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Accumulation of Pb, Cu, and Zn in native plants growing on contaminated sites and their potential accumulation capacity in Heqing, Yunnan

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Abstract

Phytoremediation is one of the cost-effective and environmental friendly technologies used to remove contaminants from contaminated soils, which has been intensively studied during the last decade. Presently, few economical and effective remediation methods are available for the remediation of Pb contaminated sites. This study was conducted to assess the potential of 19 plants growing on contaminated sites in Pb mine area. Plants and associated soil samples were collected and analyzed for total metal concentrations. While total soil Pb, Cu and Zn concentrations varied from 1,239 to 4,311, 36 to 1,020 and 240 to 2,380 mg/kg, those in the plant shoots ranged from 6.3 to 2,029, 20 to 570, and 36 to 690 mg/kg, respectively. Among the plants, we found that one cultivated crop (*Ricinus communis* L.) and two native species (*Tephrosia candida* and *Debregeasia orientalis*) have a great potential for phytoremediation of Pb contaminated soils, the Pb hyperaccumulation capacity of the 3 plants was found as the order: *R. communis* > *D. orientalis* > *T. candida* in the investigated area.

Key words: accumulation of Pb, Cu and Zn; cultivated crops; accumulator; lead mine; remediation

Introduction

The remediation of heavy metal contaminated sites is still a big challenge for researchers because of the non-degradability of these metals in the environment. Phytoremediation, a biological technology using plants to remove contaminants from soils has been intensively studied during the past decade due to its cost-effectiveness and environmental harmonies (Reeves and Baker, 2000; Krämer, 2005). This natural process accelerates sorption, precipitation and complexation reactions that occur naturally in soils to reduce the mobility (Bolan and Duraisamy, 2003). Chinese brake fern and Cretan brake fern were found to be hyperaccumulators for Arsenic (Wei and Chen, 2006) in China, but published reports regarding Pb accumulation by plants in China are few (Wei and Chen, 2001). Pb contamination is one of the major environmental problems in the world, particularly, in developing countries. Currently, no efficient and cost-effective method is available for the remediation of Pb contaminated soils (Ye *et al.*, 2001). Based on Baker and Brooks (1989), hyper accumulators are defined as plants that accumulate >1,000 mg/kg of Pb, Cu, Co, Cr or Ni, or >10,000 mg/kg of Mn or Zn. Hyperaccumulators of Co (26 species), Cu (24), Mn (8), Ni (145), Pb (5), and Zn (4) have been reported (Baker and Brooks, 1989). The five hyper accumulators of Pb include *Armeria martima*, *Thlaspi rotundifolium*, *Thlaspi alpestre*, *Alyssum wulfenianum*, and *Polycarpaea synandra*. However, Shen and Liu (1998) defined a plant as a hyper accumulator in which the Pb concentrations in the above ground part is 10–500 times more than that in plants from non-contaminated sites, and enrichment coefficient >1. In the past, only a few species with the ability to bioconcentrate Pb, Cu and Zn have been reported, in both tropical and temperate climatic zones (Ernst, 1996; Baker and Brooks, 1989). Similarly, Sieghardt (1987) has also reported the Pb concentrations in plant roots and shoots of five species grown on Pb/Zn contaminated mining deposits in Bleiberg, South Austria, but he found no hyper accumulators among the studied plants, as defined by Baker and Brooks (1989).

Most of the works focus on the phytoremediation of the metallic pollutants in soil, particularly on the metal hyper accumulation, which is the area of major scientific and technological interest in past years (Cunningham *et al.*, 1995; Cunningham and Ow, 1996). Although some Pb,

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Cu, and Zn hyperaccumulators have been found recently in temperate as well as in tropic regions throughout the plant kingdom, but it is generally restricted to endemic species growing on mineralized soils and related rock types (Baker and Brooks, 1989).

There are abundant metal resources in Yunnan Province, China, including Pb, Zn, and Cu, whereas Pb-Zn mines are dominant. The total reserves of Pb-Zn mines are 26.053 million tons in Yunnan. On the other hand, plant species are also very diverse in metal mining area, including various native herbaceous and cultivated crops. A significant part of the published work regarding the metal hyper accumulators is only based on the analysis of native specimens (Reeves and Baker, 1984).

This study was conducted to collect plant and soil samples from the Pb mining area and cultivated lands in Heqing County, Yunnan Province, China. Soil and plant samples were analyzed to investigate the Pb, Cu and Zn accumulation capability of 19 native and cultivated crop species, to understand the potential for phytoremediation of Pb contaminated soils

1 Materials and methods

1.1 Site description

The study area is located at Beiya Pb mine area, Heqing County, Yunnan Province, China, with north latitude of 26°16′40″ and east longitude of 100°01′36″. The altitude 2,197–2,740 m, annual temperature 13.5°C, annual rainfall 970 mm. The area is famous in China for its high Pb content in mining deposits. The geological structures are very complex with qaternary residual and talus material, teniary sandy conglomerate, limestone gravel in karst caves, syenlteporphyry, intrusive breccia, auriferous iron orebody, suerosie altered limestone, limestone gravel with calcareous cements (Xiao *et al*., 2003). Vegetation is subtropical alpine forest, including broadleaved trees, needle-leaved trees, bushes and herbaceous.

1.2 Sampling

On the basis of the existing information about agricultural and previous mining activities, we selected 8 locations for soil sampling, including 3 mining bank, 4 cultivated lands and 1 river bank. The plant samples were also collected from these locations. The samples of *Pteridium* var. *latiusculum*, *Polygonum chinense*, *Pteris ensiformis* and *Polygonum rude* were collected at site 1 with 1 km^2 as mining area. The samples of *Pterisfauriei* Hieron, *Bauhinia variegate*, *Artemisia lactiflora* Wall were collected at site 2 with 1 km^2 as mining area. The samples of *Osyris wightiana*, *Smilax china* L., *Aster subulatus* Michx, *conyza canadensia* (L.) Cronq. *Buddleia officinalis* Maxim were collected at site 3 with 1 km^2 as mining area. The sample of *Colocasia esculenta* was collected at site 4 with 667 m^2 as cultivated land. The samples of green vegetables, tender garlic shoots were collected at site 5 with 0.2 km^2 as cultivated land. The samples of *Ricinus communis* L. was collected at site 6 with 667 m^2 as cultivated land. The samples of *Rumex hastatus* and *Tephrosia candida* were collected at site 7 with 0.2 km^2 as cultivated land. The sample of *Debregeasia orientalis* was collected at site 8 with 1 m^2 as river side. Composite soil samples were collected from 0 to 20 cm soil layer, mainly from the root zone (rhizosphere) from each site. In total, 80 plant samples, including roots, shoots, leaves, stems, seeds, and blooms were collected in spring and summer, 2005. The samples collected only in spring were analyzed in the laboratory; the samples collected in summer were not analyzed. The plants for this study were based on the samples collected in spring, 2005 (Table 1).

1.3 Soil analysis

After transportation to lab, large stones and plant debris were removed from the soil samples, air dried at room temperature for 6 d, and sieved through a 2-mm mesh. The concentrations of Pb in soils were determined in the Environmental Laboratory of Yunnan Institute of Environmental Science, Kunming, China. For analysis of total metals, 0.5 g soil samples were wet digested with 15 ml of concentrated HNO_3/HCl/$HClO_4$ (1:2:2) mixture on a thermo block and then were diluted with 5 ml of 0.2% HNO_3. The total concentrations of Pb were determined in the acid extracts using Inductively coupled plasma emission spectroscopy (Optima 5300DV, Perkin Elmer Grop., USA).

1.4 Plant analysis

After collection, the plants were separated into shoots and roots. Plant shoots were rinsed thoroughly in deionized water while roots were properly washed with tap water and finally with deionized water to remove all visible soil particles. The washed plant samples were oven-dried at 70°C for 48 h to a constant weight. The dry weights were determined and the samples were ground. Sub-samples (1.0 g) were subsequently digested with 15 ml ultrapure mixture of concentrated HNO_3/$HClO_4$ (3:1; *V/V*) on a thermo block. After cooling down, the suspensions were filtered and filtrate was adjusted to 50 ml with double deionized water. Concentrations of Pb in the digested sam-

Table 1 Location of the sampling sites and pant species collected in this study

Sampling site	Site type	Species
1	Mining deposits	*Pteridium* var. *latiusculum*, *Polygonum chinense*, *Pteris ensiformis*, *Polygonum rude*
2	Mining deposits	*Pteris fauriei* Hieron, *Bauhinia variegate*, *Artemisia lactiflora* Wall
3	Mining deposits	*Osyris wightiana*, *Smilax china* L., *Aster Subulatus* Michx, *Conyza Canadensia* (L.) Cronq., *Buddleia officinalis* Maxim
4	Cultivated land	*Colocasia esculenta*
5	Cultivated land	Green vegetables, tender garlic shoots
6	Cultivated land	*Ricinus communis* L.
7	Cultivated land	*Rumex hastatus*, *Tephrosia candida*
8	River banks	*Debregeasia orientalis*

ples were determined using Inductively coupled plasma emission spectroscopy (Optima 5300DV, Perkin Elmer Grop., USA) in the Environmental Laboratory of Yunnan Institute of Environmental Science, Kunming, China.

1.5 Metal translocation factor and enrichment coefficient

Root-to-shoot translocation factor was described as the ratio of heavy metals in plant shoot to that in plant root, while enrichment coefficient (*R*) was calculated as follows:

$$R = C_{\text{aboveground}}/C_{\text{soil}} \quad (1)$$

where, $C_{\text{aboveground}}$ and C_{soil} represent the metal concentrations in the above ground parts of the plant and soil on dry weight basis, respectively. Enrichment coefficient basically depends on the soluble fraction of metals and organic matters in soils (Khan *et al*., 2006).

1.6 Statistical method

Correlation was made using bi-variation method, with one-tailed significance, and Pearson correlation coefficients were calculated using SPSS software.

1.7 Quality of control

Reagent blank and standard reference soil and plant materials were included to verify the accuracy and precision of the digestion and subsequent analysis procedure. The instruments were calibrated daily with calibration standards and the relative percent differences between the five-point calibration and the daily calibrations were < 20% for all of the garget analyses.

2 Results and discussion

2.1 Pb concentrations in soils

Table 1 shows the detail description of sampling sites and plant species. In this study, soil samples were highly contaminated with Pb due to mining activities. Table 2 summarizes Pb concentrations of rhizosphere horizons in the soils collected from mine and cultivated sites. The average concentration of Pb in soils was 2,687.1 mg/kg, with the lowest concentration of 1,239 mg/kg in the sample of rice cultivated land, and with the highest Pb concentration of 4,311 mg/kg, particularly, in the sample of mining deposits. The results of this study were higher than those reported in the previous studies (Pruvot *et al*., 2006; Marguí *et al*., 2006). For cultivated soils near mine site, Pb concentrations were 50 times lower than the mining deposits. However, it is cleared that the soil in mine area and cultivated lands near mining site were highly disturbed by mining activities of the Pb smelting factories in this area.

2.2 Cu and Zn concentrations in soils

Although the site was predominantly contaminated with Pb in this study, it also contained elevated concentrations of Cu and Zn, ranging from 36 to 1,020 mg/kg for Cu (Table 3), and from 240 to 2,380 mg/kg for Zn (Table 4). For cultivated soils near mine site, Cu average concentrations were 5.9 times lower than the mining deposits; Zn average concentrations were 2.1 times lower than the mining deposits. However, it is cleared that the cultivated lands near mining site were small distributed by mining Pb smelting activities.

2.3 Pb concentrations in plants

Table 2 presents the average values of Pb contents in native and cultivated plants (dry weights). The results showed a great variability of Pb concentrations according to plant species and sites of plant collection. The highest Pb concentration was measured in the sample of *R. communis* (2,029 mg/kg in shoot), while the lowest concentration was detected in the sample of *P. rude* (6.3 mg/kg in shoot, 8.6 mg/kg in root). The concentrations of Pb in

Table 2 Concentration of Pb in soils and plant shoots and roots (mg/kg), enrichment coefficient and translocation factor

Species	Shoot	Root	Soil	Times[a]	Enrichment coefficient	Translocation factor
Pteridium var	10.7	150.8	3,157	2	0.003	0.07
Polygonum chinense	40.1	160.1	3,008	8	0.01	0.25
Pteris ensiformis	9.2	210.8	3,219	2	0.003	0.04
Polygonum rude	6.3	8.6	2,978	1	0.002	0.73
Pteris fauriei Hieron	12.3	115.2	2,370	2	0.005	0.11
Bauhinia variegate	10.2	13.3	2,480	2	0.004	0.77
Artemisia lactiflora Wall	834	892	2,236	167	0.37	0.93
Osyris wightiana	16.8	22.3	4,230	3	0.004	0.75
Smilax China L.	9.8	460	3,980	2	0.002	0.02
Aster subulatus Michx	270	1,630	4,120	54	0.07	0.17
Conyza canadensia (L.) Cronq.	270.8	690	4,033	54	0.07	0.39
Buddleia officinalis Maxim	70.3	120	4,311	14	0.02	0.59
Colocasia esculenta		638	1,670	–		
Green vegetables	442	–	1,562	88	0.3	
Tender garlic shoot	189	–	1,544	38	0.1	
Ricinus communis L.	2,029	–	2,267	405	0.9	
Rumex hastatus	870	920	1,948	174	0.4	0.96
Tephrosia candida	1,689	1,980	2,207	338	0.8	0.85
Debregeasia orientalis	1,763	1,903	2,217	353	0.8	0.93

[a] Shoot concentration time levels compared to plants from non-contaminated sites. Concentration of plants from non-polluted environments: Pb 5 mg/kg (dw) (Shen and Liu, 1998).

Table 3 Concentration of Cu in soils and plant shoots and roots (mg/kg)

Species	Shoot	Root	Soil	Times[a]	Enrichment coefficient	Translocation factor
Pteridium var	250	280	408	8	0.61	0.89
Polygonum chinense	125	198	375	4	0.33	0.63
Pteris ensiformis	109	220	536	4	0.20	0.50
Polygonum rude	570	620	1,020	19	0.56	0.91
Pteris fauriei Hieron	120	270	297	4	0.40	0.44
Bauhinia variegate	111	300	426	4	0.26	0.37
Artemisia lactiflora Wall	152	200	279	5	0.54	0.76
Osyris wightiana	278	341	840	9	0.33	0.82
Smilax China L.	260	301	760	9	0.34	0.86
Aster subulatus Michx	205	283	611	7	0.34	0.72
Conyza canadensia (L.) Cronq.	41	57	156	1	0.26	0.72
Buddleia officinalis Maxim	20	29	36	1	0.56	0.69
Colocasia esculenta	40	48	98	1	0.40	0.83
Green vegetables	42	61	128	1	0.33	0.69
Tender garlic shoots	38	71	167	1	0.23	0.54
Ricinus communis L.	43	55	89	1	0.48	0.78
Rumex hastatus	47	57	132	2	0.36	0.82
Tephrosia candida	36	47	89	1	0.40	0.77
Debregeasia orientalis	35	42	53	1	0.66	0.83

[a] Shoot concentration time levels compared to plants from non-contaminated sites. Concentration of plants from non-polluted environments: Cu 30 mg/kg (dry weight) (Yoon *et al*., 2006).

Table 4 Concentration of Zn in soils and plant shoots and roots (mg/kg)

Species	Shoot	Root	Soil	Times[a]	Enrichment coefficient	Translocation factor
Pteridium var	255	293	630	9	0.40	0.87
Polygonum chinense	158	207	772	5	0.20	0.76
Pteris ensiformis	178	230	834	6	0.21	0.77
Polygonum rude	263	560	568	9	0.46	0.47
Pteris fauriei Hieron	690	938	2,380	23	0.29	0.74
Bauhinia variegate	127	366	480	4	0.26	0.35
Artemisia lactiflora Wall	107	249	496	4	0.22	0.43
Osyris wightiana	189	253	466	6	0.40	0.75
Smilax China L.	131	366	739	4	0.18	0.36
Aster subulatus Michx	150	264	497	5	0.30	0.57
Conyza canadensia (L.) Cronq.	42	48	323	1	0.13	0.88
Buddleia officinalis Maxim	36	53	270	1	0.13	0.68
Colocasia esculenta	44	67	360	1	0.12	0.66
Green vegetables	39	52	266	1	0.15	0.75
Tender garlic shoots	38	71	306	1	0.12	0.54
Ricinus communis L.	43	55	277	1	0.16	0.78
Rumex hastatus	47	57	295	1	0.16	0.82
Tephrosia candida	36	47	256	1	0.14	0.77
Debregeasia orientalis	35	42	240	1	0.15	0.83

[a] Shoot concentration time levels compared to plants from non-contaminated sites. Concentration of plants from non-polluted environments: Zn 30 mg/kg (dw) (Yoon *et al*., 2006).

plant shoots were ranged from 6.3 to 2,029 mg/kg with an average value <1,000 mg/kg, which were 2–405 times higher than in plants collected from non-contaminated sites (Table 2). This study showed that cultivated crops were also significantly contaminated by Pb, particularly near the mine site. These results are inconsistent with those observed by Liu *et al*. (2005). According to the criteria set by Baker and Brooks (1989), there were 3 plant species, including *R. communis*, *T. candida* and *D. orientalis*, to be considered as Pb hyper accumulators in the study area. Among these plants, the *R. communis* is a cultivated crop, while the other 2 plants *T. candida* and *D. orientalis*, are native plants. The concentrations of Pb in green vegetables and tender garlic shoots have exceeded the maximum permissible limit (0.2 mg/kg) set for vegetable and fruits in China, with 442 and 189 mg/kg, respectively, grown in Pb contaminated soils. The accumulating capacity of Pb for hyperaccumulators were in the order of *R. communis* > *D. orientalis* > *T. candida*.

2.4 Cu and Zn concentrations in plants

Tables 3 and 4 present the average values of Cu and Zn contents in native and cultivated plants (dw). The results showed that the variability of Cu and Zn concentrations was not as a great variability of Pb concentrations according to plant species and sites of plant collection. The results also showed that the concentrations of Cu and Zn in plant at the mining deposits were higher than those in the cultivated lands, the Cu and Zn concentrations were greater in the roots than in shoots.

Copper concentrations in the plant shoots varied from 20 to 570 mg/kg (Table 3), the maximum value was found in the root of *P. rude*, no plant species accumulated Cu above 1,000 mg/kg. Cu concentrations of 6.4–160 mg/kg in the plant biomass were reported by Stoltz and Greger (2002), which were lower than those in our research. Shu *et al*. (2002) reported Cu concentrations of 7–198 mg/kg in plant biomass of *Paspalum distichum* and *C. dactylon*.

Zn concentrations in the plants shoots ranged from 35 to 690 mg/kg (Table 4), the maximum value was found in the root of *Pteris fauriei* Hieron and no plant species accumulated Zn above 1,000 mg/kg. Research conducted by Stoltz and Greger (2002) showed Zn concentrations of 68–1,630 mg/kg in plant biomass while those by Shu *et al*. (2002) showed 66–7,607 mg/kg in plant biomass.

2.5 Pb, Cu, and Zn enrichment coefficient

The enrichment coefficient values of all studied samples for Pb are given in Table 2. Enrichment coefficients varied between 0.002 and 0.9, but always < 1. The maximum value (0.9) was observed for *R. communis*, while minimum (0.002) for *P. rude*. Enrichment coefficient is an important factor when considering the phytoremediation potential of a plant species (Zhao *et al*., 2003). In the present, there are 4 rules for hyper accumulator. One of the standard is enrichment coefficient, the enrichment coefficient > 1 shows a special ability of the plant to absorb from soils and transport metals and store them in their above-ground part (Baker and Brooks, 1989; Brown *et al*., 1995). But some research indicate that although the enrichment coefficient < 1, some plant were heavy metal hyper accumulator (Zu *et al*., 2005).

The enrichment coefficient values of all studied samples for Cu are given in Table 3. Enrichment coefficients varied between 0.20 and 0.66, but always < 1. The maximum value (0.66) was observed for *D. orientalis*, while minimum (0.20) for *P. ensiformis*.

The enrichment coefficient values of all studied samples for Zn are given in Table 4. Enrichment coefficients varied between 0.12 and 0.46, but always < 1. The maximum value (0.46) was observed for *P. rude*, while minimum (0.12) for *C. esculenta*.

2.6 Pb, Cu and Zn translocation factor

Soil-to-root and root-to-shoot concentration factors of metals are considered as important factors for selection of plants for phytoremediation. Translocation factors higher than 1 indicated a very efficient ability to transport metal from roots to shoots, most likely due to efficient metal transport systems (Zhao *et al*., 2002). The translocation factors for Pb ranged from 0.02 to 0.93, with the highest value in the sample of *A. lactiflora* and the lowest for *S. china*. The results indicated that the translocation factors of plant species were lower than 1 in this study (Table 2). Low translocation of Pb from roots to shoots is possibly owing to Pb toxicity. Lead can be toxic to photosynthetic activity, chlorophyll synthesis and antioxidant enzymes (Kim *et al*., 2003). Baker and Brooks (1989) also discussed the restriction of metal uptake by plants from contaminated soils and the presence of exclusion mechanisms in plant species.

As expected, the Cu concentrations in the roots were greater than those in the shoots, the Cu concentrations ranged from 20 to 570 mg/kg and in shoots from 29 to 620 mg/kg in roots (Table 3). Translocation factors for Cu ranged from 0.37 to 0.91, with the highest value in the sample of *P. rude* and the lowest in *B. variegate* Wall. The results indicated that the translocation factors of plant species were lower than 1 in this study (Table 3).

Like Cu, the Zn concentrations in the roots were also greater than those in the shoots, the Zn concentrations ranged from 36 to 690 mg/kg in shoots and 42 to 938 mg/kg in roots (Table 4). Translocation factors for Zn ranged from 0.35 to 0.87. The maximum translocation factor was again found in the sample of *P. rude*. The lowest translocation was found in the sample of *B. variegate*. The results indicated that the translocation factors of plant species were lower than 1 in this study (Table 4).

In general, all the three heavy metals occurred at elevated levels in plant biomass collected from the site. Normal and phototoxic concentrations of Pb, Zn and Cu were reported by Levy *et al*. (1999), which were 0.5–10 and 30–300 mg/kg for Pb, 3–30 and 20–100 mg/kg for Cu, and 10–150 and > 100 mg/kg for Zn. Almost all collected plant species showed heavy metals concentration higher than the normal or phytotoxic levels. These results may indicated that plant species growing on the site contaminated with the heavy metals were tolerant of these metals. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism.

3 Conclusions

On the basis of findings from this study, it is concluded that the cultivated crop (*R. communis*) has a great potential value for phytoremediation of Pb contaminated soils because of the fact that the Pb concentration in the plant shoots was > 1,000 mg/kg, this value is 405 folds of the value detected in the plants grown in non-contaminated soils. Similarly, two native species (*T. candida* and *D. orientalis*) also showed the phytoremediation potential for Pb-contaminated soils, with Pb concentrations in the plant shoots > 1,000 mg/kg and 326–396 folds of the values in the plants grown in non-contaminated sites. In this study, the Pb accumulation capacity of 3 hyperaccumulators was found as *R. communis* > *D. orientalis* > *T. candida*.

Among the 19 plant samples, no plant species were identified as Cu and Zn hyperaccumulators.

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