

Heavy metal accumulation by poplar in calcareous soil with various degrees of multi-metal contamination: implications for phytoextraction and phytostabilization

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Received: 15 January 2013 / Accepted: 1 April 2013 / Published online: 17 May 2013
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Abstract The object of this study was to assess the capacity of *Populus alba* L. var. *pyramidalis* Bunge for phytoremediation of heavy metals on calcareous soils contaminated with multiple metals. In a pot culture experiment, a multi-metal-contaminated calcareous soil was mixed at different ratios with an uncontaminated, but otherwise similar soil, to establish a gradient of soil metal contamination levels. In a field experiment, poplars with different stand ages (3, 5, and 7 years) were sampled randomly in a wastewater-irrigated field. The concentrations of cadmium (Cd), Cu, lead (Pb), and zinc (Zn) in the poplar tissues and soil were determined. The accumulation of Cd and Zn was greatest in the leaves of *P. pyramidalis*, while Cu and Pb mainly accumulated in the roots. In the pot experiment, the highest tissue concentrations of Cd (40.76 mg kg^{-1}), Cu (8.21 mg kg^{-1}), Pb (41.62 mg kg^{-1}), and Zn (696 mg kg^{-1}) were all noted in the multi-metal-contaminated soil. Although extremely high levels of Cd and Zn accumulated in the leaves, phytoextraction using *P. pyramidalis* may take at least 24 and 16 years for Cd and Zn, respectively. The foliar concentrations of Cu and Pb were always within the normal ranges and were never higher than 8 and 5 mg kg^{-1} , respectively. The field

experiment also revealed that the concentrations of all four metals in the bark were significantly higher than that in the wood. In addition, the tissue metal concentrations, together with the NH_4NO_3 -extractable concentrations of metals in the root zone, decreased as the stand age increased. *P. pyramidalis* is suitable for phytostabilization of calcareous soils contaminated with multiple metals, but collection of the litter fall would be necessary due to the relatively high foliar concentrations of Cd and Zn.

Keywords Heavy metals · Phytoremediation · Woody plant · Contaminated soils · Cadmium · Agricultural soil

Introduction

Large areas of agricultural land in the vicinity of mining and smelting sites has been contaminated by multiple metals as a result of deposition of particulate matter, dumping of solid wastes, and, especially, discharge of waste waters (Duaka and Adriano 1997; Ernst 2005; Keller 2006; Van Nevel et al. 2007). This is due to the ore being only a small fraction of the total volume of the mined material, and some additional metals occur naturally within the raw ore becoming inevitable by-products, for example, zinc (Zn) and lead (Pb) smelters release large amounts of cadmium (Cd) into the environment (Adriano 2001). Due to their nondegradable nature, heavy metals accumulate in the upper layer of soil and remain for long periods of time, which means they can pose a risk to food production through degradation of soils and a risk to human and animal health (Kayser et al. 2000; Liu 2003; Zhuang et al. 2009). Therefore, developing efficient techniques to remediate such multi-metal-contaminated agricultural land is urgent and imperative.

Phytoextraction has been proposed as a possible alternative to the destructive and costly techniques currently used

Responsible editor: Zhihong Xu

Electronic supplementary material The online version of this article (doi:10.1007/s11356-013-1711-0) contains supplementary material, which is available to authorized users.

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to clean up soils contaminated with heavy metals. Three groups of plants are possible candidates for phytoextraction of heavy metal-contaminated soil: hyperaccumulators, crop plants, and bioenergy plants (Keller 2006). Due to concerns regarding the use of hyperaccumulators and crop plants (Pulford and Watson 2003), a great deal of attention has been recently focused on determining the potential of bioenergy plants, such as trees, for phytoextraction (Robinson et al. 2000; Mertens et al. 2004, 2006). The major advantages of using trees are their relatively large biomass (both root and shoot) and fast growth rate (Pulford and Watson 2003; Wuana and Okieimen 2011). Among potential trees, poplars have reportedly shown high tolerance to and uptake of Cd and Zn (Vandecasteele et al. 2003; Laureysens et al. 2004; Mertens et al. 2004; Wu et al. 2010; Lettens et al. 2011). Under field conditions, the highest Cd concentration in poplar tissues can reach 209 mg kg⁻¹ noted in the leaves of *Populus trichocarpa* × *Populus deltoides* grown on a contaminated site containing more than 300 mg Cd kg⁻¹ (Robinson et al. 2000). The highest Zn concentration can reach 2,500 mg kg⁻¹, noted in the leaves of *P. alba* (AL35) grown on an acidic soil containing up to 950 mg Zn kg⁻¹ (Castiglione et al. 2009). However, some authors state that phytoextraction using poplars is doubtful (Robinson et al. 2000; Mertens et al. 2004; Komárek et al. 2008), since this technique may require an excessive amount of time to be effective, regardless of how large the achievable biomass might be. At the same time, coexisting metals may have antagonistic or synergic effects on their toxicity and uptake (Keller 2006; Castiglione et al. 2009). Due to poplars being a deciduous perennial plant, their use might result in the possible dispersion of the metals in the environment, through the shedding of leaves (Mertens et al. 2004, 2007; Van Nevel et al. 2007; Langer et al. 2009).

Phytostabilization, aimed at the fixation of heavy metals in the soil, seems to be the most suitable technology for remediating sites contaminated with multi-metal (Van Nevel et al. 2007), since few plants can extract high concentrations of more than one element in their tissues (Ernst 2005; Van Nevel et al. 2007). In particular, in the case of highly metal-contaminated soils that are too expensive to remediate by conventional means or are too long to remediate by phytoextraction, phytostabilization by revegetation may be the most relevant technology in order to stabilize the metals in the soil (Ernst 2005). Trees are well suited to phytostabilization due to their deep root systems, high transpiration rate, high metal tolerance, and ability to grow on nutrient-poor soils (Pulford and Watson 2003; Van Nevel et al. 2007; Mendez and Maier 2008). Trees can stabilize metals in the soil by physically preventing migration via wind or water erosion, leaching, and soil dispersion; alternatively, they can immobilize the metals through uptake and accumulation by the roots into the

plant, absorption on the root, or precipitation in the root zone (Pulford and Watson 2003; Mertens et al. 2004; Mendez and Maier 2008).

Whether phytoextraction or phytostabilization is the goal, the plants used need to be suited to the local climate and soil characteristics. In the case of calcareous soil in arid zones, the ideal plants must be both drought- and salt-tolerant (Keller et al. 2003; Mendez and Maier 2008). *Populus alba* L. var. *pyramidalis* Bunge is a variety of *P. alba* of which only male specimens have been identified. It is found widely in desert and arid regions of northwest China and is used for lumber production and ecological protection in terms of its fast growth rate, high biomass production, and elevated tolerance to the stresses of drought, salt, and insects (Zhang et al. 2008). Nevertheless, to the best of our knowledge, there are not any reports on the potential of *P. pyramidalis* for heavy metal uptake and accumulation on calcareous soils. In this paper, we aim to address three questions: (1) does the uptake and accumulation of heavy metals by *P. pyramidalis* on calcareous soils differ from other *Populus* species, based on either soil contamination level or soil type (calcareous and acid soils)? (2) How does *P. pyramidalis* respond to various degrees of multi-metal contamination? (3) Is *P. pyramidalis* more suited to phytoextraction or phytostabilization? A pot experiment and field experiment were performed to determine the capability of *P. pyramidalis* to take up and accumulate heavy metals from calcareous soils contaminated with various degrees of multiple metals.

Materials and methods

Soil

A sandy loamy soil collected in the 0–20 cm soil layer from arable land that had been irrigated with wastewater discharged from a Pb and Zn smelter near Shapogang in Baiyin of Gansu Province, China, was used as the source of the Cd-, Cu-, Pb-, and Zn-contaminated soil (see Liu (2003) for a site description). An uncontaminated, sandy loamy soil was collected from the 0–20 cm soil layer from arable land near Yuzhong located in the central part of Gansu province, China. At both sites, located in northern China, Chinese loess was the parent material and they were from a calcareous zone. Prior to use, soil samples were sieved through a 10-mm mesh.

Pot experiment

A gradient of soil multi-metal contamination (Table 1) was established by mixing contaminated soil with uncontaminated soil in the ratios of 0:100 (CK), 6.25:93.75 (T1), 12.5:87.5

Table 1 Mixing ratios of the polluted soil with unpolluted soil to create a range of heavy metal concentrations in the pot experiment (based on dry weight)

Treatment	Polluted soil (%)	Cd (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Zn (mg kg ⁻¹)
CK	0	0.60	27	48	60
T1	6.25	5.92	66	112	225
T2	12.5	11.0	88	185	348
T3	25	20.3	159	254	546
T4	50	39.3	274	589	1295
T5	100	80.0	530	970	2350

(T2), 25:75 (T3), 50:50 (T4), and 100:0 (T5) % (based on dry weight). After mixing, subsamples were taken from the six bulk soils to determine the total soil metal concentrations. Then, 7.5 kg of mixed soil (based on dry weight) was placed in plastic pots ($H=26.5$ cm, $D=22.5$ cm) and left outside to equilibrate for 2 months. Bulk soils were watered with tap water (no metal detected) up to 50 % of the maximal water holding capacity (WHC). After equilibration, soil in each pot was fertilized with 0.25 g N kg⁻¹ as NH_4NO_3 and 0.10 g P kg⁻¹ as K_2HPO_4 . In order to prevent anaerobic conditions in the potted soils, six small needle holes were made in the bottom of the pots and anti-rooting mats were placed to prevent roots from growing out of the pots. Two 1-year-old plantlets of *P. pyramidalis* with identical growth phases were planted in each pot in April 2011. Plants in the pots were allowed to grow under natural field conditions, and loss of water by evaporation was made up using tap water (no metal detected) to approximately 60 % of WHC to maintain optimal plant growth. Each treatment was carried out in triplicate and harvested 185 days after planting.

Field experiment in wastewater-irrigated area

Dongdagou, an escape canal in Baiyin, Gansu Province, collected not only domestic sewage but also industrial wastewater discharged by nonferrous metals mining and smelting plants as well as several other factories located in the mid to upper reaches of the canal. Agricultural land along this escape canal has been irrigated with wastewater for 40 years, from the 1960s, due to the lack of rainfall caused by the semiarid climate. It has been estimated that more than 1.33×10^3 hm² of agricultural land in this region has been contaminated to various degrees with heavy metals, one third of which has been heavily contaminated. Minqin village ($104^\circ 16'$ E, $36^\circ 29'$ N), located in the middle reach of the Dongdagou escape canal, was selected as the site of the field experiment. The site is 1,572 m above sea level and has a temperate semiarid climate. The mean annual air temperature is $\sim 8^\circ\text{C}$, and the mean annual precipitation is 198 mm, accompanied with a high evapotranspiration potential (1997 mm) during the growing season. After 2000, *P. pyramidalis* was planted on abandoned land (approximately 3.67 hm²) in the village for revegetation and

beautification, due to concerns regarding the adverse effects of heavy metals on crops and human health (Liu 2003).

Five complete plants from each stand age (3, 5, and 7 years) were sampled randomly on the site in October 2010. This was the first time the poplars from this site were cut and they were separated into individual parts (roots, stems (wood+bark), braches, and leaves) to identify the metal concentrations in each part. Pieces of the stem were sampled at the bottom, middle, and top of the total height and were subsequently pooled. Soils in the root zone (0–30 cm depth) of each plant were also sampled in order to determine the total and extractable metal concentrations. To determine variations in metal concentrations with depth, soil cores were taken from the bulk soils at five different locations with a 3.5-cm-diameter soil sampler in 20-cm increments to a 100-cm depth.

Chemical analysis

Plant samples were washed with deionized water, and then oven-dried at 75°C to a constant weight (after about 48 h) and finely ground in a titanium grinder before analysis. Soil samples were air-dried, and then sieved to pass through a 2-mm mesh prior to analysis. The soil pH was determined using a pH meter (PHS-4, Jiangsu Manufactory of Electrical Analysis Instruments, Jiangyin, China) in a soil/water solution (1:2.5, w/v). The particle size distribution was determined using a MS-S3500 Microtrac sample delivery controller (Malvern Instruments Ltd., UK). The total carbonate content was determined by the gasometric method, and the soil organic matter content was determined using the $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method (Bao 2000). Total soil N was measured with a Kjeltex System 2300 distilling unit (Tecator AB, Hoganas, Sweden). Total soil P was measured by the ammonium molybdate method using a spectrophotometer (Nanodrop, Wilmington, Delaware USA). Approximately 1 g of each plant sample and 0.5 g of each soil sample were digested separately with a mixture of 70 % HClO_4 and 66 % HNO_3 , in the ratio 1:4 (v/v) on a hot plate at 160°C for 5 h (Langer et al. 2009). Bioavailable metal concentrations in field soils were extracted by a 1-M NH_4NO_3 solution (Langer et al. 2009). Soil suspensions were centrifuged, and then passed through a 0.45- μm filter membrane to

separate the solution from the soil particles before determination. Metal concentrations in the digests and extracts were determined using an atomic absorption spectrophotometer (SOLAAR M6; Thermo Fisher Scientific, Waltham, MA, USA). The certified standard reference materials GBW 07603 (bush branches and leaves) and 07401 (soil) were obtained from the Institute of Geophysical and Geochemical Exploration, CAGS (Hebei, China), and were used to evaluate the accuracy and precision of the analysis. The experimental results presented here showed recoveries of 87–105 % for the plant samples and 95–103 % for the soil samples when compared with the certified values.

Data calculation and analysis

The leaf-to-soil ratio is the ratio of the mean concentration of the metal in the leaves of *P. pyramidalis* compared to the total concentration of the metal in the soil. The leaf-to-root ratio is the ratio of the metal concentrations in the leaves and roots. The data were transformed using $\log X$ when the assumptions of normality and homogeneity variances deviated markedly. To test whether the metal contamination levels in the pot experiment or whether metal concentrations in different plant tissues in the poplar stands with different ages in the field experiment differed, a two-way ANOVA was performed with the plant tissue, metal contamination level/stand age, and the interaction between these two factors as the fixed factors. To test whether the response of biomass production in the pot experiment differed among metal contamination levels, a one-way ANOVA was used to separate means for a given plant tissue. Separation of means was performed using the Duncan's multiple-range test, at the 0.05 significance level. All values were expressed as mean $\pm$ SD (standard deviation) on a dry weight basis.

Results and discussion

Soil properties

In Table 2, selected properties of the contaminated and uncontaminated soils are presented. In the pot experiment, the main parameters of the cation exchange capacity such as organic matter (OM), clay, and pH, were similar for the contaminated soil from Shapogang and the uncontaminated soil from Yuzhong. These properties are expected to influence the solubility of the metals and therefore variation should be small so that they do not influence the uptake of the metal by the plant (Koopmans et al. 2008). The contaminated soil from Minqin in the field experiment showed similar properties as the two soils in the pot experiment as far as texture, pH, carbonate, and electrical conductivity (EC); this was largely because they were all gray calcareous

Table 2 Selected soil properties of the studied sites in both the pot and field experiments

	Minqin	Shapogang	Yuzhong
Particle size distribution (%)			
<0.002 mm	9.46	8.71	7.44
0.002–0.05 mm	46.07	45.71	47.51
0.05–2.00 mm	44.47	45.58	45.05
pH (H ₂ O)	7.48	7.31	7.50
CaCO ₃ (g kg ⁻¹)	69.68	81.03	72.04
EC (dS m ⁻¹)	2.31	1.99	2.22
OM (g kg ⁻¹)	15.31	12.30	12.42
Total P (g kg ⁻¹)	1.46	0.89	0.79
Total N (g kg ⁻¹)	1.04	0.87	0.92

soil that developed from the same parent material, Chinese loess. Total metal concentrations in the contaminated soil from Shapogang (Table 1) were 80 (Cd), 1.25 (Cu), 1.94 (Pb), and 4.7 (Zn) times greater than the Chinese target values for arable soils in the vicinity of smelters (CEPAS 1995), i.e., 1 mg Cd, 400 mg Cu, 500 mg Pb, and 500 mg Zn kg⁻¹ soil for the target values. In the uncontaminated soil from Yuzhong (Table 1), the total concentrations of Cd, Cu, Pb, and Zn were all lower than the Chinese target values.

Pot experiment

Plant growth

The biomass production by *P. pyramidalis* in the pot experiment is presented in Fig. 1. No visual symptoms of metal toxicity were noted on aboveground tissues of *P. pyramidalis* during the course of the experiment, which indicates a strong tolerance to soil metal contamination. However, the root of *P. pyramidalis* in the metal-contaminated treatments was shorter

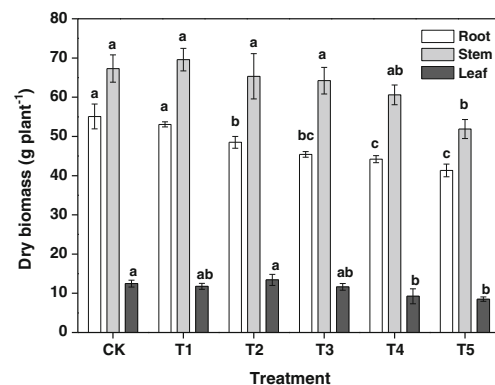


Fig. 1 Biomass production (expressed as dry matter) under different treatments in the pot experiment (mean $\pm$ SD, $n=3$). Means among the treatments for a given plant part with the same letter are not significantly different ($P<0.05$)

and smaller in diameter when compared with that in the control. Therefore, dry weight of the root was significantly less, apart from the T1 treatment, when compared with the control ($P<0.05$). In contrast, the dry weight of the stems and leaves decreased only in the heavily contaminated soils (T4 and T5 treatments) compared with the control ($P<0.05$). According to Vandecasteele et al. (2003), the acceptable soil concentrations of Cd and Zn for poplar growth were 12 and 2,275 mg kg⁻¹, respectively. The significantly reduced root biomass of *P. pyramidalis* was most likely induced by Cd toxicity. This is mainly due to coexisting metals, such as Pb, possibly competing more effectively for exchange sites on the colloidal surfaces than Cd, releasing Cd into the soil solution. In addition, Cd is known to be much more available and toxic to plants than other metals such as Cu, Pb, and Zn, even in calcareous soils (Adriano 2001; Jensen et al. 2009).

Metal concentrations in plant tissue

The result from the two-way ANOVA for the concentrations of Cd, Cu, Pb, and Zn in different plant tissues showed that the single effects of treatment and plant tissue on metal concentrations were highly significant ($P<0.001$), and that the interaction effect was highly significant as well ($P<0.001$) (Table 3). The concentrations of Cd, Cu, Pb, and Zn in the roots, stems, and leaves of *P. pyramidalis* are presented in Fig. 2. The Cd concentration in the tissues of *P. pyramidalis* increased gradually as the level of soil metal contamination increased ($P<0.05$). In addition, the general trend of Cd concentration decreasing in the order of leaves>roots>stems was observed, with the highest concentration (40.76 mg kg⁻¹) noted in the leaves in the T5 treatment. This value was much higher than the normal range of Cd concentration in deciduous foliage (4–17 mg kg⁻¹) collected from polluted areas around the world (Adriano 2001). Nevertheless, the highest Cd concentration was lower than 100 mg kg⁻¹, the critical concentration required for Cd-hyperaccumulators (Baker et al. 2000); *P. pyramidalis* is thus not considered to be a Cd-hyperaccumulator. In the case of Zn, its concentration in poplar tissues gradually

increased, similar to the Cd concentration, as the level of soil metal contamination increased ($P<0.05$). Furthermore, the leaves of *P. pyramidalis* were found to take up exceptionally high levels of Zn compared with the stems and roots, with the highest Zn concentration (696 mg kg⁻¹) observed in the T5 treatment as well. High levels of Cd and Zn were found in the leaves of *P. pyramidalis*, which is consistent with previous results obtained for *Populus* species clones screened by pot culture experiments (Robinson et al. 2000; Komárek et al. 2008; Langer et al. 2009). It is possible that poplars' high water use is the reason that they accumulate larger amounts of Cd and Zn in their leaves when compared to other trees (Robinson et al. 2000).

Unlike Cd and Zn, extremely high levels of Cu and Pb accumulated mainly in the roots of *P. pyramidalis* compared with the levels in the stems and leaves. The highest concentrations of Cu and Pb were observed in the roots in the T5 treatment, i.e., 48.21 mg kg⁻¹ for Cu and 41.62 mg kg⁻¹ for Pb. The concentrations of Cu and Pb in the aboveground tissues, however, were consistently less than 8 and 5 mg kg⁻¹, respectively. Accordingly, an extremely low leaf-to-soil ratio was found for Cu and Pb, i.e., ≤ 0.18 for Cu and <0.1 for Pb (see Table S1 of the Electronic Supplementary Material, ESM).

Field experiment

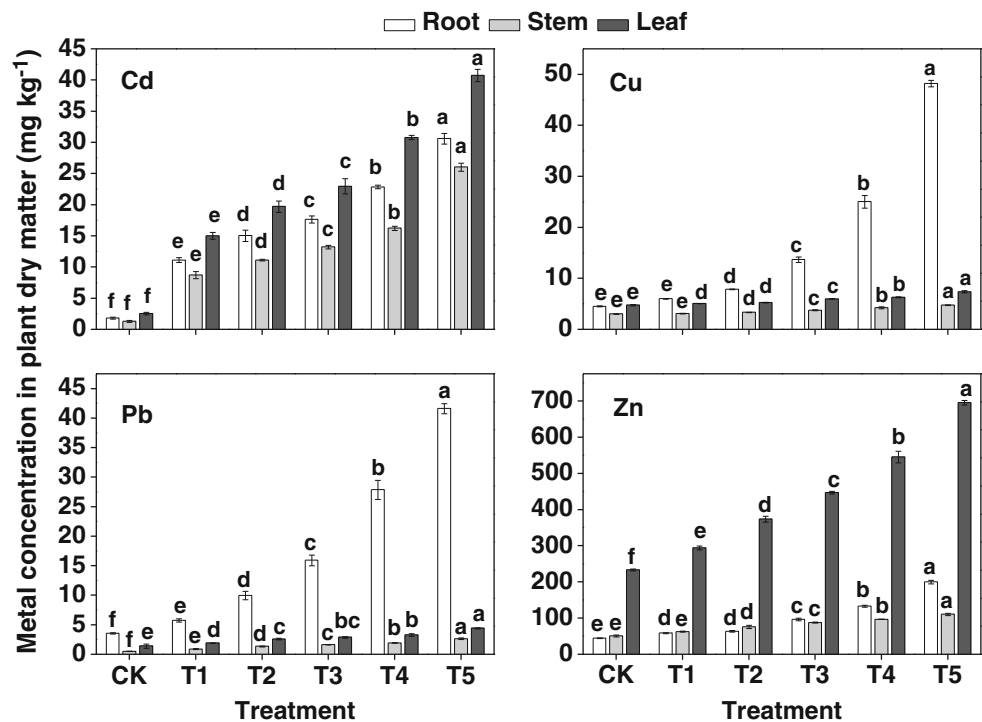
Changes of metal concentrations in soil

Table 4 shows the total and NH₄NO₃-extractable concentrations of Cd, Cu, Pb, and Zn in the root zone (0–30 cm) of *P. pyramidalis* grown in a wastewater-irrigated field. It was found that the soil was mainly contaminated with Cd and Zn, as their mean concentrations in the soil were 30 (Cd) and 1.44 (Zn) times greater than the Chinese target values for arable soils near smelters (CEPAS 1995). Contamination in this field was heterogeneous, including spatial variation as well as variation with depth. More specifically, the total metal concentrations in the surface soil across this site ($n=15$) ranged from 20 to 39 mg kg⁻¹ for Cd, 183–497 mg kg⁻¹ for

Table 3 Results of the two-way ANOVA on heavy metal concentrations in the pot and field experiments

Experimental type	Source of variation	Cd		Cu		Pb		Zn	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Pot experiment	Treatment (T)	1712	<0.001	1696	<0.001	659	<0.001	1447	<0.001
	Plant organ (P)	678	<0.001	5040	<0.001	3448	<0.001	1632	<0.001
	T×P	36.521	<0.001	1284	<0.001	465	<0.001	424	<0.001
Field experiment	Stand age (S)	8.575	0.001	5.595	0.007	6.070	0.004	7.693	0.001
	Plant organ (P)	40.048	<0.001	9.182	<0.001	13.678	<0.001	37.440	<0.001
	S×P	0.559	0.760	1.933	0.095	1.534	0.187	1.062	0.398

Fig. 2 Heavy metal concentrations in parts of *P. pyramidalis* under different treatments in the pot experiment (mean±SD, $n=3$). Means among the treatments for a given plant part with the same letter are not significantly different ($P<0.05$)



Cu, 202–568 mg kg⁻¹ for Pb, and 417–1,148 mg kg⁻¹ for Zn. In addition, the concentrations of Cd, Cu, and Zn decreased sharply with depth, and these metals were preferentially enriched in the upper 20 cm of the soil (see Fig. S1, ESM). This is likely due to the high carbonate content in the soil (Table 2). The Pb concentration, however, remained relatively constant throughout the soil profile, most likely due to the high background concentration in the parent material. In the case of the NH₄NO₃ extraction (Table 4), the concentrations of Cd, Cu, and Zn were found to decrease with an increase in the stand age, whereas, Pb was not detected in all stands. What is more, the percentage of total soil Cd, Cu, and Zn extracted by NH₄NO₃ decreased as the stand age increased, i.e., from 5.6, 0.97, and 1.25 % in the 3-year-old stand to 4.02, 0.84, and 0.83 % in the 7-year-old stand, respectively.

Metal concentrations in plant tissue

Generally, the results of the two-way ANOVA for the concentrations of Cd, Cu, Pb, and Zn in different plant tissues

showed that the effects of stand age and plant tissue on metal concentrations were significant ($P<0.01$), whereas the interaction effect was not significant ($P>0.05$) (Table 3). There were no statistical links shown between the age of the stand and the distribution of metals in the tissues. As shown in Table 5, mean concentrations of Cd and Zn in the parts of the poplar trees were in the descending order of leaves and bark>branches>roots>wood, whereas the corresponding concentrations of Cu and Pb decreased from roots and bark>branches>wood>leaves, regardless of the stand age. Previous field-based studies have shown that Zn is easily translocated to the leaves, most likely due to it being an essential micronutrient (Laureysens et al. 2004; Madejón et al. 2004; Castiglione et al. 2009), this has also been shown to be true on soil amended with different concentrations of ZnSO₄ (Langer et al. 2009). High levels of Cd were found in the leaves of *P. pyramidalis* as well, showing similar uptake and storage mechanisms for Cd and Zn in poplar species (Lettens et al. 2011; Van Nevel et al. 2011). In the case of Cu and Pb, and in contrast to Cd and Zn, they were mainly

Table 4 Total and extractable concentrations of heavy metals in soil samples in the field experiment (mean±SD, $n=5$)

Stand age (year)	Total (mg kg ⁻¹)				Extractable (mg kg ⁻¹)			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
3	31.26±4.84	309±129	334±156	739±253	1.75±0.44	3.01±0.72	ND	9.24±3.75
5	30.48±5.11	380±75	459±55	790±237	1.27±0.38	3.00±0.47	ND	5.70±2.62
7	29.12±6.87	306±77	338±98	624±170	1.17±0.35	2.57±0.60	ND	5.21±2.90

Table 5 Heavy metal concentrations in tissues of *P. pyramidalis* in the field experiment (mean±SD, $n=5$)

Stand age (year)	Plant tissue	Cd (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Zn (mg kg ⁻¹)
3	Root	11.34±3.22	19.65±11.59	11.99±7.42	110.63±55.97
	Wood	7.05±2.04	3.85±1.62	2.95±1.99	53.35±12.10
	Bark	24.90±4.44	18.22±4.92	39.98±19.91	284.12±107.56
	Branch	19.46±6.31	10.73±3.24	26.27±10.98	174.91±61.20
	Leaf	20.97±5.80	4.91±2.90	2.16±1.34	553.87±131.60
5	Root	11.43±3.08	13.24±8.66	15.04±11.54	107.34±45.77
	Wood	7.78±2.66	4.24±3.51	3.30±2.21	44.66±18.13
	Bark	22.64±5.28	10.01±5.05	25.39±16.55	197.74±22.82
	Branch	20.87±8.51	7.25±1.35	16.31±12.11	148.57±50.58
	Leaf	22.17±6.63	3.31±2.17	2.71±2.08	477.37±102.48
7	Root	6.72±1.69	7.68±2.48	5.02±3.72	48.99±17.18
	Wood	4.48±1.26	6.71±4.54	4.00±5.92	34.26±6.18
	Bark	19.39±4.57	11.06±2.62	17.27±10.02	193.76±29.71
	Branch	12.15±4.15	6.31±3.88	7.84±9.42	101.64±61.62
	Leaf	13.37±2.98	1.92±0.62	0.90±0.67	335.02±77.84

concentrated in the roots of *P. pyramidalis*. This is in agreement with previous investigations on other poplars grown in fields contaminated with multiple metals (Laureysens et al. 2004; Madejón et al. 2004; Vollenweider et al. 2011), showing the different abilities of the metals to translocate from the root to leaf (high vs. low leaf-to-root ratio of Cd, Zn vs. Cu, Pb).

Bark and wood are the two basic components of the stems. It was observed that the concentrations of all four metals in the bark were significantly higher than the concentrations in the wood ($P<0.05$), regardless of the stand age. More specifically, on average, the Cd and Zn concentrations in the bark were 3.6- and 5.1-fold the concentrations in the wood, respectively; likewise, the bark concentrations of Cu and Pb were, respectively, 2.9 and 8.5 times higher than the wood concentrations. These results are consistent with several other experimental studies (Laureysens et al. 2004; Mertens et al. 2006, 2007) and the review by Pulford and Watson (2003) when investigating metal compartmentation in stem components of older woody plants including poplars and willows. A possible explanation might be the rapid lateral movement of metals from the wood to the bark driven by the transpiration stream after entering the stems (Pulford and Watson 2003; Laureysens et al. 2004).

Changes of metal concentrations with stand age

Significant stand age differences in levels of accumulation were found for the four metals (Table 5). Generally, in the case of the 3-, 5-, and 7-year-old stands, the concentrations of Cd, Pb, and Zn in tissues of *P. pyramidalis* were significantly lower in the 7-year-old stand than in the 3-year-old stand ($P<0.05$). Whereas the Cu concentration in both the 5- and 7-year-old stands were significantly less than that in the 3-year-old stand ($P<0.05$), regardless of the plant tissue.

Accordingly, it was observed that the highest concentrations of Cd, Cu, Pb, and Zn in the 3-year-old stand were 24.90 (Cd) and 39.98 (Pb) mg kg⁻¹ in the bark, 19.65 (Cu) mg kg⁻¹ in the roots, and 553.87 (Zn) mg kg⁻¹ in the leaves. The general trend of the metal uptake decreasing as the tree age increases, as reported in this research, has also been found in previous studies (Mertens et al. 2006; Lettens et al. 2011). This trend may be caused by a combination of multiple factors including the following three ways: firstly, rapid biomass production results in a biological dilution effect (Pulford and Watson 2003). This effect would be more important for young trees, as the metal uptake rate cannot match the biomass development rate at this stage (Lettens et al. 2011). Li et al. (2009) found that a 2-year-old *Averrhoa carambola* stand showed the highest metal removal efficiency among the 1-, 2-, 3-, and 4-year-old stands in a field trial. Secondly, persistent plant uptake reduced the pool of bioavailable metals, particularly in terms of leaf metal concentration. For example, Greger and Landberg (1999) found that 25.6–34.6 % of the ammonium acetate extractable Cd was removed by willow grown on soils containing 0.46–5.57 mg Cd kg⁻¹ in a 90-day pot trial. Mertens et al. (2006) reported that the proportion of total soil Cd extracted by ammonium acetate was lower in a 6-year-old willow stand (6 %) than in a 1-year-old stand (17 %). Lastly, the roots of perennial woody plants can progressively extend downwards to the deeper soil layer that is usually less contaminated, resulting in a decrease in metal uptake with time, particularly in terms of the older trees. Lettens et al. (2011) presented a 7-year data series of foliar Cd and Zn concentrations in *P. trichocarpa*×*P. deltoides*; they found a significant decrease in foliar concentrations of Cd and Zn with time. Hammer et al. (2003) observed that Cd and Zn concentrations in the shoots of *Salix viminalis* decreased

with time in field trials and this was probably attributed to root development in deeper soil layers that were less contaminated (Keller et al. 2003). Thus, to obtain the maximum effect of phytoextraction, when using woody plants, determining the optimal rotation period is very important.

Implications for phytoextraction and phytostabilization

Significant plant tissue differences in levels of accumulation were found in both the pot and field experiments for the four metals. *P. pyramidalis* had the highest accumulation of Cd and Zn in its leaves compared with the highest accumulation of Cu and Pb that was in its roots. This trend from this research is in line with previous results obtained for *Populus* species clones screened by pot culture (Robinson et al. 2000; Komárek et al. 2008; Langer et al. 2009) and field experiments (Laureysens et al. 2004; Madejón et al. 2004; Castiglione et al. 2009). The foliar concentrations of Cu and Pb measured in both the pot and field experiments were always within the normal ranges in terrestrial plants (Alloway 1995) and the values found in other poplars (Madejón et al. 2004; Chang et al. 2005; Mertens et al. 2007). Nevertheless, *P. alba* AL35 and *Populus nigra* L. × *Populus maximowiczii* Henry showed exceptionally high foliar Cu (240 mg kg⁻¹) and Pb (50 mg kg⁻¹) concentrations, respectively (Komárek et al. 2008; Castiglione et al. 2009). This is largely due to the relatively high metal concentrations in the soil as well as the differences in clones and soil properties. Extremely low levels of Cu and Pb were translocated to the leaves of *P. pyramidalis*, which indicates that *P. pyramidalis* is therefore suitable for phytostabilization of Cu and Pb on calcareous soils.

The accumulation of Cd and Zn in the leaves of *P. pyramidalis* was always higher than Cu and Pb, since a relatively high leaf-to-soil ratio was noted for Cd and Zn in both the pot and field experiments (see Table S1, ESM). This trend is in agreement with the results obtained in other poplars grown on multi-metal-contaminated soils, regardless of whether the soil was acid (Chang et al. 2005; Mertens et al. 2007; Komárek et al. 2008; Vollenweider et al. 2011) or calcareous (Laureysens et al. 2004; Madejón et al. 2004; Mertens et al. 2004). However, considering both the dry biomass yield and metal concentrations in plant tissues, the Cd extraction ratio in the pot experiment (calculated as (shoot Cd content/soil Cd content) × 100 %) decreased from 5.3 % in the control to 3.5 % in the T1 treatment and to 0.6 % in the T5 treatment. Similarly, the Zn extraction ratio decreased from 2.8 % in the control to 1 % in the T1 treatment and to 0.1 % in the T5 treatment. The decreased metal extraction ratio was most likely determined by the decrease in the leaf-to-soil ratio induced by increasing soil metal concentrations (Robinson et al. 2000; Langer et al. 2009), as well as the reduction in shoot biomass impacted

by particularly high levels of soil metal concentrations (Fig. 1). Based on this extraction rate, it would take 24 years to reduce the soil Cd burden in the T1 treatment (5.92 mg kg⁻¹) down to 1 mg kg⁻¹; similarly, it would take 16 years to reduce the soil Zn burden in the T3 treatment (546 mg kg⁻¹) down to 500 mg kg⁻¹. According to Keller (2006), 10 years is an optimal remediation period, as this is an economically acceptable phytoextraction period. Thus, phytoextraction of Cd and Zn using *P. pyramidalis* would not be a realistic option regardless of how large their achievable shoot biomass was, due to the amount of time required for effective remediation.

The foliar Zn concentration in both the pot (Fig. 2) and field experiments (Table 5) were generally within the values observed in other poplars grown on contaminated soils (Laureysens et al. 2004; Madejón et al. 2004; Mertens et al. 2004) and were slightly higher than the normal ranges in terrestrial plants (1–400 mg kg⁻¹) (Alloway 1995). In the case of foliar Cd concentration in the pot experiment, its values ranged from 2.5 to 40.8 mg kg⁻¹. The values were generally lower than that reported by Robinson et al. (2000), where the foliar Cd concentration ranged from 6 to 75 mg kg⁻¹ in two poplars (Kawa and Argyle) grown in an acid soil containing a range of 0.6–60.6 mg Cd kg⁻¹ dry soil. The difference may be due to different soil properties; that is, the calcareous soil in this research in contrast to the acidic soil reported (pH=5.7). Generally, in calcareous soils, comparatively low metal uptake levels, by plants, are recorded compared to acidic soils (Adriano 2001). This is due to the adsorption by CaCO₃ as well as the low desorption induced by high pH that attributes relatively low metal bioavailability in calcareous soils (Sanchez-Camazano et al. 1998; Kayser et al. 2000). However, at low soil Cd levels (< 15 mg kg⁻¹), the foliar Cd concentrations in this research (Fig. 2) were found to be generally higher than those reported in other clones of *Populus* species, regardless of the soil being calcareous (Vandecasteele et al. 2003; Madejón et al. 2004; Mertens et al. 2004; Wu et al. 2010) or acidic (Mertens et al. 2007; Wu et al. 2010; Vollenweider et al. 2011). Additionally, in the field experiment, although a significant decrease was observed in foliar Cd concentrations as the stand age increased (Table 5), the leaves still contained more than 10 mg Cd kg⁻¹ after 7 years, with an average value of 13.4 mg kg⁻¹. Elevated concentrations of Cd in the leaves of *P. pyramidalis* might therefore result in the risks of surface contamination and wildlife exposure (Mertens et al. 2001, 2007). According to Mendez and Maier (2008), plants used for phytostabilization should contain less than 10 mg Cd kg⁻¹ in shoots, based on US domestic animal toxicity limits determined from the maximum tolerable levels for cattle. Therefore, *P. pyramidalis* is not suitable for phytostabilization of Cd-only-contaminated calcareous soils considering the

long-term habitat development of the poplar plantation. Nevertheless, soil near smelters is always contaminated with multiple metals; therefore, a thorough collection of the litter fall will have to be performed in the process of phytostabilization using *P. pyramidalis*.

The uptake of the four metals found in the field experiment was significantly lower than those from the pot experiment (see Fig. 2 and Table 5). This may be explained by several factors: (1) the root in the pot contacted efficiently with the substrate in a limited amount of space and soil (Kayser et al. 2000; Keller et al. 2003; Keller 2006), while the root colonization in the field might avoid the contaminated spots, due to heterogeneous soil contamination (Table 4). (2) The root in the field can progressively extend downwards as stand age increases to the uncontaminated deeper soil layers (see Fig. S1, ESM). (3) Unlike the short-term simulation represented by pot culture experiment, the extractable metal concentrations in the root zone in the field can decrease with increasing stand age (Table 4). Although this effect on the concentrations of the total metal in the soil was negligible, the metal uptake might be greatly inhibited, especially for older plants, due to the decreased extractable metal concentrations most likely represented by the most soluble fraction (Mertens et al. 2006; Jensen et al. 2009). Therefore, appropriate site-specific plant management is especially important in phytoremediation of metal-contaminated sites.

Conclusions

Based on the pot culture and the field experiments in this research, plant tissue differences in accumulation were found for the four metals of Cd, Cu, Pb, and Zn. *P. pyramidalis* accumulated relatively high concentrations of Cu and Pb in its roots with both the leaf-to-soil and leaf-to-root ratios being less than 1. The foliar Cu and Pb concentrations were always within the normal ranges for terrestrial plants and were less than 8 and 5 mg kg⁻¹, respectively. Relatively high leaf-to-soil and leaf-to-root ratios were noted for Zn and especially Cd in contrast to Cu and Pb, but phytoextraction would need at least 24 and 16 years for Cd and Zn, respectively. Therefore, *P. pyramidalis* has a high phytostabilization potential for remediating multi-metal-contaminated calcareous soils, but a thorough collection of the litter fall will have to be performed in the long-term habitat development of poplar plantations due to the high foliar concentrations of Zn and particularly Cd.

Acknowledgments This study was financially supported by the National Natural Science Foundation of China (NSFC 51178209, 91025015) and the Scholarship Award for Excellent Doctoral Student granted by Lanzhou University.

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