Environ Geol (2007) 51: 1043–1048
DOI 10.1007/s00254-006-0373-3

ORIGINAL ARTICLE

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Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China

Received: 5 April 2006
Accepted: 6 June 2006
Published online: 28 July 2006

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Abstract The absorption and accumulation of Pb, Zn, Cu and Cd in some endurant weed plant species that survived in an old smeltery in Liaoning, China, were systematically investigated. Potential hyperaccumulative characteristics of these species were also discussed. The results showed that metal accumulation in plants differed with species, tissues and metals. Endurant weed plants growing in this contaminated site exhibited high metal adaptability. Both the metal exclusion and detoxification tolerance strategies were involved in the species studied. Seven species for Pb and four species for Cd were satisfied for the concentration time level standard for hyperaccumulator. Considering translocation factor (TF) values, one species for Pb, seven species for Zn, two species for Cu and five species for Cd possessed the characteristic of hyperaccumulator. Particularly, *Abutilon theophrasti* Medic, exhibited strong accumulative ability to four heavy metals. Although enrichment coefficients of all samples were lesser than 1 and the absolute concentrations didn't reach the standard, species mentioned above were primarily believed to be potential hyperaccumulators.

Keywords Heavy metal · Hyperaccumulator · Phytoremediation · Smeltery · Soil contamination · China

Introduction

Heavy metal contamination usually results from anthropogenic activities such as mining, smelting, mineral processing, metalliferous electroplating, internal combustion engine operating, energy and fuel production (Kim et al. 2003), causing severe damage to ecosystems including plants, animals, micro-organism and human health. Smelting activities generate a great deal of particulate emissions and waste slag enriched in heavy metals that contaminate the surrounding—soil, water and air. Such effects are particularly serious and pose a severe ecological and human health risk when smelting works are located in the vicinity of urban environments. Recently many smelteries have been moved out of cities in view of the environmental problems; however, the abandoned land surface is contaminated by heavy metals and is in great need of remediation.

Traditional techniques of soil remediation are costly and may cause the secondary pollution. Phytoremediation is a newly evolving field of science and technology to clean up polluted soil, water or air (Meagher 2000). It may be defined as the using of green plants to remove, destroy or sequester hazardous substances from environment (Zhou and Song 2004; Zu et al. 2005). A series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, low-cost and environmentally friendly technology (Zhou and Song 2001; Wei et al. 2003; Eapen and Dsouza 2005; Krämer 2005), especially for the discovery of hyperaccumulators (Wei and Zhou 2004). Hyperaccumulators which are often found growing in polluted areas can naturally accumulate higher quantities of heavy metals in their shoots than roots. In view of the fact that metal removal from soil can be greatly enhanced by the judicious selection of plant species, the knowledge about the ability of various plant species or tissues to absorb and transport metals, will thus provide an insight into choosing appropriate plants for phytoremediation (Deng et al. 2004; Zhou and Song 2004). Furthermore, the identification of hyperaccumulators is an imperious and important task as the key to successful implementation of phytoremediation (Zhou 2002; Zhou and Song 2004).

In China, there are numerous metal smelteries which cause severe heavy metal contamination, especially with Pb, Zn, Cu and Cd. However, a small number of plant species could survive and reproduce when most of plants died off due to the increasing contamination of heavy metals in soil (Zhou et al. 2001). Although these plant species surviving in the smelting areas can evolve into endurant species, whether these endurant species are of great significance in phytoremediation, is still a riddle to be explored. We collected eight weed species that were surviving in an abandoned smeltery site in Liaoning and studied the concentrations of Pb, Zn, Cu and Cd in them. The main objectives were to find out the ability of endurant plant species to accumulate and tolerate Pb, Zn, Cu and Cd, then try to identify hyperaccumulators that could be applied to the remediation of metal-contaminated soils. These informations then could be helpful in the selection of appropriate species for future phytoremediation.

Materials and methods

Site description

The Shenyang Smeltery (123°49′277″–123°49′655″ E, 42°07′476″–42°07′793″ N) was founded in 1936 and its area was 3.6×10^5 m^2. It was located in the heavy industrial center of Shenyang, which is the capital of Liaoning Province, northeast China. The main product of the smeltery was Pb, Zn, Cu, Au and Ag. However, the centuries-old smeltery was responsible for more than 40% sulfur dioxide (SO_2) and 98% lead particulate emissions within the city. As a result of pollution problems, it was demolished in 2002 and most of the site is seriously contaminated with heavy metals, but some endurant plants grow very well there.

Sampling

Sampling was carried out during late August 2004 and eight species were collected from within the smeltery. At least three individual plants from each species were randomly collected, then mixed to give a composite whole plant sample. Simultaneously, soil samples were collected from between the roots of each plant and well mixed into a soil sample for analysis.

Plant analysis

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 roots, stems and leaves were separated and oven-dried at 105°C for 30 min, then at 70°C to constant weight. The dried tissues were weighed and ground into powder for the determination of metal concentration. Metal (Pb, Zn, Cu and Cd) analysis, which was carried out by acid [conc. HNO_3 + conc. $HClO_4$ (3:1, v/v)] (Wang et al. 2003) digestion followed by measurement of total concentrations of all elements of interest using inductively-coupled plasma-atomic emission spectrometry (ICP-AES). A standard reference plant material (GBW07602) from the Ministry of Geology and Mining Industry, People's Republic of China, was used to verify the accuracy of metal determination. The recovery rates for all heavy metals (Pb, Zn, Cu and Cd) in plant samples were within $90\pm10\%$.

Soil analysis

Soil samples were air-dried for 2 weeks, and then sieved through a 2 mm mesh. The pH value was determined in a 1:2.5 (w: v) soil: deionized water slurry. The loss on ignition (LOI) (organic matter content) was determined by ashing 1 g dry soil in muffle furnace for 3 h at 600°C. Samples were then sieved through a 0.149 mm mesh and analyzed for total metals. Total metal contents [digested with conc. HNO_3 + conc. $HClO_4$ (3:1, v/v)] (Wang et al. 2003) were measured by the ICP-AES for Pb, Zn, Cu and Cd.

Data processing and statistical analysis

Translocation factor (TF) of heavy metals from roots to shoots and enrichment coefficient (EC) of heavy metals in a plant are calculated as follows:

$$TF = [\mathrm{Metal}]_{shoot}/[\mathrm{Metal}]_{root} \quad (1)$$

$$EC = [Metal]_{shoot}/[Metal]_{soil} \quad (2)$$

shoot concentration time level was defined as the ratio of heavy metals in plant shoots to that in plants from non polluted environments (Zu et al. 2005).

The principal component analysis (PCA) was performed to assess the correspondences among the different components of Pb, Zn, Cu, Cd, pH and LOI in soils. Dimension reduction of the data can be achieved by using a smaller number of principal components than original variables (Heberger et al. 2003). The statistical package used throughout this study was SPSS 11.0.

Results

Accumulation of metals in plants

As shown in Table 1, accumulation of the four heavy metals in these plant species varied greatly. Considering concentrations in weed roots, Pb were ranged from 38.7 mg/kg in *Abutilon theophrasti* Medic. to 527.3 mg/kg in *Physalis angulata* L.; Zn were ranged from 20.9 mg/kg in *Conyza canadensis* (L.) Cronq. to 310.5 mg/kg in *Physalis angulata* L.; the concentration range of Cu was from 18.0 mg/kg in *Conyza canadensis* (L.) Cronq. to 191.3 mg/kg in *Helianthus tuberosus* L.; Cd concentrations in roots were ranged from 2.1 mg/kg in *Abutilon theophrasti* Medic. to 28.4 mg/kg in *Physalis angulata* L..

As for heavy metal concentrations in weed shoots, the analytical data showed that the accumulating range of Pb was from 18.7 mg/kg in *Conyza canadensis* (L.) Cronq. to 331.3 *Physalis angulata* L.; Zn was ranged from 74.4 mg/kg in *Conyza canadensis* (L.) Cronq. to 264.6 mg/kg in *Ambrosia trifida* L.; Cu concentrations in roots were from 10.2 mg/kg in *Conyza canadensis* (L.) Cronq. to 47.9 mg/kg in *Physalis angulata* L.; Cd concentrations in roots were ranged from 5.4 mg/kg in *Abutilon theophrasti* Medic. to 19.1 mg/kg in *Solanum nigrum* L..

Soil properties

The concentrations of Pb, Zn, Cu and Cd in soil from the smeltery were extremely high, up to 1,004.3–9,385.1, 736.8–9,161.7,376.4–12,531.0 and 11.2–197.3 mg/kg, respectively. As shown in Table 2, metal concentrations in soil samples collected from the smeltery were higher than those in normal soils. The maximum concentration of Pb, Zn, Cu and Cd in soil was 19, 18, 31 and 197 times as much as the third level of the National Soil-Environmental Quality Standard of China (GB 15618, 1995).

The pH value of soil samples were shown in Table 2. The soils from the smeltery were slightly alkaline except sample 3 and the pH conditions were propitious to plant growth. The organic matter content (LOI) was ranged from 2.3%~14.7%. The correspondence among the total metal contents (Pb, Zn, Cu and Cd), pH and LOI in soil was examined using the principal component analysis (PCA), which provides a data structure study in a reduced dimension, covering the maximum amount of information present in the data (Terrab et al. 2004). The relationships among the soil variables are illustrated in Fig. 1. Each variable is placed in a three-dimensional space in which the axes represent the three components. The three components explained 97.0% of the total variance in the data, with component 1, i.e. Zn, Cu and Cd, accounting for 49.0%; component 2, i.e. pH and LOI, accounting for 37.3%; component 3, i.e. Pb, accounting for 10.7%. According to the principal component analysis, Pb wasn't included in the same component with other heavy metals. In the smeltery located in the west of Shenyang, the urban heavy industrial center, many factors may therefore, cause the distinct variation of Pb concentration in soils, such as gasoline lead emissions or fuel exhaust emissions by surrounding factories (Ren et al. 2004).

Translocation factor and enrichment coefficient

Translocation factors of four heavy metals for weeds were shown in Fig. 2 (a). Only the TF_{Pb} of *Abutilon theophrasti* Medic, TF_{Cu} of *Chenopodium acuminatum*

Table 1 Pb, Zn, Cu and Cd accumulation in roots and shoots of weed species collected from the smeltery (mg/kg) and shoot concentration time levels compared to plants from non-polluted environments[a]

Species	Pb			Zn			Cu			Cd		
	Root	Shoot	Times	Root	Shoot	Times	Root	Shoot	Times	Root	Shoot	Times
Physalis angulata L.	527.3	331.3	66.2	310.5	180.8	1.8	49.5	47.9	4.8	28.4	12.2	12.2
Ambrosia trifida L.	516.5	111.9	22.4	103.6	264.6	2.6	71.2	26.4	2.6	14.0	12.8	12.8
Helianthus tuberosus L.	490.3	54.2	10.8	200.4	205.9	2.1	191.3	21.1	2.1	13.0	9.7	9.7
Solanum nigrum L.	183.8	87.4	17.5	50.4	94.7	0.9	34.4	16.8	1.7	4.0	19.1	19.1
Chenopodium acuminatum Willd	101.9	97.9	19.6	104.7	154.9	1.5	27.7	28.1	2.8	2.6	8.5	8.5
Polygonum lapathifolium L.	64.8	56.3	11.3	69.9	168.5	1.7	19.0	14.3	1.4	3.4	5.5	5.5
Conyza canadensis (L.) Cronq.	43.8	18.7	3.7	20.9	74.4	0.7	18.0	10.2	1.0	8.2	18.7	18.7
Abutilon theophrasti Medic.	38.7	61.4	12.3	56.1	158.2	1.6	32.5	37.2	3.7	2.1	5.4	5.4

[a] Concentration of plants from non-polluted environments: Pb 5 mg/kg, Zn 100 mg/kg, Cu 10 mg/kg, Cd 1 mg/kg DM

Table 2 Soil properties of the old smeltery

Sample No.	Species	Heavy metal oncentration in soil (mg/kg)				pH	LOI (%)
		Pb	Zn	Cu	Cd		
1	*Ambrosia trifida* L.	9385.1	4775.7	3944.4	85.9	7.2	10.6
2	*Solanum nigrum* L.	7121.3	1785.9	1831.6	54.4	7.5	7.6
3	*Polygonum lapathifolium* L.	4828.9	1434.2	788.4	20.9	6.2	14.7
4	*Physalis angulata* L.	3705.8	4116.0	2719.6	130.3	7.5	2.3
5	*Helianthus tuberosus* L.	3044.0	9161.7	12531.0	197.3	7.4	4.5
6	*Conyza canadensis* (L.) Cronq.	2069.8	736.8	895.8	50.8	7.8	3.8
7	*Abutilon theophrasti* Medic.	1004.3	1234.2	711.5	14.6	7.7	5.3
8	*Chenopodium acuminatum* Willd	1004.3	900.0	376.4	11.2	7.6	2.9
Average		4020.4	3018.1	2974.8	70.7	7.4	6.5
Minimum		1004.3	736.8	376.4	11.2	6.2	2.3
Maximum		9385.1	9161.7	12531.0	197.3	7.8	14.7

Willd and *Abutilon theophrasti* Medic. were higher than 1, which indicated that the transportation of Pb and Cu to the shoots of plants was not easy. As for the TF value for the other two heavy metals, TF_{Zn} of seven species and TF_{Cd} of five species were higher than 1, which showed that it is easier for Zn and Cd to move in plants than for Pb and Cu.

Considering the enrichment coefficient, all samples were lower than 1 (Fig. 2b). The maximum value for Pb, Zn, Cu and Cd was 0.10, 0.17, 0.08 and 0.76, respectively.

Discussion

Adaptability strategy of plants enduring heavy metals

The general trend from Table 2 showed that these plant species could vegetate within a broad range of metal (Pb, Zn, Cu and Cd) concentrations in the soil. The total Pb, Zn, Cu and Cd contents in the soil of the smeltery greatly exceeded the ranges which were considered toxic to normal plants (Kabata-Pendias and Pendias 1984), so these plants growing in the polluted site exhibited strong metal adaptability.

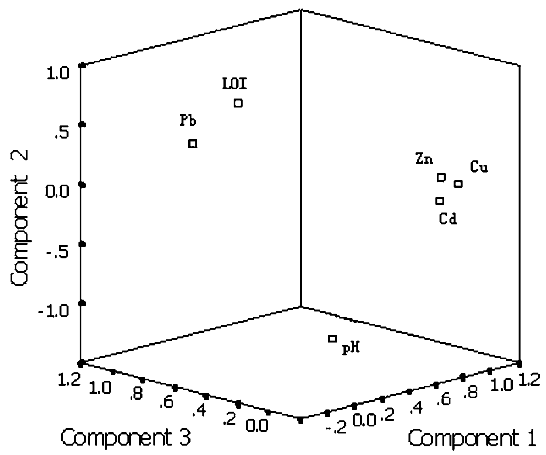

Fig. 1 Principal Component Analysis (PCA) of Pb, Zn, Cu, Cd, pH and LOI in soils. Each variable is placed in three-dimensional space in which the axes represent the three components. The three components explained 97.0% of the total variance in the data, with component 1 accounting for 49.0%, component 2 accounting for 37.3%, and component 3 accounting for 10.7%. The first component includes Zn, Cu and Cd, the second component consists of pH and LOI, then the third component is Pb

The exclusion of metals from aboveground tissues has been regarded as a metal tolerant strategy (Wei et al. 2005). These species that do not appear to be affected by excessive heavy metals may possess metal resistance capability (Pichtel et al. 2000). Resistance of plants to heavy metals can be achieved by an avoidance mechanism, which includes mainly the immobilization of a metal in roots and in cell walls (Garbisu and Alkorta 2001). As shown in Fig. 2, Pb accumulated by these species was retained in roots and TF values < 1 except *Abutilon theophrasti* Medic., which demonstrates the limited mobility of Pb in these plants. Pichtel et al. (2000) reported that 65–70% plants tested contained higher root Pb compared to shoot Pb, indicating the limited mobility of Pb once absorbed by the roots. Fitzgerald et al. (2003) also found that there was a clear partitioning of Pb within *Agrostis stolonifera*, *S. tabernaemontani* and *Spartina spp*., which had much higher concentrations of Pb in the roots than in the shoots. The elevated metal concentrations in roots and low translocation to the aboveground tissues in some investigated species might also suggest that they are capable of rather well-balanced uptake and translocation of metals under heavily metal-polluted conditions (Deng et al. 2004).

The high metal accumulation in some investigated species indicates that the internal metal detoxification tolerance mechanisms might exist in them, in addition to their exclusion strategies. Pb and Cd are toxic elements to all plants, while Zn and Cu are essential to plant growth, but will cause toxic effects when plant organs accumulate such metals exceeding their normal levels. The mean concentrations of Pb, Zn, Cu and Cd in shoots of a normal plant are 5, 100, 10, 1 mg/kg (Zu et al. 2004). Our results showed that the concentrations of Pb, Cu and Cd in the shoots of the investigated species were higher than the

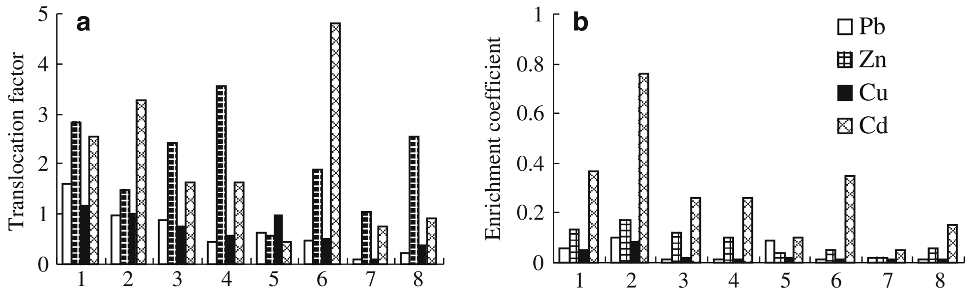

Fig. 2 Translocation factor (a) and enrichment coefficient (b) of weed species collected from the smeltery.1: *Abutilon theophrasti* Medic.; 2: *Chenopodium acuminatum* Willd; 3: *Polygonum lapathifolium* L.; 4: *Conyza canadensis* (L.) Cronq.; 5: *Physalis angulata* L.; 6: *Solanum nigrum* L.; 7: *Helianthus tuberosus* L.; 8: *Ambrosia trifida* L.

mean concentrations in a normal plant (Table 1), which showed that the investigated species had a strong ability to tolerate these heavy metals. Detoxification tolerance to heavy metals is based on the sequestration of heavy metal ions in vacuoles, on binding them by appropriate ligands like organic acids, proteins and peptides and on the presence of enzymes that can function at high levels of metallicions (Garbisu and Alkorta 2001).

Hyperaccumulative trends and potential applications to phytoremediation

The standard of a hyperaccumulator is still under dispute. At present, the standard is described as 4 rules: 1) the concentrations of heavy metals in plant shoots reach hyperaccumulating levels, i.e. Pb and Cu > 1,000 mg/kg, Zn > 10,000 mg/kg and Cd > 100 mg/kg DM; 2) the concentrations of heavy metals in shoots are 10–500 times as much as those in a normal plant, i.e. Pb 5 mg/kg, Zn 100 mg/kg, Cu 10 mg/kg and Cd 1 mg/kg DM; 3) the metal concentrations in shoots are invariably greater than those in roots; 4) enrichment coefficient > 1 (Zu et al. 2004, 2005).

According to the concentration time level (Table 1), 7 species for Pb (*Physalis angulata* L., *Ambrosia trifida* L., *Helianthus tuberosus* L., *Solanum nigrum* L., *Chenopodium acuminatum* Willd, *Polygonum lapathifolium* L. and *Abutilon theophrasti* Medic.) and 4 species for Cd (*Physalis angulata* L., *Ambrosia trifida* L., *Solanum nigrum* L. and *Conyza canadensis* (L.) Cronq.) were satisfied for this hyperaccumulator-identifying standard. Considering TF values (Fig. 1), one species for Pb (*Abutilon theophrasti* Medic.), seven species for Zn (*Conyza Canadensis* (L.) Cronq., *Ambrosia trifida* L., *Polygonum lapathifolium* L., *Abutilon theophrasti* Medic., *Solanum nigrum* L., *Chenopodium acuminatum* Willd and *Helianthus tuberosus* L.), two species for Cu (*Chenopodium acuminatum* Willd and *Abutilon theophrasti* Medic.) and five species for Cd (*Abutilon theophrasti* Medic., *Chenopodium acuminatum* Willd, *Polygonum lapathifolium* L., *Conyza Canadensis* (L.) Cronq. and *Solanum nigrum* L.) possessed the characteristic of hyperaccumulator. Particularly, *Abutilon theophrasti* Medic. exhibited potential hyperaccumulative ability to all these four heavy metals. Although the enrichment coefficient (EC) of all these species was lower than 1 (Fig. 2) and the absolute concentrations could not reach the standard, these plant species could be primarily considered as potential hyperaccumulators.

There are two distinct strategies in soil phytoremediation, phytoextraction and phytostabilization (Salt et al. 1998). Phytoextraction is the utilization of metal accumulating plants that can transport and concentrate metals from the contaminated soils to shoots, and then the aboveground tissues can be gathered by conventional methods. In phytostabilization, plants can stabilize pollutants in the soil by rendering them harmless (Eapen and Dsouza 2005). According to the field investigation, different plant species showed different accumulating ability. Considering the TF values, *Abutilon theophrasti* Medic. and *Chenopodium acuminatum* Willd. would be appropriate for extracting Cu; *Conyza Canadensis* (L.) Cronq., *Ambrosia trifida* L., *Polygonum lapathifolium* L., *Abutilon theophrasti* Medic., *Solanum nigrum* L., *Chenopodium acuminatum* Willd and *Helianthus tuberosus* L. for Zn; *Abutilon theophrasti* Medic., *Chenopodium acuminatum* Willd., *Conyza canadensis* (L.)Cronq., *Solanum nigrum* L. and *Polygonum lapathifolium* L. for Cd; *Abutilon theophrasti* Medic. for extracting Pb from contaminated soils, respectively. In a word, they were suitable for phytoextraction to remedy polluted soil. On the other hand, metal translocation into shoots appeared to be very restricted in other plants. Those plants could play an important role in metal removal from metal-polluted soils by metal immobilization in roots, which would be suitable as phytostabilizers for the remediation of metal-contaminated lands.

Conclusions

According to the field investigation, some plants could vegetate at heavily metal-polluted areas and absorb a wide range of soil metals (Pb, Zn, Cu and Cd). Seven species for Pb and four species for Cd were tested for the concentration time level standard for hyperaccumulator. Considering translocation factors, one species for Pb, seven species for Zn, two species for Cu and five species for Cd possessed the characteristic of hyperaccumulator. Although enrichment coefficients of all samples were lower than 1 and the absolute concentrations could not reach the standard, plant samples mentioned above were primarily believed to be potential hyperaccumulators. Contaminanted soils often contain several pollutants, so it is necessary to screen out plants that can accumulate different pollutants simultaneously. In our survey, *Abutilon theophrasti* Medic. exhibited strong accumulative ability to all these four heavy metals. Meanwhile, some species can be good choice for phytoextraction and others for phytostabilization.

Acknowledgment The authors acknowledge the financial support from the Natural Science Foundation of China as a project for Distinguished Young Scholars (No. 20225722). This work is also a component part of the key basic research development and planning (973) project (No. 2004CB418503) that was supported by the Ministry of Science and Technology, the People's Republic of China.

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