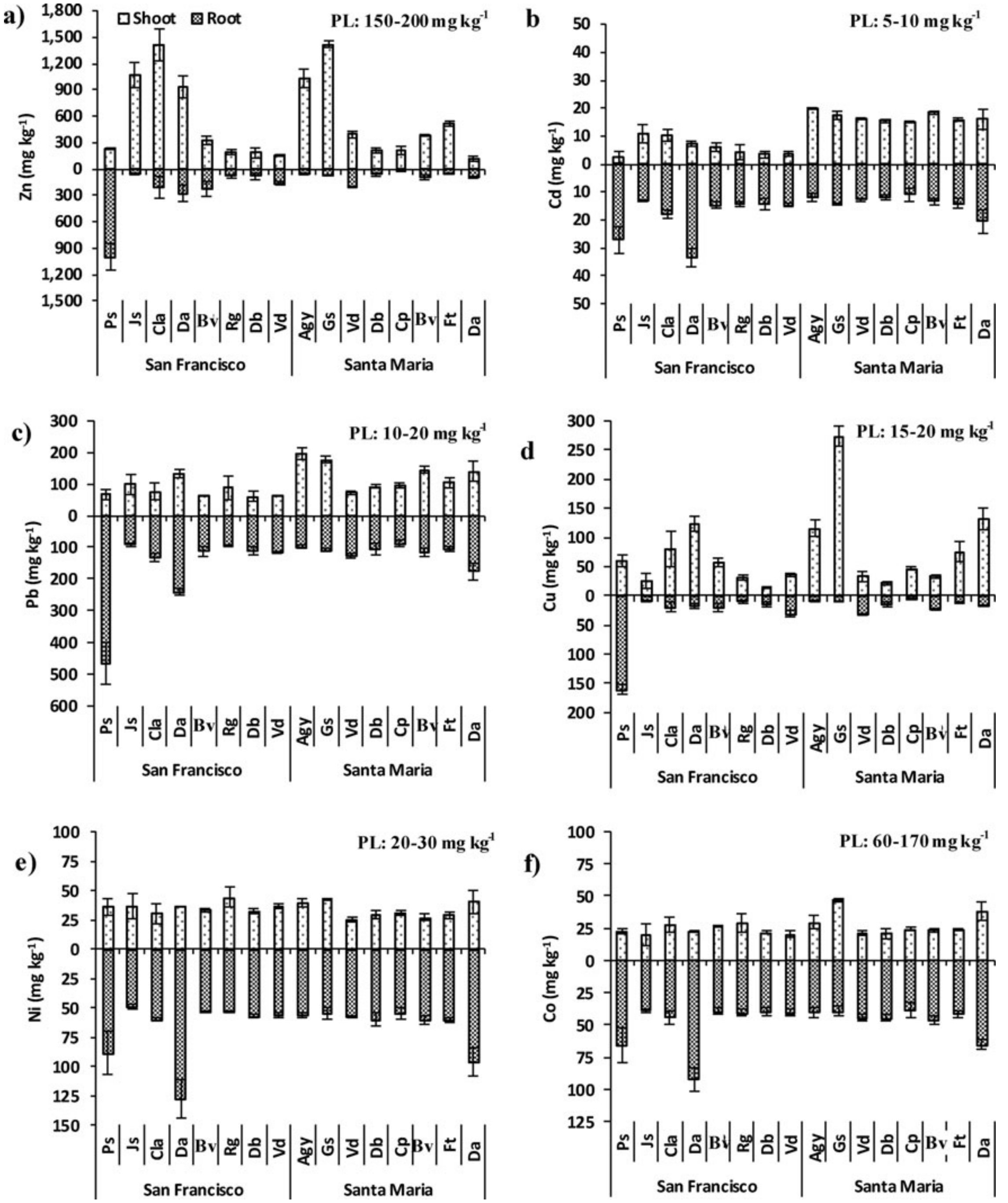

Fig. 1. Concentrations of Zn (a), Cd (b), Pb (c), Cu (d), Ni (e), and Co (f) in shoots and roots of wild plants growing on two mine tailings at Zimapan, Mexico. Mean (n = 3), error bars represent standard deviation. Identification species similar to those in Table 1. PL: phytotoxicity level (Vamerali *et al.* 2010).

for Zn. Only *D. bicolor* did not reach the Cu-PL. The PLs are those mentioned by Vamerali *et al.* (2010).

Cadmium concentrations among plants from SM were homogeneous. These values, were higher than those observed in SF. The same trend was identified for Pb. Plant species at both sites exceeded the PL for shoot Pb concentrations (Figure 1). All species collected from SM and *Juniperus* sp. from SF showed Cd shoot concentrations above the PL.

Shoot concentrations of Ni and Co were similar in all species at both sites. While all species investigated had Ni concentrations above PL; none species reached the PL for Co.

For all species, concentrations of Pb, Ni and Co were higher in roots than in shoots (Figure 1c, e, f), except for *A. gymnocephalus* and *Gnaphalium* sp. In species from the SF site Cd concentration were highest in roots, whereas for plants collected at SM, concentrations in roots and shoots were similar (Figure 1b). Concentrations of Zn and Cu in all species were higher in shoots than in roots (Figure 1a, d). Only *Pteridium* sp. roots contained about 5 times more Zn and Cu than the shoots of this species.

Bioaccumulation Factors

BCFs were different when calculated from total and DTPA-extractable concentrations; the formers were obviously lower than the second ones. In many species and elements the BCFTs were zero, especially for Pb in SF and Ni in SM (Table 2). Total concentrations do not reflect bioavailable concentrations; therefore, the BCFDs are described here. In all elements and species from SM BCFDs were >1. Plants from SF had BCFD >1 in the case of Cu, Ni and Co; but the values were variable for Zn, Cd and Pb.

It is noteworthy that for individuals of the same species but originated from the two different sites under investigation, clearly distinct BCFDs were observed. *Dichondra argentea*, when growing at SM, had the highest BCFDs for Pb and Ni, being respectively 8 and 4 times higher than those from the SF tailing. In *B. veronicifolia*, *D. bicolor* and *V. dentata* from the SM, Zn-BCFDs were >1, in those from the SF tailings <1. Similar trends were observed for Pb in *B. veronicifolia* and Cd in *D. bicolor.*

In general, the order of TFs was $Zn>Cu>Pb>Cd>Ni = Co$ (Table 2). The highest TFs for Zn and Cu were observed in *Gnaphalium* sp. (>20). *Aster gymnocephalus* demonstrated high TFs for Cd, Zn, Cu and Pb. In case of Cd, all plants originating from the SF tailing showed a TF <1, whereas the TFs for plants collected from SM were ≥ 1. For all species the Ni-TF was <1. Co-TF for all species, except *Gnaphalium* sp., was <1. *Pteridium* sp. was the only species with a TF<1 for all elements studied.

Discussion

Some of the species identified in this study were already found on mine tailings in different Mexican semi-arid regions: *B. veronicifolia* (Flores-Tavizón *et al.* 2003), *D. bicolor* (González-Chávez *et al.* 2009; Ortega-Larrocea *et al.* 2010), *A. gymnocephalus*, *Juniperus* sp. (González and González-Chávez 2006), *F. trinervia* and *V. dentata* (Franco-Hernández *et al.* 2010). The reported concentrations of PTEs in vegetal tissues and the BCFs are variable, but the presence of these plants in several mine tailings suggests that they possess a high PTEs tolerance and are adapted to the arid conditions prevalent at these sites. There were also identified other species that have not been reported previously as PTEs tolerants: *C. lanceolata, D. argentea, R. graveolens, and C. pumila.*

There were no hyperaccumulator species according to the criteria mentioned either by Brooks (2000) or more recently by van der Ent *et al.* (2012), but some species could be appropriate for use in phytoextraction or phytostabilization of PTEs contaminated substrates. Efficient phytoextraction depends basically on high PTEs concentrations in harvestable plant tissues (generally leaves and stems) (Vangronsveld *et al.* 2009; Barrutia *et al.* 2011), and high plant biomass production (Cortés-Jiménez *et al.* 2013). In this study there were species with relatively high PTEs concentrations (Figure 1) that might be suitable for phytoextraction purposes. However, Vangronsveld *et al.* (2009) mentioned important limitations of PTEs phytoextraction: (1) it can only be used for low to moderately contaminated soils, (2) its applicability is limited to soil surface (at rooting depth) which varies with the species used, but on average is not more than 50 cm. In case of some woody plants, the target zone can be in the range of one to several meters. Thus, phytoextraction does not sound a practical applicable technique for mine tailings. The application of fast growing high biomass species, also offers the possibility to combine PTEs extraction with the production of biomass for bioenergy production (Ruiz-Olivares *et al.* 2013; Schröder *et al*. 2008).

Aster gymnocephalus and *Gnaphalium* sp. showed high BCFDs and had concentrations of Zn, Cd, Pb, Ni and Cu higher than PL; thus they may be useful for phytoextraction purposes. As well as *F. trinervia* and *C. pumila* for Zn, Cd, Pb and Ni. Though, keeping in mind the limitations of phytoextraction mentioned before. It is also recommended that more studies on biomass production, extraction mechanisms, extraction efficiency and propagation techniques are performed in order to successfully apply this strategy in the field.

According to Arthur *et al.* (2005), phytostabilization establishes a plant cover in order to prevent PTEs dispersion by wind or water erosion, or by leaching. Phytostabilization does not necessarily depend on biomass production or PTEs accumulation in aerial plant tissues. The basis of phytostabilization is to decrease PTEs bioavailability by ‘fixing’ elements in the rhizosphere or in roots. Metal immobilizing soil amendments can also contribute to this process (Vangronsveld *et al.* 2009). *Pteridium* sp. was the only plant where TFs for all PTEs were <1; by consequence it might be a useful species for phytostabilization of Zn, Cd, Pb, Ni, Cu and Co contaminated substrates.

It is remarkable that the same species growing on the two study sites has different BCFDs for certain elements (Table 2). For example, the BCFD for Zn of *D. bicolor* growing at SM was 2.7 whereas at SF it was 0.3. Similar observations were done in *V. dentate,* and in *D. bicolor* and *B. veronicifolia* for Cd. Like for the BCFDs also the TFs for the same species but collected at different sites were distinct. *Dalea bicolor* and *V. dentata* growing at SM had a Cd-TF >1 but <1 when growing at SF. With this perspective, the same species can behave as an accumulator or as an excluder depending on the site characteristics where it is growing. The behavior of PTEs accumulation in plants seems to be affected by the environment (van der Ent *et al.* 2012). This is support by the results of correlation analysis. Shoot and roots PTEs concentration were significant correlated with different variables at each site, SF (Table S3) and SM (Table S4).

Taking into account the differences in total and extractable metal concentration in rhizosphere and bulk substrate, it is recognized the importance of rhizospheric processes in phytoremediation (Wenzel 2009); but at the same time it is accepted their complexity and heterogeneity. Through the release of different compounds it is possible that plants, and their associated microorganisms, modify their rhizospheres. Additionally,

Table 2. Bioconcentration and translocation factors of wild plants growing on two mine tailings in Zimapan, Hgo

	Zn			Cd			Pb			Cu			Ni			Co		
Species	BCFT[a]	BCFD[b]	TF[c]	BCFT	BCFD	TF	BCFT	BCFD	TF	BCFT	BCFD	TF	BCFT	BCFD	TF	BCFT	BCFD	TF
San Francisco																		
Pteridium sp.	0.1	0.7	0.2	0.0	1.1	0.1	0.0	2.6	0.2	0.2	10.2	0.4	0.1	7.7	0.4	0.1	14.0	0.4
Juniperus sp.	0.2	0.9	17.0	0.1	1.6	0.8	0.0	0.5	1.1	0.0	7.4	3.0	0.1	5.3	0.7	0.1	19.7	0.5
Cuphea lanceolata	0.8	7.7	6.7	0.3	4.6	0.6	0.1	2.4	0.6	0.4	29.6	3.9	0.0	1.6	0.5	0.1	169.5	0.6
Dichondra argentea	0.2	1.0	3.4	0.1	1.7	0.2	0.1	2.3	0.6	0.2	35.4	7.1	1.1	4.1	0.3	0.1	16.3	0.3
Brickelia veronicifolia	0.1	0.4	1.4	0.1	1.0	0.4	0.0	0.5	0.6	0.2	9.3	2.8	1.0	3.0	0.6	0.1	4.1	0.7
Ruta graveolens	0.0	0.4	2.5	0.1	2.4	0.3	0.1	2.2	1.0	0.1	10.6	2.9	0.8	4.8	0.8	0.1	19.5	0.7
Dalea bicolor	0.0	0.3	2.3	0.0	0.4	0.3	0.2	1.9	0.6	0.0	4.1	1.0	0.1	1.6	0.6	0.1	10.6	0.5
Viguiera dentata	0.1	0.6	0.9	0.1	2.0	0.2	0.0	2.6	0.5	0.2	10.2	1.2	0.1	2.6	0.6	0.1	10.1	0.5
Range	0.0–0.8	0.3–7.7	0.2–17	0.0–0.3	0.4–4.6	0.1–0.8	0.0–0.2	0.5–2.6	0.2–1.1	0.0–0.4	4.1–35.4	0.4–7.1	0.0–1.0	1.6–7.7	0.3–0.8	0.1–0.1	4.1–169.5	0.3–0.7
Santa Maria																		
Aster gymnocephalus	0.2	5.7	20.5	0.4	23	1.7	0.3	4.6	2.0	0.2	28.4	12.7	0.1	8.2	0.7	0.1	372.5	0.7
Gnaphalium sp.	0.4	8.6	20.8	0.4	27.2	1.2	0.1	1.7	1.6	0.2	125.0	27.7	0.1	7.3	0.8	0.1	30.5	1.2
Viguiera dentata	0.1	2.3	2.0	0.3	17.1	1.3	0.1	2.8	0.6	0.1	8.6	1.0	0.0	7.5	0.4	0.1	16.2	0.5
Dalea bicolor	0.1	2.7	3.3	0.4	32.4	1.3	0.0	4.6	0.9	0.0	8.0	1.6	0.1	8.6	0.5	0.1	7.8	0.5
Crotalaria pumila	0.9	26.9	11.6	0.6	19.7	1.4	0.1	12.3	1.1	2.0	6.6	9.2	0.0	7.4	0.6	0.1	9.8	0.6
Brickelia veronicifolia	0.1	2.2	4.2	0.3	16.4	1.4	0.1	4.2	1.3	0.0	3.8	1.4	0.0	4.7	0.5	0.4	12.4	0.5
Flaveria trinervia	1.7	4.8	10.9	0.5	74.6	1.1	0.1	8.6	1.0	0.1	55.0	5.6	0.0	10.7	0.5	0.1	44.7	0.6
Dichondra argentea	0.4	25.7	1.3	0.5	51.6	1.0	0.1	18.4	0.8	0.3	69.7	7.2	0.1	17.8	0.4	0.8	56.1	0.6
Range	0.1–1.7	2.2–26.9	1.3–20.8	0.3–0.6	17.1–74.6	1.0–1.7	0.0–0.3	1.7–18.4	0.6–1.6	0.0–2.0	3.8–125	1.0–27.7	0.0–0.1	4.7–17.8	0.4–0.8	0.1–0.8	7.8–372.5	0.5–1.2

BCF: bioconcentration factor calculated from [a]Total and [b]DTPA-extractable concentration of PTE in rhizosphere; [c]: translocation factor.

mine tailings have specific mineralogical and physicochemical characteristics leading to different PTEs exposure to plants. As a result, each plant species, its associated microorganisms, and the different PTEs formed a complex system with characteristics different from the bulk substrate.

Our results support the thesis that PTEs uptake and translocation from root to shoot not only depends on the plant species but that other factors. The differences in the plant strategy (accumulator or excluder) need further studies in order to determine which environmental, chemical, physical and biological factors are involved and determine which strategy is employed.

Comparing both BCF and TF is the main way to decide if a plant is a metal accumulator or excluder. The general consent is BCF and TF are higher than 1 is an accumulator plant, and with values lower than 1 is a metal excluder. However, it was observed that TF values (<1) indicate that a species does not translocate PTEs to the aboveground biomass; while the BCFD (>1) may indicate that the same species behaves as a PTEs accumulator. It is even more contrasting in the case of BCFT. Additionally, it was observed that the highest BI did not correspond with the highest values of BCFD and PTEs concentrations in plant tissues.

It is therefore required to specify and validate which of both concentrations, total or extractable and by which methodology, is the best for BCF calculation. The different behavior of the same species under different conditions and the lack of an unambiguous approach for their quantification limit their usefulness as criteria for plant selection in phytoremediation work.

Conclusions

The species studied could grow on mixed/multiple PTEs contaminated sites. The plants able to grow on these sites involve annual species, shrubs, perennial herbs, and one creeping perennial species; all of them are adapted to semi-arid conditions. This allows the establishment of a plant cover that combats dispersion of PTEs from bare mine tailings throughout the year. *Dalea bicolor, A. gymnocephalus, V. dentata* and *Juniperus* sp. are all promising plants because they were also reported growing on mine tailings in other Mexican regions. *C. lanceolata*, *D. argentea*, *R. graveolens*, and *C. pumila* are species not reported previously.

Considering the BCFDs, TFs and PL, *Pteridium* sp. shows very suitable for phytostabilization of Zn and Cd. *Aster gymnocephalus* can be a candidate for Zn, Cd, Pb, and Cu phytoextraction in PTEs contaminated susbtrates, as well as *Gnaphalium* sp. for Cu, and *C. pumila* for Zn. Further work is needed regarding potential biomass production and propagation techniques of the aforementioned species.

For a better evaluation of plant potential for use in phytoremediation it is recommended that the methodology and approach for calculation and interpretation of BCFs and TFs is standardized. Further, more fundamental research still is needed to better exploit the metabolic diversity of the plants themselves, but also to better understand the complex interactions between contaminants, soil, plant and micro-organisms (bacteria and mycorrhiza).

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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