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Effects of EDTA on Lead Uptake by *Typha orientalis Presl*: A New Lead-Accumulating Species in Southern China

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Abstract A series of field investigations have been conducted at Yongzhou Pb/Zn/Cu mine tailings, Hunan Province, southern China. The specific aim was to search for new lead accumulators with fast growth rate and large biomass. The results of tissue analyses identified *Typha orientalis Presl* has a strong accumulation of lead. The average lead concentrations in the leaves and roots are 619 and 1,233 mg/kg, respectively. The growth and Pb content of the plant were also studied by hydroponic culture with different concentrations of $Pb(NO_3)_2$. Growth of the plant was not affected by Pb up to 300 mg/L. The Pb concentrations in the leaves and roots increased with increasing of Pb level in the modified Hoagland's nutrient solution. The maximum concentrations of Pb in the leaves and roots were 16,190 and 64,405 mg/kg, respectively. The study also demonstrated that EDTA not only increased the amount of Pb taken up by plants but also speeded up the metal translocation from roots to leaves.

Keywords EDTA · Lead · Accumulator · *Typha orientalis Presl*

Lead is one of the most frequently encountered heavy metals in polluted environments (Seaward and Richardson 1990). Lead is also one of the most persistent metals with a soil retention time of about 150–5,000 years in the environment (Shaw 1990). Severe Pb contamination in soils and in ground and surface waters may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and Pb toxicity in plants, animals, and humans (Huang and Chen 1997). The use of biological materials to cleanup heavy metal contaminated soils has been focused on as an efficient and affordable form of bioremediation. Phytoremediation is of growing interest because of its low environmental impact and cost-effectiveness, even if a longer time is required for treatment (Shivendra and Natalie 2002). Hyperaccumulators are the basis of phytoremediation. But hyperaccumulators are usually small, native plants, such as those that belong to the genus *Thlaspi* and several others (Cunningham and Ow 1996). Full-scale applications have yet to be achieved. Therefore, some researches think that these plants will not fulfill all expectations related to removal of metals from the environments (Aneta et al. 2003). We conducted investigations to find Pb-accumulating and Pb-tolerant plants with fast growth rate and large biomass. The plant in the present study, *Typha orientalis Presl*, satisfied these requirements. In addition to natural plant adaptations, the addition of synthetic chelators, soil acidifiers, or commercial nutrients can enhance phytoremediation. It has been demonstrated that Pb is rapidly accumulated in the roots if Pb is bioavailable in the plant growth media; however, only a small proportion of absorbed Pb is translocated to shoots (Huang and Cunningham 1996; Kumar et al. 1995). Numerous reports on the use of synthetic chelators in enhancing uptake and transport of heavy metals by plants have been published in recent years. It was demonstrated that EDTA was the most effective for Pb (Michael et al. 2007; Lasat 2002; Huang and Cunningham 1996). One of the most intensely studied subject at the moment is the possibility of

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using commercial plants exhibiting tolerance to heavy metal ions and high biomass growth in induced phytoremediation e.g., corn, barley, bean, pea or wheat. Enhanced uptake and accumulation of metals by plants was obtained due to introducing a proper chelator into the environment.

There were two objectives of this study: one was to identify that *T. orientalis Presl* is a new Pb-tolerant and Pb-accumulating plant, the other was to demonstrate how the presence of EDTA affected the uptake of lead by *T. orientalis Presl*.

Materials and Methods

The old Pb/Zn/Cu mining area has been mined for more than 100 years in Hunan province, mid-south south of China. The long-time mining activities produced a mass of unsettled spoil heaps, which included excavation wastes, washing wastes, and smelting wastes. The mine resides in the area featuring typical semi-tropical continental climate. The annual temperature is 17.4°C and the annual rainfall is 1,431.4 mm.

Typha orientalis Presl, an easy-to-grow plant with high biomass production, was collected from the mine tailings, as well as the tailings pond around it in the autumn of 2005. These plants were washed thoroughly with distilled water and then were separated into leaves, roots, and underground stems, dried for 2 h at 105°C and 48 h at 80°C. The dried samples were powdered by a metal-free mill, and passed through 2 mm sieve. Lead contents of 200 mg plant tissue were extracted by HNO_3 (10 mL) and $HClO_4$ (0.5 mL). The tailings pond samples were air-dried and ground. Total Pb in soil was estimated by digestion with HCl:HNO_3:$HClO_4$ (4:1:1); pH was measured using 1:1 soil/water ratio and organ mattercontent was measured by the Walkley Black method (Nelson and Sommers 1982). The samples were dried at 105°C for 6 h to determine the moisture absorption value, and then placed in a muffle furnace and ignited at 900°C for 2 h to determine the loss on ignition. The concentrations of lead were quantified with flame atomic absorption spectroscopy using a Perkin Elmer AAnalyst 700. The selected physical and chemical properties of the Pb-contaminated soil are presented in Table 1.

Germination of *T. orientalis presl* Seedlings

The caudices were collected from the mine in the following early spring. The stems were washed under running tap water for 30 min, then soaked in distilled water for 5 h, followed by three rinses with sterile distilled water. They were soaked in 1/2 Hoagland's nutrient solution to germinated under a light/dark regime of 16/8 h at 25°C for 7 days.

Table 1 Physical and chemical characteristics of the mine in Hunan province, China

Parameters	Tailings
Mining element	Cu, Pb, Zn
Bedrock type	Sandstone
pH (1:1 soil/water ratio)	3.8
Loss on ignition	11.13%
Moisture absorption	1.187%
Organic matter	6.37%

Nutrient Solution and Treatments

Modified Hoagland's medium (115 mg/L ammonium nitrate, 2.86 mg/L boric acid, 656 mg/L calcium nitrate, 0.08 mg/L cupric sulfate, 5.32 mg/L ferric tartrate, 240.7 mg/L magnesium chloride, 1.81 mg/L manganese chloride, 0.016 mg/L molybdenum trioxide, 300 mg/L potassium nitrate, and 0.22 mg/L zinc sulfate) (Shivendra and Natalie 2002). The basal nutrient medium was supplemented with $Pb(NO_3)_2$ (Sigma, analytical grade) at the rate of 0–1,000 mg/L. The pH of the medium was adjusted to 5.5 ± 0.3 with 1 mmol/L HCl or 1 mmol/L NaOH.

EDTA Treatment

Disodium salt of ethylenediamine-tetraacetic acid (0.1 or 0.5 mmol/L) was added in the nutrient solution containing 300 and 500 mg/L $Pb(NO_3)_2$, and pH was adjusted to 6.8 to allow maximum dissolution of EDTA.

T. orientalis Presl was grown in the following experimental variations:

I. with 300 and 500 mg/L $Pb(NO_3)_2$
II. with 300 and 500 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA
III. with 300 and 500 mg/L $Pb(NO_3)_2$ + 0.5 mmol/L EDTA

Incubation of Seedlings and Pb Analysis

Healthy and equal-sized seedlings were chosen and aseptically transferred to plastic pots (500 mL) containing 300 mL of nutrient solution with or without $Pb(NO_3)_2$ in the presence or absence of EDTA. Seedlings were incubated at 25/20°C under 16/8 h of light/dark regime. Plants were harvested 30 days after treatment. Each Pb treatment was replicated three times. Observations for three replicated were recorded in each treatment.

The harvested plants were washed thoroughly with distilled water and soaked in 10 mmol/L $CaCl_2$ for 5 min, separated into leaves, roots, and underground stems, and then oven-dried. The dry samples were digested with HNO_3 (10 mL) and $HClO_4$ (0.5 mL) and then diluted to 100 mL with 2% HNO_3 in volumetric flasks. The concentrations of lead were also quantified with flame atomic absorption spectroscopy using a Perkin Elmer AAnalyst 700.

Results and Discussion

Pb Accumulation in Plants Grown in Mine Tailings

The results of field investigations analyses were showed in Table 2. The tailings pond from the mining area had high Pb content. For the four sites, the highest total Pb was 6,871 mg/kg, with the average amount of 5,797.5 mg/kg. The average Pb concentration in leaves and roots (including underground stems) was 619 and 1,233 mg/kg, respectively. The bioconcentration factors were 0.1–0.26 and the values were relatively low, because the total Pb content is too high in the mine. However, most of the Pb concentrations of contaminated sites needing remediation are lower than the mine wastelands. The translocation factors were 0.48–0.52, which were higher than non-accumulating plants (He et al. 2002).

Effects of Pb Treatments on Biomass of *T. orientalis Presl*

Lead did not significantly affect the growth and appearance of the species until 300 mg/L and the biomass of plants was similar with the control. In 500 mg/L Pb treatment, the leaves showed a serious desiccation and dry weight was even half of the control. When Pb concentration exceeded 500 mg/L, the biomass decreased significantly. In 1,000 mg/L Pb treatment, the growth was inhibited thoroughly, and the seedlings died (Fig. 1).

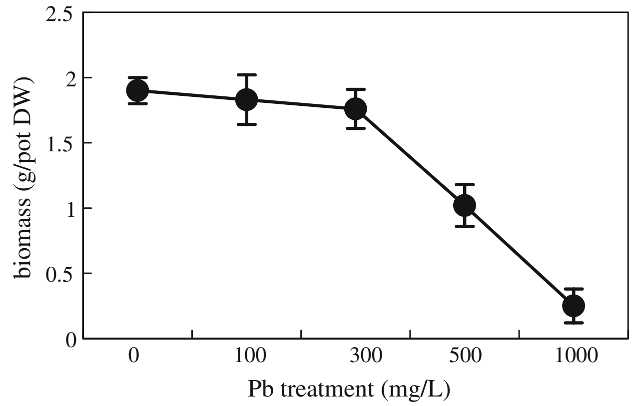

Fig. 1 Effects of different concentrations of Pb on *T. orientalis Presl*

Pb Accumulation in Plants Grown in Hydroponic Culture

The Pb concentrations in the leaves and roots increased with increasing of Pb level in the nutrient solution of 0–500 mg/L. The highest Pb concentrations in the leaves and roots were 16,190 and 64,405 mg/kg, respectively, at 500 mg/L Pb treatment (Fig. 2). At 1,000 mg/L $Pb(NO_3)_2$, the seedlings were dead which was the direct reason for the decreased Pb contents in plants.

The mobility of Pb from roots to shoots of plant is usually low (Begonia et al. 1998). When Pb enters the plant root, it encounters the neutral pH, high phosphate, and high carbonate environment of the intercellular spaces. Under these conditions, Pb precipitates as phosphate or carbonate and does not reach the xylem for translocation (Malone et al. 1974; Gabrielle and Patrick 1996). However, a higher shoot/root ratio of heavy metal concentration in plants is important in practical phytoremediation of heavily metal-contaminated environment. It has been shown that Pb hyperaccumulators usually have a higher shoot/root ratio of 0.04–0.10 of Pb concentration in plant than the non- hyperaccumulators (Kumar et al. 1995). In this study, *T. orientalis Presl* treated with 100–500 mg/L

Table 2 Lead bioconcentration and translocation of *T. orientalis Presl* under field conditions in YongZhou Pb/Zn/Cu Mine, Hunan, China

No.	Soils (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)	Caudices (mg/kg)	Translocation factor[a] (F/R)	Bioconcentration factors	
						Fronds[b]	Undergrounds[c]
1	6,871	697	647	739	0.50	0.10	0.20
2	5,846	673	607	681	0.52	0.12	0.22
3	3,940	488	493	531	0.48	0.12	0.26
4	6,533	702	639	746	0.51	0.11	0.21

[a] Ratios of Pb concentration in fronds to that in roots

[b] Ratios of Pb concentrations in fronds to that in soils

[c] Ratios of Pb concentrations in undergrounds to that in soils

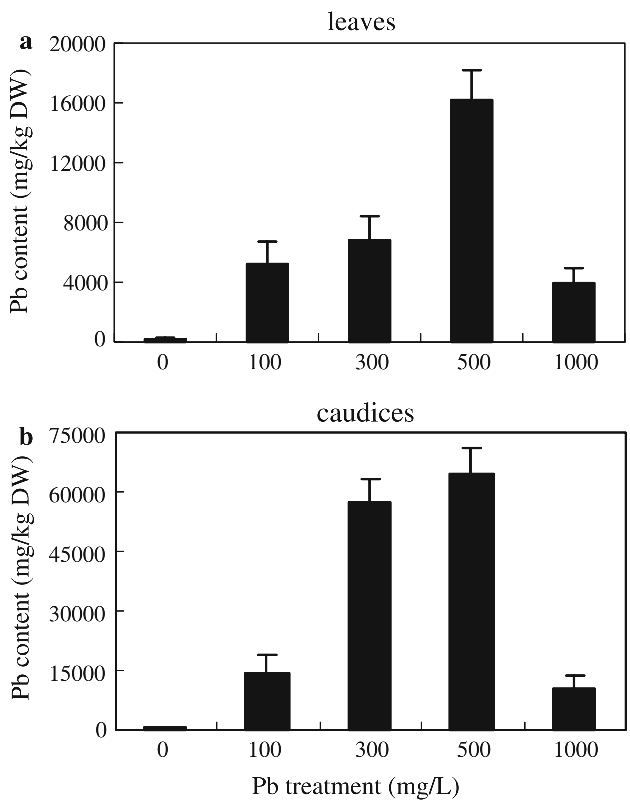

Fig. 2 Accumulation of Pb in (**a**) leaves and (**b**) caudices of *T. orientalis Presl* grown on modified Hoagland's medium containing 0–1,000 mg/L $Pb(NO_3)$

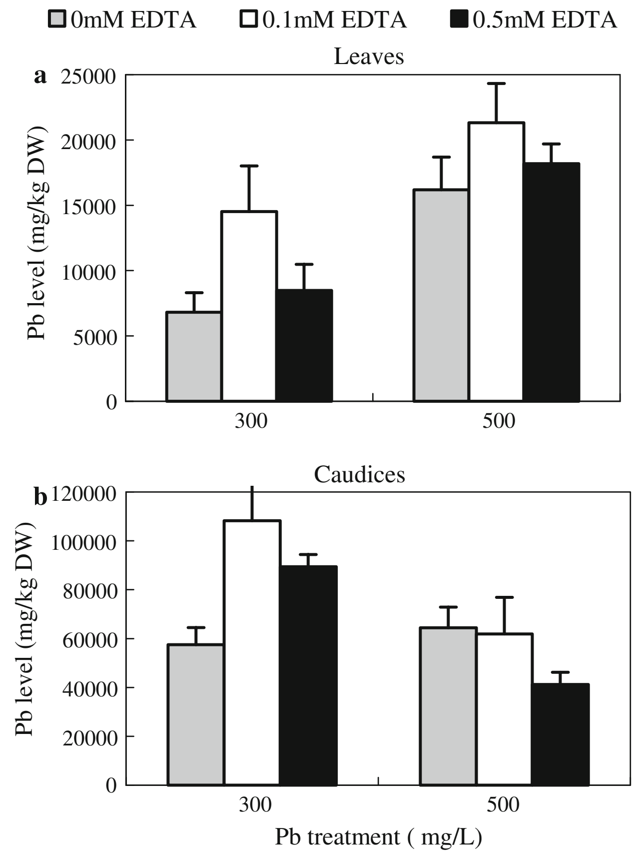

Fig. 3 Changes in Pb content in (**a**) leaves and (**b**) caudices of *T. orientalis Presl*, grown hydroponically in modified Hoagland medium in the presence of $Pb(NO_3)_2$, $Pb(NO_3)_2$ + 0.1 mmol/L EDTA and $Pb(NO_3)_2$ + 0.5 mmol/L EDTA

showed 0.12–0.25 shoot/root ratio, while those of non-accumulating ones did not exceed 0.04 (He et al. 2002).

The limit of detection is defined as the concentration equivalent to three times the standard deviation (N = 10) of the reagent blank. Detection limit value of the element Pb as in flame AAS was 0.010 mg/L.

The Effect of EDTA on Pb Accumulation in Various Plant Parts

It was determined that addition of 300 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA leads to an increase in the total amount of Pb taken up by plants by 91%, while 0.5 mmol/L EDTA increases Pb by 52.3% (Fig. 3). About 0.1 mmol/L EDTA supplied with the metal in 300 mg/L had the greatest effect on the growth of accumulation with Pb content in leaves and underground parts increasing 113% and 88.4%, respectively. With the addition of 0.5 mmol/L EDTA, the respective increase was changed to 24.5% and 55.6%. It was determined that leaves of *T. orientalis Presl* cultivated with the addition of 300 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA accumulated 113% more Pb (14,520 mg/kg DW) than leaves of plants treated with only 300 mg/L $Pb(NO_3)_2$ (6,815 mg/kg DW) (Fig. 3a). Under the condition of 500 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA or 0.5 mmol/L EDTA, 31.7% or 1.2% more Pb were accumulated in leaves of the treated plants. But there was a decrease in the underground parts, 3.9% and 36%, respectively. It indicated that the translocation of Pb to the aboveground parts was improved. It was also determined that the translocation factor was highly improved with the addition of EDTA, especially when 0.5 mmol/L EDTA was added to 500 mg/L $Pb(NO_3)_2$, with a 19% increase, which became 9% at 0.1 mmol/L EDTA. Under the condition of 300 mg/L $Pb(NO_3)_2$, the changes were not distinct (Table 3). Results from this study demonstrated that the plants had the highest accumulation and translocation under the condition of 500 mg/L + 0.5 mmol/L EDTA.

The Effect of EDTA on the Tolerance of *T. orientalis Presl*

No negative effect of EDTA presence on the rate of root elongation growth of *T. orientalis Presl* plants or on plant biomass growth was observed. During cultivation period in a medium with the addition of 0.1 and 0.5 mmol/L EDTA,

Table 3 Translocation factors of *T. orientalis Presl* grown hydroponically in modified Hoagland medium

Treatment	Translocation factor
300 mg/L $Pb(NO_3)_2$	0.12
300 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA	0.13
300 mg/L$Pb(NO_3)_2$ + 0.5 mmol/L EDTA	0.09
500 mg/L $Pb(NO_3)_2$	0.25
500 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA	0.34
500 mg/L$Pb(NO_3)_2$ + 0.5 mmol/L EDTA	0.44

root elongation growth remained similar to the control, i.e., 98% and 89%, respectively (Table 3). There was the same trend in biomass. Differences of the habits were observed between plants exposed to 500 mg/L $Pb(NO_3)_2$ and treated with $Pb(NO_3)_2$ supplemented with 0.1 or 0.5 mmol/L EDTA. Inhibition of root elongation growth and browning of roots occurred in plants cultivated only with lead nitrate addition. However, when the chelator was added to the medium, a clear decrease in the Pb toxic effect on plants was observed. The addition of EDTA eliminated to a great degree the inhibition of root elongation growth, lowered roots browning and resulted in a growing number of side roots. Moreover, the fresh weight of plants treated with Pb–EDTA was much higher in comparison with plants treated only with Pb and remained at the slightly lower level than the biomass of the control plants, which were grown only in Hoagland medium. When 500 mg/L Pb + 0.1 mmol/L or 0.5 mmol/L EDTA was used, the fresh weight of plants was 80% and 68% of the control plant biomass, respectively.

Indexes of tolerance were also determined for plants cultivated only with EDTA application. IT values of these plants were above 85% (Table 4). These data indicate that EDTA addition, especially at 0.1 mmol/L concentration, does not have a negative effect on plant growth.

The obtained data prove that *T. orientalis Presl* plants cultivated with the addition of Pb–EDTA show high resistance to the phytotoxic effect of metals. The results obtained from the cultivation with EDTA addition were compared with the results obtained after only exposing to 500 mg/L Pb. Adding 0.1 mmol/L EDTA raised the IT value 84%, which was close to the control plants, whereas after adding a higher concentration of the chelate (0.5 mmol/L) root elongation growth of *T. orientalis Presl* raised 66%. Their fresh biomass was 20% and 32%, respectively, lower than the control, which was relatively higher in comparison with plants treated only with $Pb(NO_3)_2$.

Table 4 Index of tolerance (IT) – estimated by means of the Wilkins' test (1957) for *T. orientalis Presl* roots grown hydroponically in modified Hoagland medium

Treatment	IT[a] (%)
0.1 mmol/L EDTA	98.0
0.5 mmol/L EDTA	89.2
500 mg/L $Pb(NO_3)_2$ + 0.1 mmol/L EDTA	93.7
500 mg/L $Pb(NO_3)_2$ + 0.5 mmol/L EDTA	84.6
500 mg/L $Pb(NO_3)_2$	50.9

[a] IT = Average length of roots in tested solution/(averagelength of roots in control) × 100%

Until recently, no Pb-hyperaccumulating plants have been reported in China. So finding hyperaccumulators and accumulating plants with fast growth rate and large biomass are both good ways to solve Pb contamination problems. In our survey, *Typha orientalis Presl* was found to be able to accumulate 16,190 mg/kg Pb in the leaves. Besides, the increase of plant dry matter of leaves and roots did not decrease even when treated with Pb up to 300 mg/L. Our data showed that *T. orientalis Presl* not only has high accumulation and strong tolerance ability of Pb, but also has fast growth rate and relatively large amount of biomass. *Typha orientalis Presl* has great potential for Pb removal from contaminated soils, wetlands, and rivers.

Plant characteristics change according to the environmental conditions, plant age and its general physical condition and the vegetation period (Aneta et al. 2003). There are several possible areas through which lead can penetrate plants. However, it is common that roots are the main pathway through which trace metal ions penetrate plants. It was determined that in the examined *T. orientalis Presl* plants roots are the main accumulation site of Pb^{2+} (Table 2 and Fig. 2b). The Pb concentration in the underground parts is 2–8-fold higher than it in the leaves. Comparatively, plants of the Brassicaceae family, which are thought to be good lead accumulators, the metal content in the roots was over 100 mg/g DW and in the aboveground parts about 10 mg/g DW, i.e., 10 times less than in the roots (Kumar et al. 1995). *Typha orientalis Presl* plants treated with different contents of $Pb(NO_3)_2$ showed low ability to translocate Pb to the aboveground organs. Only 11%–34% of Pb taken up by plants was deposited in leaves of *T. orientalis Presl*. It was demonstrated that the layer of cuticle and waxes in leaves usually forms an effective barrier against atomic Pb penetration. This protective layer stops Pb^{2+} ions on the root surface and does not let them into the leaves (Little and Martin 1972; Piechalak and Tomaszewska 2002).

EDTA application made it possible to raise the level of metal translocation to aboveground parts from 25% to 44%. Under the condition of 500 mg/L $Pb(NO_3)_2$ + 0.5 mmol/L EDTA, the translocation factor had the optimal increase. The biomass of plants was also increased. In our experiments *T. orientalis Presl* plants were grown with addition of 300 or 500 mg/L $Pb(NO_3)_2$ + 0.1 or 0.5 mmol/L EDTA. The application of EDTA at the concentration of 0.1 or

0.5 mmol/L together with Pb ions into medium resulted in a significant limitation of the metal phytotoxic effect. This concerns inhibition of root elongation growth, root coloring, and the appearance of mucus (Piechalak and Kasierska 2000). Results from this study demonstrated that the plants had the highest accumulation and translocation under the condition of 500 mg/L + 0.5 mmol/L EDTA.

Differences in growth rate of root length are an important indicator of plant resistance to heavy metals – one of the most commonly used measurement methods once described by Wilkins (1957). This method consists in comparing the growth of roots growing with addition of the stress factor with the growth of roots of control plants. The obtained result is described as the Index of tolerance (IT). The lowered root elongation growth of *T. orientalis Presl* plants observed after application of 500 mg/L $Pb(NO_3)_2$ corresponds with the results described earlier by many other authors. In our study, the value of IT was close to the control with the addition of EDTA, especially 0.1 mmol/L content.

In the field, dramatic increases in soil solution Pb could result in contamination of groundwater with consequent environmental impacts. The time of chelate application is important to avoid possible chelate-induced metal movement. It is also important to avoid rain days for the chelate application. Thus, the practical use of chelates for enhancement of metal removal in phytoremediation strategies may require careful site-specific evaluation to minimize the risk of serious secondary environmental contamination. Further research is needed before the chelate-assisted phytoextraction technique can be widely used at contaminated sites.

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