Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process

Yahua Chen[1,2], Xiangdong Li[1]* and Zhenguo Shen[1,2]

[1]*Department of Civil & Structural Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong*

[2]*College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China*

Abstract

In a pot experiment, the potential use of ten plant species, including six dicotyledon species and four monocotyledon species, was investigated for the EDTA-enhanced phytoextraction of Pb from contaminated soil. Mung bean and buckwheat had a higher sensitivity to the EDTA treatment in soils. In the 2.5 and 5.0 mmol kg^{-1} EDTA treatments, the Pb concentrations in the shoots of the six dicotyledon species ranged from 1000 to 3000 mg/kg of dry matter, which were higher than those of the monocotyledon species. The highest amount of phytoextrated Pb (2.9 mg Pb pot^{-1}) was achieved in sunflowers, due to the high concentration of Pb in their shoots and large biomass, followed by corns (1.8 mg Pb pot^{-1}) and peas (1.1 mg Pb pot^{-1}). The leaching behavior of heavy metals as a result of applying EDTA to the surface of the soil was also investigated using short soil-leaching columns (9.0 - cm diameter, 20 - cm height) by the percolation of artificial rainfall. About 3.5%, 15.8%, 13.7% and 20.6% of soil Pb, Cu, Zn and Cd, respectively, were leached from

*Corresponding author (X. D. Li). E-mail address: cexdli@polyu.edu.hk; Fax: (852) 2334-6389; Tel: (852)

the soil columns after the application of 5.0 mmol kg^{-1} of EDTA. The growth of sunflowers in the soil columns had little effect on the amount of metals that were leached out. This was probably due to the shallowness of the layer of soil, the short time-span of the uptake of metals by the plant and the plant's simple root systems.

Key words: Phytoextraction; EDTA; Heavy metals; Lead; Plant species; Leaching; Soil column

1. Introduction

The contamination of soils by heavy metals is one of the most serious environmental problems and has significant implications for human health. Numerous efforts have been made to develop technologies for the remediation of contaminated soils, including *ex-situ* washing with physical-chemical methods, and the *in-situ* immobilization of metal pollutants (Rulkens et al., 1995). These methods of cleanup are generally very costly, and often harmful to properties of soil (i.e., texture, organic matter, microorganisms) that are desirable for the restoration of contaminated sites. Recently, the phytoextraction of heavy metals from contaminated soils has attracted attention for its low cost of implementation and many environmental benefits (McGrath et al., 1993; Salt et al., 1998).

Two approaches have been generally proposed for the phytoextraction of heavy metals: the use of natural hyperaccumulator plants with exceptional

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metal-accumulating capacity, and the utilization of high biomass plants with a chemically enhanced method of phytoextraction (Salt et al., 1998). Hyperaccumulator plants usually accumulate only a specific element, tend to grow slowly and to have a low biomass. Many of them are not suitable for use in phytoextraction in the field. Moreover, some metals, such as Pb and Cr, are largely immobile in soils, and the efficient uptake of metals by plants is often limited by the solubility and diffusion of heavy metals to the root surface of plants. Most of the metal accumulated by plants is often retained in the roots, with relatively limited amounts being translocated to the more easily harvestable shoots. It has been suggested that high-biomass crops be used, such as maize (*Zea mays* L.), peas (*Pisum sativum* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), Indian mustard (*Brassica juncea* L.) and cabbage (*Brassica rapa* L. subsp. *chinensis*), with appropriate chemical treatments to enhance the solubility of metals in soil and translocation from the roots to the shoots (Huang and Cunningham, 1996; Blaylock et al., 1997; Huang et al., 1997; Ebbs and Kochian, 1998; Begonia et al., 2002; Shen et al., 2002). Several chelating agents, such as CDTA, DTPA, EDDHA, EDDS, EDTA, EGTA, HEDTA and NTA, have been studied for their ability to dissolve metals and enhance the uptake of metals by plants (Blaylock et al., 1997; Huang and Cunningham, 1996; Huang et al., 1997; Ebbs and Kochian, 1998; Cooper et al., 1999; Wu et al., 1999; Kayser et al., 2000; Kos and Leštan, 2002).

Apart from *in situ* phytoextraction, synthetic chelates are used to increase the removal of metal in the *ex-situ* washing methods. That chelates enhance the leaching of heavy metals in soils has been extensively documented using batch and column-leaching experiments (Papassiopi et al., 1999; Römkens et al., 2002; Kos and Leštan, 2002; Madrid et al., 2003). However, there have been few studies to assess the effects of the growth of plant roots on the chelate-induced metal leaching in soils. In soil columns where cabbage plants were being grown, Grčman et al. (2001) observed that 38, 10 and 56% of the initial total Pb, Zn and Cu, respectively, in soil were leached down the soil profiles during the 10 mmol kg^{-1} EDTA treatment. Kos and Leštan (2002) found that 2.5 – 10 mmol kg^{-1} of EDTA caused the leaching of a large portion of the total Pb initially present in soil, and suggested that uncontrolled EDTA treatments may not be acceptable for environmentally safe phytoextraction.

The success of a phytoextraction process is dependent on adequate plant yields and high metal concentrations in the shoots of plants. Research is still needed to determine the most successful combinations of plant species and types of chelates to be used. Some detailed assessment of the potential environmental impact of applying chelates is also required since adding synthetic chelates to soil has been shown to increase the risk of spreading contaminants and to cause additional health, safety and environmental concerns due to the high solubility and toxicity of metal-chelate complexes.

The objectives of this study were: (i) to compare the potential use of the ten plant species for the EDTA-enhanced phytoextraction of Pb from contaminated soil; (ii) to evaluate the possible effects of the application of EDTA on the leaching of heavy metals in the soil columns with and without sunflower plants.

2. Materials and Methods

2.1. Experiment 1

Soil samples were collected from a disused agricultural field in Yuen Long in Hong Kong in August 2001. The samples were air-dried, crushed to pass through a 1-cm diameter sieve, then artificially amended with 800 mg kg^{-1} soil Pb in the form of $PbCO_3$ (lead carbonate). The Pb-amended soils were allowed to undergo five cycles of saturation with de-ionized water and air-drying process, each lasting one week. The results of the sequential extraction showed that there was no significant change in the chemical partitioning of Pb between soil samples collected 36 days and 64 days after the addition of Pb, indicating that the added Pb achieved equilibration in the soils. The selected chemical and physical properties of the amended soils are given in Table 1.

At the 78^{th} day after the Pb amendment, soil basal fertilizers were applied at 100 mg K kg^{-1} of dry soil and 80 mg P kg^{-1} of dry soil as KH_2PO_4. Additional N was added at 100 mg kg^{-1} of dry soil as $CO(NH_2)_2$. After the application of soil basal fertilizers, the soil samples (580 g of dry soil) were placed in plastic pots (8 cm diameter x 10 cm in height). The level of moisture in the soils was maintained to near field water capacity (40%) and allowed to equilibrate for 7 days.

Ten crop species, including six dicotyledon and four monocotyledon, were used in this study: corn (*Zea mays* L. cv. Yedan No. 2), sorghum (*Sorghum bicolor* L. cv. Jinza No. 12), mung beans [*Vigna radiat* (L.) R. Wilczek var. *radiata* cv. VC-3762], oil sunflowers (*Helianthus annuus* L. cv. S61), common buckwheat (*Fagopyrum esculentum* Moench. cv. Pingqiao No. 2), cabbage [*Brassica rapa* L. subsp. *chinensis* (L.) Hanelt cv. Xinza No. 1], Chinese mustard (*Brassica juncea* L. Czern. et Coss. cv. Liyangkucai), pea (*Pisum sativum* L. cv. Qinxuan No. 2), wheat (*Triticum aestivum* L. cv. Yangmai No. 11), and barley (*Hordeum vulgare* L. cv. Kepin No. 7). The seeds of all of the crops were sterilized with 0.1% $HgCl_2$ for 5 min, then washed with tap water three times. After being soaked in de-ionized water for 10 h, the seeds were sown in pots. After germination, the plants were thinned to ten plants per pot for all species. The pots were watered with de-ionized water daily, if necessary.

On the 16th day after sowing, EDTA was applied to the surface of the soil at rates of 0 (control), 2.5 and 5.0 mmol EDTA kg^{-1} of soil as a 50 mmol/L Na_2-EDTA solution for each pot. All of the experiments were conducted in a greenhouse under conditions of natural light. Plants were watered as needed and plastic saucers placed below each pot to prevent leaching from the pots. The temperature of the air ranged from 18 - 30ºC. Each treatment was replicated three times, and was in a completely randomized block design.

Plant shoots were harvested 2 days before, and 2 and 7 days after the EDTA treatment, respectively. At each harvest, three plants were collected randomly from each pot. Plant shoots were cut approximately 0.5 cm above the surface of the soil. Shoot materials were rinsed with tap water and de-ionized water, and dried in an oven at 70ºC for 72 h. The samples were weighed, then ground in an agate mortar, and strained using a 0.85-mm diameter screen.

2.2. Experiment 2

Soil samples were collected from another site in Yuen Long in October 2001. The soils were air-dried, and crushed to pass through a 1-cm diameter sieve, then artificially contaminated with Pb (2500 mg kg^{-1} soil) in the forms of $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (lead sulfide) at a ratio of 1:1; with Cu (500 mg kg^{-1} soil) in the form of $CuCO_3$ (copper carbonate); with Zn (1000 mg kg^{-1} soil) in the forms of $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulfide) at a ratio of 1:1; and with Cd (15 mg kg^{-1} soil) in the form of $Cd(NO_3)_2 \cdot 4H_2O$ (cadmium nitrate). To prepare the contaminated soil, all of the metals that were added were first homogenized sufficiently with 250 g of uncontaminated soil in solid form in a mortar, subsequently mixed with 2 kg of uncontaminated soil in a plastic bag, and finally mixed thoroughly with 100 kg of natural soil samples in a concrete mixer (Croker mixers). The amended soils were allowed to equilibrate for a period of 15 days, undergoing five cycles of saturation with de-ionized water (with a soil water content of 13 – 14 %) and air-drying.

To create uniform bulk densities, each of the seven polyethylene leaching columns (9.0 - cm inner diameter, 20 - cm height) was packed with materials in the following order (bottom to top) : a) 1 - cm layer of acid-washed quartz sand (< 2 cm); b) a layer of fiberglass, c) 1365 g of air-dried amended soil equivalent to a layer 18 cm in thickness. De-ionized water was subsequently added to the soils to 60% of the field water capacity. Three seeds of oil-sunflower (*Helianthus annuus* L. cv. S61) were initially sown in four soil columns. After germination, one single seedling with a similar biomass was allowed to grow in the soil columns. Four soil columns with growing plants and three columns without plants were exposed to outdoor conditions at air temperatures ranging from 14 – 24 ºC and protected against precipitation using a roof. On the 25th day after the plants were sown, about 150 mg N fertilizer per kg^{-1} soil was applied to the surface of the soil of all columns as a urea solution. The soils were watered to maintain a soil moisture level of 50 – 60 % of the field water capacity. On the 50th day after the sunflower seeds were sown, 5.0 mmol of EDTA kg^{-1} soil in the form of a Na_2-EDTA solution was applied to three soil columns without plant and to three soil columns with sunflowers. The soil columns were moved into a room and irrigated to field capacity with de-ionized water three days after the application of EDTA. Artificial rainwater was then applied gradually to the top of each soil column, forming a shallow pond which was allowed to drain. The volumes of the leachate were then measured. The rate of flow ranged from 0.6 to 0.9 cm^3 min^{-1}. The application of rainwater was conducted for 16 h per day for three days. The composition of the artificial rainwater (mg L^{-1}) used in this leaching experiment was: NO_3^-: 1.94; NH_4^+: 0.49; Na^+: 1.87; Mg^{2+}: 0.25; Ca^{2+}: 0.29; Cl^-: 3.41; SO_4^{2-}: 2.65; pH = 4.40 (Hodson et al., 2001).

After the leaching experiment, sunflower shoots and roots were gently removed from the soil and carefully washed with tap water. The roots and shoots were further separated with scissors and washed with de-ionized water, and subsequently dried in an oven at 70 ºC for three days.

2.3. Chemical and physical analysis

The pH of the soil was measured with 0.01 mol/L $CaCl_2$ of solution in 1:5 (w/v) using a pH meter. The cation exchangeable capacity (CEC) of the soil was determined by the ammonium acetate saturation method. The soil texture, organic matter content, total N and field capacity were measured using the procedures described by Avery and Bascomb (1982). The total metal concentrations of the soil were determined by ICP-AES (Perkin-Elmer Optima 3300 DV) after a strong acid digestion (1:4 concentrated HNO_3 and $HClO_4$ (v/v) (Wong et al., 2002). The plant materials were wet-digested with a mixture of $HNO_3/HClO_4$ for elemental analysis using ICP-AES (Shen et al., 2002). In this study, all of the results are expressed on a dry weight basis.

The leachate solutions were filtered through Whatman No. 42 filter paper. The pH value of the leachate solutions was determined using a pH meter, and dissolved total organic carbon (TOC) was measured with a Shimadzu TOC-5000A analyser. A sub-sample was digested with concentrated HNO_3, then diluted with 5% HNO_3 and analysed for heavy metals using ICP-AES (Perkin-Elmer Optima 3300 DV).

The data reported in this paper were analysed using SPSS® 10.0 computer programme. A probability of 0.05 or less was considered to be statistically significant for the data set.

3. Results

3.1. Effects of EDTA treatment on plant growth

After the germination of the seeds, the growth of all plant seedlings appeared to be normal in the soils containing a total Pb of 860 mg kg^{-1} before EDTA was applied in Experiment 1 . No visual symptoms of metal toxicity were found (see Table 2). The application of EDTA affected the growth of the plants and the production of shoot biomass. The dry weights of the shoots of all plants decreased as the rate at which EDTA was applied increased. The biomasses of maize, sunflower and cabbage were higher than those of other plant species tested with and without the EDTA treatment. Monocotyledon species showed relatively less response to the addition of EDTA, exhibiting little chlorosis, with small reductions of shoot biomass. Barley appeared to be the least sensitive to EDTA, followed by maize and wheat. Six dicotyledon species tested (mung bean, sunflower, cabbage, buckwheat, pea and mustard) were highly sensitive to the EDTA treatment. The leaves of the plants exhibited visual symptoms (such as curling, chlorosis, necrosis and stunting) of metal or EDTA toxicity 1-2 days after the application of EDTA, followed by a rapid senescence and drying of the plant shoots. The toxic effect was most prominent on mung bean and buckwheat, resulting in the death of the plants within two days after EDTA had been applied at a rate of 5 mmol kg^{-1}. The dry weights of mung bean and buckwheat decreased from 71 and 42 mg $plant^{-1}$ in the control group to 48 and 24 mg $plant^{-1}$, respectively. When plants were grown for an additional 5 days, the inhibition of plant growth by the application of EDTA became more pronounced, particularly for the dicotyledon species.

3.2. Uptake of lead by different plant species with EDTA treatment

In the control group, the concentration of Pb in the shoots of plants varied from approximately 10 to 57 mg kg^{-1} of dry weight among the ten species of plants (Table 3). Generally, the shoots of dicotyledon species had higher Pb concentrations than the monocotyledon species. The highest concentration of Pb was found in the shoots of sunflowers (57 mg kg^{-1}).

The application of EDTA at a rate of 2.5 or 5.0 mmol/kg dramatically increased the concentrations of metals in the shoots of plants. Generally, the concentrations of Pb in the shoots of dicotyledon species were much higher than in those of monocotyledon species under the experimental conditions. Plant uptake of Pb was particularly enhanced (see Table 3). The high rate of application of EDTA (5 mmol kg^{-1}) was more efficient than the low rate (2.5 mmol kg^{-1}) for enhancing the concentration of Pb in shoots. For most species of plants, the shoots harvested 7 days after the EDTA treatment had higher concentrations of Pb than those harvested on the second day.

There were wide ranges of Pb concentration among the plant species. The differences were much greater among the plants grown on the EDTA-treated soils than among those of the control group. On the second day after the application of EDTA, increases in the concentrations of Pb in the shoots of buckwheat, mung bean and pea were most significant among the tested species. The concentrations of Pb in the shoots of buckwheat, mung bean and peas reached 1660, 1430 and 1040 mg kg^{-1} DW, respectively, in the 2.5 mmol kg^{-1} EDTA treatment. This was a 65-fold, 25-fold and 104-fold increase, respectively, over those of the control groups. On the seventh day after the 5.0 mmol kg^{-1} EDTA treatment, the most significant effect of EDTA was found in mustard, followed by sunflower. The concentrations of Pb in the shoots of mustard and sunflowers were 2900 and 1800 mg kg^{-1} DW, respectively, which were a 96-fold and 32-fold increase over those of the controls (Table 3). Compared with other crops, the increases of Pb concentrations were lower in the shoots of wheat and barley after the EDTA treatment.

3.3. EDTA effects on the Pb phytoextraction

The application of EDTA to the soil substantially increased the amount of Pb extracted by the shoots of the plants (see Table 3). The Pb phytoextraction efficiency of the ten crops varied significantly. The highest amount of Pb extracted (2.9 mg Pb pot^{-1}) was achieved by sunflower, due to the high concentration of Pb in their shoots and their large biomass, followed by corn (1.8 mg Pb pot^{-1}) and peas (1.1 mg Pb pot^{-1}). Wheat and barley extracted less than 0.4 mg Pb pot^{-1}, indicating a low efficiency in Pb phytoextraction. For the six dicotyledon species, there were no significant differences in the total amount of Pb extracted by plant shoots between the EDTA application rate of 2.5 and 5 mmol kg^{-1} (Table 3). Although the 5.0 mmol kg^{-1} of EDTA application slightly increased the concentrations of Pb in the shoots, the total amount of metals extracted by shoots remained largely unchanged due to a reduction in the yield of the shoots as the rates at which EDTA was applied increased (Table 3).

3.4. The leaching patterns of metals from soil columns

After the addition of EDTA, the amounts of DOC and metals leached from each of the soil columns were measured after 1000 ml of artificial rainwater were applied (Experiment 2). The leaching patterns of DOC, Pb, Cu, Zn and Cd in the leachate solutions are shown in Fig. 1.

In the leachates from the soil columns where EDTA had not been applied, the concentrations of DOC ranged from 15 to 50 mg C L^{-1}. The concentrations of Pb, Cu, Zn and Cd in the leachates were very low, indicating that only a very small amount of heavy metals had been removed by the rainwater. The cumulative amounts of Pb, Cu, Zn and Cd were less than 1% of the initial concentrations of metal in the soil.

After the EDTA treatment, the TOC in the leachates increased rapidly at the beginning of the leaching experiment (Fig. 1). The highest TOC concentrations reached 2640 and 2280 mg C L^{-1} in the leachates with sunflower growth and in those without plants at leachate volumes of about 300 ml and 400 ml, respectively. Thereafter, the concentrations of TOC in the leachate solution declined and remained at a fairly low level at a leachate volume of 800 ml (about 20 mg C L^{-1}).

Concentrations of dissolved Pb, Cu, Zn and Cd closely followed the pattern of DOC (Fig. 1). The concentrations of Pb, Cu, Zn and Cd in the leachates increased rapidly to the highest levels at leachate volumes of 300 ml and 400 ml, respectively, from the column with sunflowers and without plant. Subsequently, the concentrations of metals declined and reached an equilibrium of about 800 ml in leachate volume. The majority of the leached heavy metals occurred in the first 800 ml of leachates from the soil columns.

As for Pb, Cu, Zn and Cd, the peak concentrations of Fe appeared at a leachate volume of about 300 ml and 400 ml (Fig. 1), respectively, from the columns with and without the plant. The peak concentration of Fe in the leachate without plant was higher than that in the leachate from the columns with sunflowers. Columns with sunflower plants leached 25% less Fe than those without plants. Compared with the total Fe in the soil columns, the amount of Fe that had leached was generally small, accounting for less 0.5% of the total concentration.

Table 4 shows the total amounts of heavy metals taken up by sunflower seedlings grown in the soil columns after the application of EDTA. The total uptake of Pb, Cu, Zn and Cd by sunflower seedlings was 0.62, 0.42, 2.23 and 0.12 mg, respectively. On the other hand, the total amount of Pb, Cu, Zn and Cd leached from the soil columns planted with sunflowers was 116, 136, 237 and 3.25 mg, respectively. About 4% of the initial total Pb, 16% of Cu, 13% of Zn and 23% of Cd in soils were leached from the soil columns. The total amount of heavy metals absorbed by the roots and shoots of sunflowers only accounted for a very small proportion (<1%) of the total leached heavy metals. No significant differences were found between the amounts of Pb, Cu, Zn and Cd leached from the columns with and without sunflower plants (Table 4). Therefore, the growth of sunflower plants did not change the leaching patterns of heavy metals in columns of soil under the present experimental conditions (see Fig. 1).

4. Discussion

Heavy metals, including Pb, can cause significant damage to plants and microorganisms. The study of metal-accumulating plants has demonstrated that plants have genetic differences permitting the uptake and translocation of metals into aboveground tissues. The normal range of Pb in the shoots of most plants was 0.1 – 5 mg kg^{-1} DW (Reeves and Baker, 2000), and most crops do not possess the ability to protect sensitive cellular activity and structures from excessive levels of Pb. In Experiment 1, all ten crop seedlings were able to grow on the soil containing 860 mg kg^{-1} Pb. The concentration of Pb in shoots ranged from 10 to 57 mg kg^{-1}. The application of EDTA decreased the net production of shoot and root biomass and increased the accumulation of Pb in the shoots of the plants. Among ten plant species tested, the monocotyledon species showed limited signs of phytotoxicity and decreases of shoot biomass after the EDTA treatment (Table 2). In this case, both the direct adverse action of EDTA and the increased bioavailabilty of soil metals could depress the growth of plants. Epstein et al. (1999) reported that EDTA and Pb were taken up together by the plant, and suggested that Pb was translocated in the plant as the Pb-EDTA complex. Table 3 showed that average concentration of Pb in the shoots of six dicotyledon species was significantly greater than that in the shoots of four monocotyledon species after the application of EDTA to soils. The result could partly explain why the dicotyledon species suffered from more severe phytotoxicity than the monocotyledon species after the application of EDTA. Cooper et al. (1999) showed that the phytotoxic effects of EDTA occurred at least partly in response to the uptake of Pb by plants. Monocotyledon species are usually more tolerant to metals than dicotyledon species (Marschener, 1995). More detailed studies are needed to separately evaluate the adverse effects of EDTA and heavy metals, and the mechanisms by which different plants tolerate toxic pollutants.

The aim of phytoextraction is to reduce the levels of metals in contaminated soil to acceptable levels within a reasonable time frame. The process depends on the ability of the selected plants to grow and accumulate metals under the specific climate and soil conditions of the site being remediated. The results of our study indicate that EDTA increased the accumulation of Pb by a factor of 7 to 31 in all species of plants except for peas. The enhancing effect of EDTA observed here was smaller than that reported by Blaylock et al. (1997) and Huang et al. (1997).

Peas had a greatest ability to uptake metal after the EDTA treatment by a factor of 50. However, the plants were very sensitive to the presence of EDTA and all of the plants were dead 2 days after 5.0 mmol kg^{-1} EDTA was applied. Mustard plants had a lower shoot biomass than sunflower under our experimental conditions, although the Pb concentrations in the shoots reached 2900 mg kg^{-1}, which was the highest among all the plant species investigated. In light of recently published work, it appears that plants used for phytoextraction must first become well developed and established in contaminated soils before they are exposed to the increased stress of metals caused by the application of EDTA. For this reason, sunflowers were grown in the second experiment due to their high biomass and concentrations of Pb in their shoots (Table 3). It would be advantageous if EDTA is applied to soil at a low rate to facilitate the breakdown barriers to the uptake of metals by plants and to enable metals to be translocated into the shoots. At the same time, plants should be tolerant to the high concentrations of metal without significant inhibitions to growth or reductions in biomass. When 2.5 mmol kg^{-1} of EDTA were added to the contaminated soil for 2 days, a 25-fold to 105-fold increase in the concentration of Pb in shoots was observed in some dicotyledon species, such as mung bean, buckwheat and peas, compared with the plants without EDTA treatment. Increases in the concentration of Pb in shoots were likely due to an increase in uptake rather than to an decrease in the dry weights of the plants. However, the mass balance of Pb extracted from the soil into the plant shoots indicate that the percentage of Pb extracted in one phytoextraction cycle was less than 0.6% of the total Pb in soil, even in the treatment where the highest amount of Pb extracted was determined. The screening of more sensitive taxa to EDTA applications could help to minimize the amount of chelate applied in fields, reduce operating costs, and alleviate the potential risks of EDTA and the migration of mobilized heavy metals to ground water.

Although the total concentration of Pb in many contaminated soils may be high, the mobilizable and bioavailable fraction of Pb (water soluble and exchangeable) is usually very low due to the strong association of Pb with organic matter, Fe-Mn oxides and clays; and precipitation as carbonates, hydroxides and phosphates (McBride, 1994). Sequential extractions of soil used in the present study indicated that only a minor part of total Pb (1%) could be exchanged into the soil solution from soil compartments (data not shown). EDTA treatments may shift the distribution of soil Pb from more resistant phases to more soluble forms (Shen et al., 2002). Thus, the application of EDTA to soils has the potential risk that mobilized Pb and other heavy metals will migrate from the soil to the groundwater.

Results from our previous experiments showed that soil matrix planted with vetiver grass (*Vetiveria zizanioides*) with a long, massive and complex root systems, could effectively reduce the leaching of heavy metals downwards in an EDTA-assisted method of phytoextraction (Chen et al. 2004). In the present leaching test, significant amounts of Pb, Cd, Cu and Zn leached out from the soil column with sunflower plants, and the amount was about the same as in the control group (without plants) (see Fig. 1). In columns to which EDTA had been applied, plant growth had little effect on the amount of metal leached under current conditions. First, soil column used in this study were shorter than 20 cm. Second, the leaching was conducted 2 days after the EDTA treatment. Soil Pb could be solubilized by EDTA in a short duration (6 h) and maintained at a high level afterwards (Shen et al., 2002). The metal taken up by the plant was very minor compared with the amount leached from the soil columns (Table 4), although the concentration of Pb in the shoots of the sunflowers increased 3.8-fold 2 days after the EDTA treatment at 5.0 mmol kg^{-1} (Table 3). More importantly, continuous plant growth could decrease the water content of soil, retarding the movement of Pb through the soil column and increasing the adsorption of metal in soils (Chen et al., 2004). Alternatively, it would be advantageous to use deep-rooted plants that can consume more soil water to reduce metal leachate from soils. Third, the root systems of the sunflowers used in this study were less developed than those of the vetiver grass used in our previous experiment (Chen et al., 2004). Vetiver grass grows very quickly and has a fine, deep and penetrating root system, which is capable of reaching down to 3 - 4 m in the first year (Truong, 2000).

The chelate-assisted phytoextraction of heavy metals has potential value. The careful management of soil and the right selection and combination of plants are needed before this remediation technology can be used in field conditions. Kos and Leštan (2003) reported that the use of biodegradable chelate EDDS and permeable barriers might lead to environmentally safe induced Pb phytoextraction and the *in situ* washing of Pb. Madrid et al. (2003) discussed a system that can be used to recycle metal-laden drainage water back to land to prevent the contamination of ground water. High biomass plants that can grow in the presence of high concentrations of chelate-solubilized heavy metals will need to be further identified. With suitable metal-accumulating plant and new irrigation strategies, it should be possible to improve chelate-assisted phytoextraction techniques to minimize the potential pollution of groundwater.

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Table 1. Selected physicochemical properties of tested soils

	Experiment 1	Experiment 2
pH ($CaCl_2$)	5.06	6.04
Sand (%) >0.05 mm	34.5	54.3
Silt (%) 0.05-0.001 mm	43.1	31.1
Clay (%) <0.001 mm	22.4	14.6
Organic matter (%)	2.13	1.67
Total Pb (mg kg^{-1})	861	2400
Total Cu (mg kg^{-1})	11.9	630
Total Zn (mg kg^{-1})	46.1	1270
Total Cd (mg kg^{-1})	0.83	11.6
Total Ca (%)	0.25	0.65
Total Fe (%)	1.22	0.69

Table 2. Dry weights of the shoots of ten plant species 2 and 7 days after the application of EDTA. Error bars represent ± SE (n=3).

Crop Species	EDTA (mmol/kg soil)	Shoot dry wt (mg / plant) 2d	7 d
Dicotyledon			
Mung bean	0	71.1 ± 7.6 (N)*	---
	2.5	50.7 ± 4.8 (III)	Nd**
	5.0	48.3 ± 5.4 (IV)	Nd
Buckwheat	0	41.7 ± 9.3 (N)	78.7 ±11.3 (N)
	2.5	28.0 ± 3.2 (II)	25.6 ± 7.3 (IV)
	5.0	24.1 ± 5.4 (IV)	Nd
Sunflower	0	280 ± 41 (N)	533 ± 39 (N)
	2.5	209 ± 31 (II)	241 ± 37 (IV)
	5.0	182 ± 25 (II)	164 ± 30 (IV)
Cabbage	0	230 ± 27 (N)	303 ± 48 (N)
	2.5	210 ± 38 (II)	190 ± 28 (II)
	5.0	168 ± 36 (III)	185 ± 20 (III)
Pea	0	95.0 ± 7.2 (N)	167 ± 29 (N)
	2.5	75.0 ± 7.0 (II)	103 ± 15 (IV)
	5.0	80.0 ± 6.6 (III)	Nd
Mustard	0	35.6 ± 6.5 (N)	132 ± 28 (N)
	2.5	22.2 ± 3.4 (II)	78.9 ± 10 (III)
	5.0	22.9 ± 4.2 (III)	35.0 ± 5.3 (IV)
Monocotyledon			
Maize	0	367 ± 29 (N)	520 ± 61 (N)
	2.5	263 ± 24 (I)	430 ± 29 (I)
	5.0	245 ± 40 (I)	399 ± 49 (II)
Sorghum	0	75.0 ± 5.8 (N)	133 ± 30 (N)
	2.5	65.0 ± 5.7 (I)	128 ± 23 (I)
	5.0	63.8 ± 10.5 (I)	93.3 ± 17 (II)
Wheat	0	62.2 ± 5.7 (N)	105 ± 12 (N)
	2.5	60.0 ± 2.8 (N)	76.3 ± 9.2 (N)
	5.0	58.8 ± 5.7 (N)	76.3 ± 5.8 (I)
Barley	0	61.3 ± 3.5 (N)	124 ± 11 (N)
	2.5	56.3 ± 8.6 (N)	102 ± 4.8 (N)
	5.0	51.3 ± 4.6 (N)	106 ± 11 (I)

Note: *: The figures in the brackets stand for the degrees of harm to plant seedlings as judged by observing those the pot experiment. N: stands for no visual harm in leaf; I: stands for the area of visible necro-macula in leaves was less than 5%; II: the area of necro-macula in leaves was about 20%; III: the area of necro-macula in leaves was about 50%; and IV: stands for the oncoming death of seedlings.

**Nd denotes no data because these species of plants did not survive.

Table 3. Amounts of Pb accumulation in the shoots of ten species of plants 2 and 7 days after the application of EDTA. Error bars represent ± SE (n=3).

Crop Species	EDTA (mmol /kg soil)	Pb concentration (μg / g DW)		Amount of Pb accumulation (μg / plant)	
		2 d	7 d	2 d	7 d
Dicotyledon					
Mung bean	0	56.9 ± 7.3	---	4.1 ± 0.9	---
	2.5	1430 ± 143	Nd	72.3 ± 6.3	Nd
	5.0	1490 ± 182	Nd	72.5 ± 14.4	Nd
Buckwheat	0	25.6 ± 6.2	40.3 ± 2.9	1.0 ± 0.2	3.2 ± 0.7
	2.5	1660 ± 118	2500 ± 168	46.2 ± 2.1	63.4 ± 13.8
	5.0	1820 ± 162	Nd	43.3 ± 5.8	Nd
Sunflower	0	53.5 ± 8.9	56.9 ± 7.3	14.9 ± 2.8	30.0 ± 1.9
	2.5	389 ± 55	1160 ± 68	81.2 ± 11.4	278 ± 39
	5.0	391 ± 68	1800 ± 175	69.5 ± 8.5	286 ± 30
Cabbage	0	24.6 ± 4.3	31.7 ± 4.9	5.7 ± 1.5	9.7 ± 2.3
	2.5	262 ± 39	475 ± 47	54.3 ± 5.5	89.4 ± 3.7
	5.0	452 ± 51	459 ± 42	76.3 ± 19.1	84.7 ± 9.8
Pea	0	9.97 ± 1.50	13.7 ± 3.2	0.9 ± 0.1	2.3 ± 0.9
	2.5	1040 ± 52	1110 ± 152	78.5 ± 11.2	113 ± 13
	5.0	1390 ± 106	Nd	112 ± 14	Nd
Mustard	0	28.7 ± 4.4	30.2 ± 5.3	1.0 ± 0.2	3.9 ± 0.8
	2.5	461 ± 46	1260 ± 99	10.2 ± 1.7	99.1 ± 12.2
	5.0	761 ± 101	2900 ± 164	17.3 ± 2.7	101 ± 9.9
Monocotyledon					
Maize	0	42.3 ± 3.8	36.7 ± 5.9	15.8 ± 2.6	18.9 ± 2.5
	2.5	332 ± 45	295 ± 30	86.7 ± 6.5	128 ± 13
	5.0	649 ± 63	445 ± 48	159 ± 26	179 ± 34
Sorghum	0	24.1 ± 4.2	18.7 ± 1.8	1.8 ± 0.4	2.5 ± 0.3
	2.5	302 ± 19	271 ± 40	19.8 ± 3.0	34.5 ± 6.5
	5.0	572 ± 36	833 ± 63	36.2 ± 4.2	76.9 ± 8.7
Wheat	0	12.3 ± 1.8	14.2 ± 2.9	0.8 ± 0.2	1.5 ± 0.4
	2.5	271 ± 18	233 ± 35	16.3 ± 1.2	17.7 ± 2.9
	5.0	387 ± 25	329 ± 17	22.7 ± 2.3	25.1 ± 2.9
Barley	0	16.5 ± 2.0	18.2 ± 3.7	1.0 ± 0.1	2.3 ± 0.5
	2.5	125 ± 20	223 ± 22	6.96 ± 0.87	22.8 ± 2.8
	5.0	94.3 ± 14	360 ± 56	4.80 ± 0.46	38.1 ± 5.0

Note: *Nd denotes no data because these species of plants did not survive.

Table 4. Total amounts of metals in the leachates after the application of 1,000 ml of rainwater and in the sunflower seedlings grown in the soil columns.

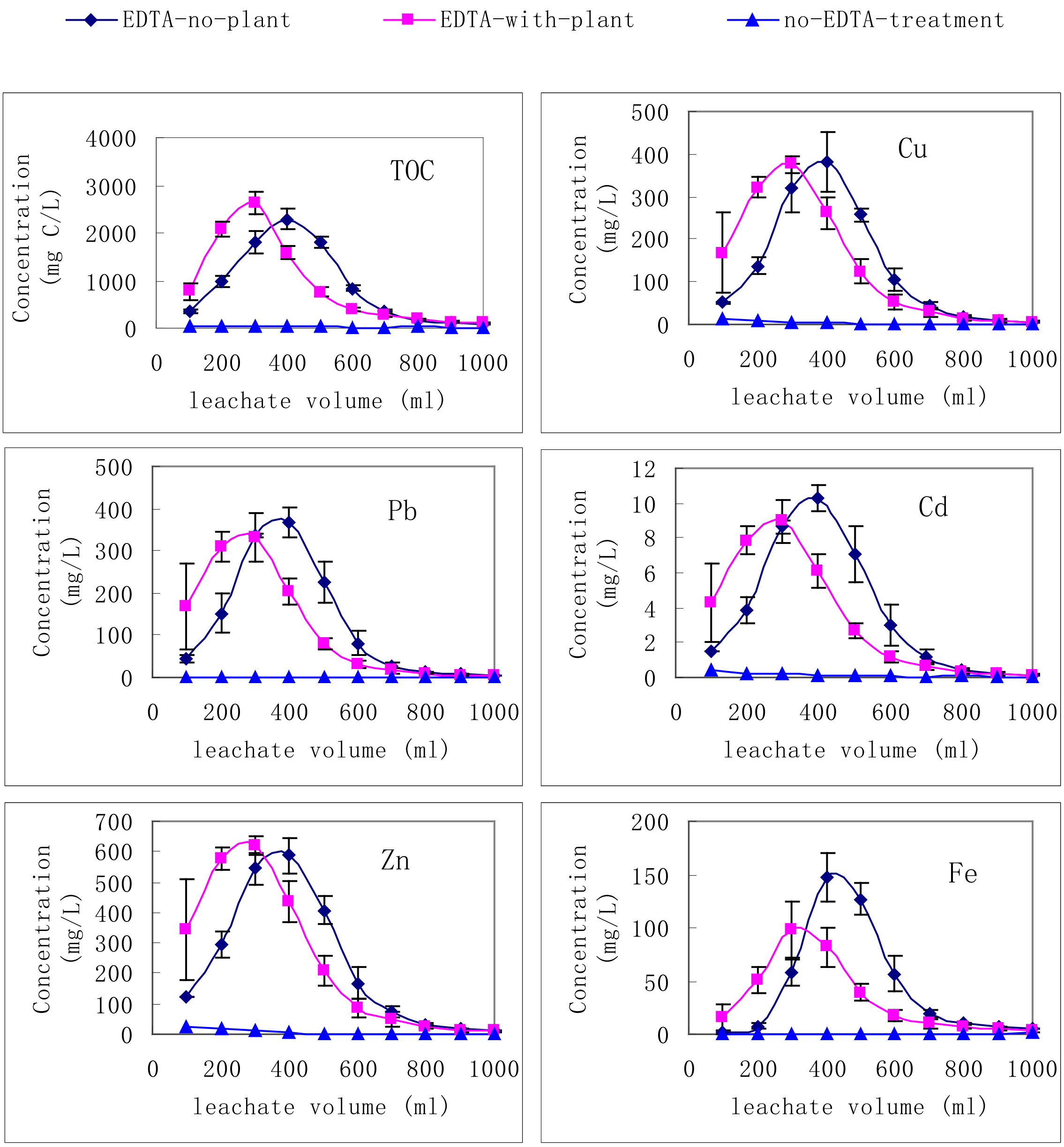
Metals	Total initial content of metals in soil columns (mg)	Leachates (mg)*			Sunflower (mg)
		Without EDTA With plants	With EDTA No plants	With EDTA and plants	With the application of EDTA
Pb	3280	1.58	126 ± 10	116 ± 8	0.62
	(100)**	(0.05)	(3.84)	(3.54)	(0.02)
Cu	860	3.19	133 ± 5	136 ± 6	0.42
	(100)	(0.37)	(15.5)	(15.8)	(0.05)
Zn	1730	6.47	225 ± 5	237 ± 3	2.23
	(100)	(0.38)	(13.1)	(13.7)	(0.13)
Cd	15.8	0.129	3.64 ± 0.29	3.25 ± 0.15	0.12
	(100)	(0.82)	(23.0)	(20.6)	(0.73)
Ca	8880	373	485 ± 29	482 ± 40	34.2
	(100)	(4.20)	(5.46)	(5.43)	(0.39)
Fe	9350	0.479	44.0 ± 0.2	33.0 ± 4.5	0.78
	(100)	(0.005)	(0.47)	(0.35)	(0.01)
K	2120	29.7	25.3 ± 0.9	26.5 ± 2.5	41.8
	(100)	(1.40)	(1.19)	(1.25)	(1.97)
Mg	671	45.8	57.9 ± 2.3	57.6 ± 5.8	7.6
	(100)	(6.83)	(8.64)	(8.58)	(1.13)

Note: * After the application of 1,000 ml of rainwater (the amount of rainwater applied was nearly equivalent to 158 mm of rainfall precipitation within 2 days).

** The figures in the brackets are the percentage (%) of the total initial content of metals in soil columns.

Figure Captions

Fig.1 Concentrations of TOC（mg C L^{-1}）, Pb, Cu, Zn, Cd and Fe（mg L^{-1}）in the leachates from the soil columns treated with EDTA and with sunflower, with EDTA without plant growth, and without EDTA and with plants.


EDTA-no-plant
EDTA-with-plant
no-EDTA-treatment
TOC
Concentration (mg C/L)
0
1000
2000
3000
4000
0
200
400
600
800
1000
leachate volume (ml)
Cu
Concentration (mg/L)
0
100
200
300
400
500
0
200
400
600
800
1000
leachate volume (ml)
Pb
Concentration (mg/L)
0
100
200
300
400
500
0
200
400
600
800
1000
leachate volume (ml)
Cd
Concentration (mg/L)
0
2
4
6
8
10
12
0
200
400
600
800
1000
leachate volume (ml)
Zn
Concentration (mg/L)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
leachate volume (ml)
Fe
Concentration (mg/L)
0
50
100
150
200
0
200
400
600
800
1000
leachate volume (ml)