The use of Vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals

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Abstract

Recent research has shown that phytoextraction approaches often require soil amendments, such as the application of EDTA, to increase the bioavailability of heavy metals in soils. However, EDTA and EDTA-heavy metal complexes can be toxic to plants and soil microorganisms and may leach into groundwater, causing further environmental pollution. In the present study, vetiver grass (*Vetiveria zizanioides*) was studied for its potential use in the phytoremediation of soils contaminated with heavy metals. In the pot experiment, the uptake and transport of Pb by vetiver from Pb-contaminated soils under EDTA application were investigated. The results showed that vetiver had the capacity to tolerate high Pb concentrations in soils. With the application of EDTA, the translocation ratio of Pb from vetiver roots to shoots was significantly increased. On the 14th day after 5.0 mmol EDTA kg^{-1} of soil application, the shoot Pb concentration reached 42, 160, 243 mg kg^{-1} DW and the roots Pb concentrations were 266, 951, and 2280 mg kg^{-1} DW in the 500, 2500 and 5000 mg Pb kg^{-1} soils, respectively. In the short soil leaching column (9.0-cm diameter, 20-cm height) experiment, about 3.7%, 15.6%, 14.3% and 22.2% of the soil Pb, Cu, Zn and Cd were leached

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from the artificially contaminated soil profile after 5.0 mmol EDTA kg^{-1} of soil application and nearly 126 mm of rainfall irrigation. In the long soil leaching experiment, soil columns (9.0-cm diameter, 60-cm height) were packed with uncontaminated soils (mimicking the subsoil under contaminated upper layers) and planted with vetiver. Heavy metals leachate from the short column experiment was applied to the surface of the long soil column, the artificial rainwater was percolated, and the final leachate was collected at the bottom of the soil columns. The results showed that soil matrix with planted vetiver, could re-adsorb 98%, 54%, 41%, and 88% of the initially applied Pb, Cu, Zn, and Cd, which may reduce the risk of heavy metals flowing downwards and entering the groundwater.

Key words: phytoremediation; heavy metals; lead; EDTA; Vetiver grass (*Vetiveria zizanioides*).

1. Introduction

Heavy metal contamination in soils is one of the world’s major environmental problems, posing significant risks to human health as well as to ecosystem. Recently, phytoremediation, using plants to remove metal pollutants from contaminated soils, is being developed as a new method for the remediation of contaminated land. This environmentally friendly, cost-effective and plant-based technology is expected to have significant economic, aesthetic, and technical advantages over traditional engineering techniques (Baker et al., 1994; Salt et al., 1998; Garbisu and Alkorta, 2001).

Because heavy metals in soils are generally bound to organic and inorganic soil constituents, or alternatively, present as insoluble precipitates, a large proportion of metal contaminants are unavailable for root uptake by field-grown plants (Raskin et al., 1994). Methods of increasing heavy metal contaminants bioavailability in soil and its transport to plant shoots are vital to the success of phytoremediation in the field (Ernst, 1996). For plant uptake, heavy metals must first be disassociated from soil compartments into a soil solution. The solubility process of soil-bound metals can be achieved by a number of approaches, including the application of soil acidifiers, commercial nutrients or some chelates such as EDTA, DTPA, CXDTA, EGTA, or citric acid (Ebbs et al., 1997; Chen and Cutright, 2001). Among these chelates, EDTA was shown to be the most efficient in mobilizing Pb from various soil compartments (Huang et al., 1997; Shen et al., 2002; Wenzel et al., 2002).

EDTA is a low toxicity multidendate chelating agent, and is able to form stable complexes with a wide variety of metals (Khan et al., 2000). When present in water percolating through polluted soils, EDTA is capable of solubilizing heavy metals into a soil solution. However, the mobilization process can increase the migration of heavy metals downwards into groundwater, causing further environmental pollution in the surrounding areas (Kedziorek et al., 1998; Barona et al., 2001; Römkens et al., 2002). Methods to prevent the leaching of mobilized heavy metals down the soil profile should be considered in the phytoremediation design, including the optimum chelate concentration, time and locations of the chelate application to soils. Apart from the soil amendment approaches, the root systems of plants may also help to reduce metal leaching in soil profiles.

Recently, many high-biomass plants have been used in phytoremediation studies. Among them the Brassicaceae (mustard) family, to which most metal-hyperaccumulator species belong, represents a potential and promising source of plants to be used in phytoremediation. Other high biomass plants, such as corn, peas, sunflowers, ragweeds, and goldenrods have also been successfully used in chelate-assisted phytoextraction studies (Blaylock et al., 1997; Chen and Cutright, 2001). However, the root systems of these plants are mainly located in the top 5 - 20 cm layers of soil and very few root systems can penetrate to deeper soil layers. Therefore, the root systems of these plants are unable to absorb the heavy metals that may possibly have leached in soil profiles.

Vetiver grass (*Vetiveria zizanioides*) is a tall (1 – 2 m), fast-growing, perennial tussock grass. It has a long (3 - 4 m), massive and complex root system, which can penetrate to the deeper layers of the soil (Dalton et al., 1996; Truong, 2000; Pichai et al., 2001). Vetiver grass is native to south and south-east Asia where it has been grown for centuries for roof thatching, fodder for livestock, and perfumery and cosmetic industries. Vetiver was first used for soil conservation and land stabilization purposes in Fiji in the early 1950s, and promoted by the World Bank for soil and water conservation in India in the 1980s (Dalton et al., 1996). Its root aromatic oil can be used as repellent for Formosan subterranean termite and represent a promising natural alternative for the control of this invasive pest (Zhu et al., 2001). It is likely that vetiver grass technology will become one of the leading biological systems of soil and water conservation, land rehabilitation, and embankment stabilization in the 21st century (Grimshaw, 1997; Truong, 2000).

Owing to its unique morphological, physiological and ecological characteristics such as its massive and deep root system, and its tolerance to a wide range of adverse climatic and edaphic conditions, including elevated levels of heavy metals， the interest in this grass is increasing in recent years (Pinthong et al., 1998; Truong, 2000; Chen et al., 2000). However, the use of vetiver grass in phytoremediation technology is not widely recognized owing to lacking of detailed investigation of its capacity in absorbing contaminants and practical field application.

The objectives of the present study were (1) to investigate the metal uptake ability of the roots and shoots of vetiver in different Pb-contaminated soils to which EDTA has been applied; (2) to study the leaching behaviors of heavy metals in soil under EDTA application; (3) to assess the ability of vetiver to retain heavy metals in soil columns.

2. Materials and Methods

2.1. Experiment 1: EDTA effects on vetiver uptake of Pb

Soil preparation

Soil was collected from the 0 - 30 cm surface layer of a vegetable garden in a suburban area of Nanjing, China. The soil was air-dried, crushed to pass through a 4 mm diameter sieve, and mixed thoroughly. The chemical and physical properties of the soils are presented in Table 1. The electrical conductivity (EC) of the soil was measured using a conductivity meter on the soil extract obtained by shaking soil with double distilled water at 1:2 (w/v) soil:water ratio. The soil’s pH was measured by 0.01 mol $CaCl_2$ at 1:5 ratio (w/v) using a pH meter. The cation exchangeable capacity (CEC) of the soil was determined using the ammonium acetate saturation method. The soil texture, organic matter content, total N and field capacity were measured by the procedures described by Avery and Bascomb (1982). The total metal concentrations were determined by ICP-AES (Perkin-Elmer Optima 3300 DV) after strong acid digestion (1:4 concentrated HNO_3 and $HClO_4$ (v/v)) (Li et al., 2001).

Lead was added at a rate of 0, 500, 2500, 5000 mg Pb kg^{-1} dry soil as an aqueous solution of $Pb(NO_3)_2$ for four different soil treatments (each treatment of soil was about 100 kg). Basal fertilizers applied to the soil were 80 mg P kg^{-1} dry soil, and 100 mg K kg^{-1} dry soil as KH_2PO_4 (Shen et al., 2002). Additional N was added to the amended 0, 500, 2500 mg kg^{-1} Pb of treatment soils, up to 676 mg kg^{-1} dry soil as $NH_4NO_{3,}$ which was equal to the application of N to the 5000 mg kg^{-1} Pb of treatment soil due to the application of more $Pb(NO_3)_2$. Each treatment soil was air-dried for one week before use in the pot experiment.

Planting vetiver grass

The soil samples (5 kg) were placed in plastic pots (15 cm diameter x 25 cm height). The moisture level of the soil was maintained to near field water capacity (35.6%) and equilibrated for two weeks. The seedlings of vetiver grass (*Vetiveria zizanioides*) were selected and pruned (the shoots were originally 20 cm high and the roots 8 cm long), and then transplanted into the pots in August 2001. The pots were watered daily to 60% of the field water capacity.

EDTA treatments

Five weeks after the seedlings were transplanted in the pots, EDTA was applied to the surface of the soil in the pots at rates of 0, 0.5, 2.5, 5.0 mmol EDTA kg^{-1} of soil in one single dose as a 400 ml Na_2-EDTA solution. Following the application of EDTA, the soil was irrigated to 60% of field water capacity on a daily basis. All of the pot experiments were conducted in a greenhouse under natural light conditions. Air temperature ranged from 18 - 36ºC. Each soil and plant treatment was replicated four times, and the pots were in a completely random block design.

Plant sampling and analysis

On the fourteenth day after the application of EDTA, the vetiver shoots (above 20 cm from the node) were cut, washed with tap water and then with D.I.W. The vetiver seedlings were carefully moved from the pots, washed in tap water, and the root samples (below 8 cm from the node) were cut (owing to remain living seedlings for reproduction). The snipped roots were gently washed using brush pen for removing remained soil particles, then soaked in 0.01 mol/L $CaCl_2$ for 30 min. and subsequently washed with D.I.W. for three times. The shoot and root samples were dried at 70ºC for 72 h, and then were milled in a coffee mill (Philips HR 2185). The plant materials were acid-digested with a mixture of $HNO_3/HClO_4$ (4:1 v/v) for elemental analysis with ICP-AES (Li and Thornton, 1993).

A certified standard reference material (SRM 1515) of the National Institute of Standards and Technology, USA, was used in the analysis as part of the QA/QC protocol. Reagent blanks and analytical duplicates were also used where appropriate to ensure the accuracy and precision of the analysis. The recovery rate was 93±8% for Pb in the plant reference material. All results were expressed on a dry weight basis.

2.2. Experiment 2: EDTA effects on the mobility of heavy metals in soils

The artificial rainwater preparation

The artificial rainwater composition (mg/L) used in the 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; and pH: 4.40. (Hodson et al., 2001).

Soil preparation

Soil samples were collected from a disused agricultural field in the area of Yuen Long in Hong Kong. The samples were sieved to pass through a 2 mm sieve and air-dried for three days. The chemical and physical properties of the soil are given in Table 1. The soils were artificially contaminated with Pb (2500 mg kg^{-1} of soil) as $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (lead sulfide - galena) at a Pb concentration ratio of 1:1; Cu (500 mg kg^{-1} of soil) as $CuCO_3$ (copper carbonate); Zn (1000 mg kg^{-1} of soil) as $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulfide) at a Zn concentration ratio of 1:1; and Cd (15 mg kg^{-1} of soil) with $Cd(NO_3)_2 \cdot 4H_2O$ (cadmium nitrate). After adding heavy metals, the soils were equilibrated for fifteen days, undergoing five cycles of saturation with de-ionized water and air-drying processes.

The soil leaching column preparation

The air-dried soil (1370g) was packed in four leaching columns (9.0-cm diameter and 20-cm height), and the soil depth in each column was about 18 cm. Each of the four soil columns was planted with one single oil-sunflower (*Helianthus annuus L.*) seedling for fifty days under outdoor conditions with air temperatures ranging from 14 - 24°C, and was protected against natural precipitation by a roof. The soil columns were watered with de-ionized water if necessary.

The heavy metal leaching process

On the fiftieth day after the sunflowers were growing in the columns, 5.0 mmol EDTA kg^{-1} of soil was applied to the soil surface. Three days after the application of EDTA, the four columns were transferred indoors and saturated with a small amount of artificial rainwater, then flushed using a large amount of rainwater. The leached solutions were collected at the bottom of each column for every 200 ml of the rain water application in four intervals. The first 200 ml of leachate from the four columns were mixed together and the total volume was 800 ml (referred to as Solution 1). The second to fourth leaching solutions from the four columns, referred to as Solution 2 - 4, were mixed subsequently according to the order of leaching.

2.3. Experiment 3: Vetiver grass on heavy metal retention in soil columns

Soil column preparation

Original soil samples from Experiment 2 were used in this experiment. Each of the five polyethylene leaching columns (9.0-cm inner diameter, 60-cm height) was packed in layers, as follows: (from bottom to top): a) 2-cm layer of acid-washed quartz sand (< 2 cm); b) a circle of fiberglass (2 mm), c) 4900 g of air-dried soil (8.7% water content) equivalent to a layer of 56 cm in the column. Three seedlings of vetiver grass (the root length was about 20 cm) were transplanted into three of the soil columns (Column No. 1, No. 2 and No. 3). Another two columns (Column No. 4 and No. 5) without vetiver grass were used as the control group. The soil columns were exposed to outdoor conditions with air temperatures ranging from 14 - 26ºC, and protected against natural precipitation using a roof. The soil columns with vetiver grass were watered with artificial rainwater if necessary. On the forty-fifth day after the vetiver grass was transplanted, the five columns were flushed with artificial rainwater.

Rainfall leaching experiments

On the sixtieth day after the vetiver grass was transplanted, the first 800 ml of heavy metal leachate solution (Solution 1 obtained from Experiment 2) were applied to the four soil columns (No. 1, No. 2, No. 4 and No. 5), which was equal to 31.4 mm of rainfall per column. Subsequently, other heavy metal leachate solutions (Solution 2 - 4 from Experiment 2) were added to these columns at two-day intervals. As for soil column No.3, artificial rainwater was added instead at the same volume. After two days, the artificial rainwater was applied to each column at 55 mm precipitation every day for eleven days (equal to 350 ml of rainwater applied to each column per day).

The leachate solutions were collected at the bottom of the soil columns and measured for volume on a daily basis. A sub-sample of the leachate solutions was filtered through Whatman No. 42 filter paper, digested with concentrated HNO_3, then diluted with 5% HNO_3 and analysed for heavy metals by ICP-AES. During the leaching experiment, the vetiver grass shoots (in columns No. 1, 2 and 3) were sampled and analysed for element concentration by ICP-AES, and the vetiver roots were collected and analysed at a later stage, as previously described. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® statistical software.

3. Results

3.1. Uptake of Pb by Vetiver in Pot Experiment

In the pot experiment, the vetiver grass grew well, and there were no visual signs of phytotoxicity in any of the treatments during the first six weeks after transplanting. After the application of EDTA (0.5, 2.5 and 5.0 mmol kg^{-1} of soil), vetiver seedlings under all different treatments showed no adverse effects, such as wilting, changing colour and necrosis for fourteen days. The results showed that vetiver is able to grow in highly contaminated soil, even after EDTA application.

Table 2 depicts the Pb uptake patterns of the vetiver grown in the 500, 2500 and 5000 mg kg^{-1} Pb amended soils under different EDTA application programmes. In the control group without EDTA treatment, the shoot and root Pb concentrations increased significantly with increasing Pb concentration in the soils, in the shoot from 0.82 to 43.0 mg kg^{-1} of dry weight, and in the root from 60 to 556 mg kg^{-1} DW in the soils. Pb in the roots accounted for the majority of the total Pb in the plant. The translocation of Pb from roots to shoots was low (see Table 2).

The application of EDTA to the soils resulted in a surge of Pb concentrations in the shoots and roots of vetiver (Table 2). For example, in the 5000 mg kg^{-1} Pb of treatment soil, the shoot Pb concentrations reached 69, 127, 243 mg kg^{-1} after 0.5, 2.5, 5.0 mmol kg^{-1} of EDTA was applied, compared with 43 mg kg^{-1} Pb without the EDTA treatment. The root Pb concentrations were 871, 1440, 2280 mg kg^{-1} after the application of 0.5, 2.5, 5.0 mmol kg^{-1} of EDTA, compared with 556 mg kg^{-1} Pb without the EDTA application. The translocation ratio of Pb from roots to shoots grew as larger amounts of EDTA were applied (see Table 2). Similar patterns were obtained in other soil treatments (500 and 2500 mg kg^{-1}). These results indicated that the application of EDTA increased the Pb accumulation of vetiver roots from the rhizosphere of the soil, and also facilitated Pb translocation from roots to shoots.

3.2. The soil leaching column experiment

In the soil leaching columns, the leaves of oil sunflowers showed slight etiolation before the EDTA application. Some visual necro-maculae were observed in the old leaves, accounting for nearly 5% of the whole leaves. Two days after a 5.0 mmol EDTA kg^{-1} soil application, there were distinct phytotoxicity symptoms in the sunflower seedlings. The amounts and area of necro-macula in leaves increased significantly. On the third day after the application of EDTA, the area of dead macula accounted for approximately 25% of the whole leaves, and some leaves were crinkled and withered at the tips.

The concentrations of heavy metals in the leachate solutions from the soil columns are presented in Table 3. The largest amount of leaching of heavy metals from the soil columns occurred in the first 1600 ml of the leaching solution, which was equivalent to about 63 mm of rainfall. After this, the leached heavy metals decreased sharply, and nearly reached a plateau at the 94 mm rainfall level (see Table 3).

The total amounts of heavy metals leached from the soil columns are listed in Table 4. The results showed the effects of EDTA on the mobility of heavy metals after artificial rainwater irrigation (equivalent to 126 mm of precipitation). About 3.73%, 15.6%, 14.3% and 22.2% of Pb, Cu, Zn and Cd were leached from the soil columns (see Table 4). The most mobile metal was Cd, followed by Cu and Zn. Pb was the metal that was leached least from the soils.

3.3. The retention of heavy metals by vetiver in soil columns

In the soil columns, vetiver grass grew rapidly. Before the application of the heavy metals leachate solution (obtained from Experiment 2), the roots of vetiver grass had nearly penetrated to the bottom of the soil column (56 cm). Compared with the initial root length, the roots elongated about 36 cm in sixty days.

After the initial application of 200 ml of the heavy metals leachate solution to the surface of the soil columns, leachates solutions could be collected at the bottom of Column No. 4 and No. 5, where vetiver had not been planted (see Table 5). A further 600 ml of heavy metal solutions were applied to the columns in the next six days. Approximately 500 ml of leachate samples were collected from Column No. 4 and 5, respectively. However, there was no leachate in Columns No.1 and 2, which had been planted with vetiver, even after the application of 800 ml of heavy metal solution. This showed that vetiver could effectively adsorb soil water (leachate solution) in the soil profile probably by its transpiration and root system.

The different patterns of Pb, Cu, Zn and Cd leaching behaviors from the soil columns are shown in Fig. 1 - 4. Heavy metals leached from the soil columns without plants were much higher than from those planted with vetiver. Most of the leached heavy metals reached a plateau when rain precipitation approached about 500 mm (see Fig. 1 - 4). In the no-plant soil columns, about 38%, 78%, 81% and 61% of the initial Pb, Cu, Zn and Cd were leached from the soil profile (see Table 6). In the planted vetiver soil columns, however, the leaching of heavy metals was much lower, only accounting for 2% of Pb, 46% of Cu, 59% of Zn and 12% of Cd, respectively. The results indicated that the vetiver was capable of trapping the leached heavy metals from upper soil layers, especially for Pb and Cd.

The concentrations of Pb, Cu, Zn and Cd in the shoots of vetiver grass grown in the soil columns are shown in Table 7. The concentrations of heavy metals except Pb in vetiver shoots increased with the application of a heavy metals leachate solution (Columns 1 and 2). The results showed that the additional heavy metals (Cu, Zn and Cd) in the leachate solutions could be adsorbed by the roots of vetiver grass and be transported to the shoots. The shoot Cd concentration in the soil columns with leachate application was about four-fold of that of the control. There was no significant difference in Pb concentration in the shoots between the heavy metal leachate solution application and the control group (Table 7). This may be due to the low mobility and bioavailability of Pb in the soil columns.

4. Discussion

The shoot metal concentration of plants can partially reflect the efficiency of plants on the remediation of soil heavy metals. Thus, the ratio of shoot metal concentration to total soil metal concentration can also partially reflect the ability of plants to absorb soil heavy metals and transport them to shoots. Such the ratio was proposed by Baker et al. (1994) and Dahmani-Muller et al. (2001). Baker et al. (1994) defined the accumulation factor by dividing shoot concentration to total soil metal concentration, and Dahmani-Muller et al. (2001) used the term of bioaccumulation factor (BF) defined metal concentration in shoots versus initial metal concentration in substrates. Without the application of EDTA, the ratio of shoot Pb to total soil Pb of vetiver grass ranged from 0.002~0.009 (see Table 8), which was smaller than that of *Zea mays* (0.016~0.09) (Huang and Cunningham, 1996), *Brassica juncea* (0.025~0.055) (Huang and Cunningham 1996; Blaylock et al., 1997), *Pisum sativum* (0.02) (Huang et al., 1997) and *Thlaspi caerulescens* (0.003~0.023) (Baker et al. 1994, Huang and Cunningham 1996) used in the phytoremediation of lead contaminated soils. The results showed that the uptake of Pb by vetiver shoots was low under normal soil conditions.

According to the results of Kabata-Pendias and Pendias (1992), Pb concentrations in mature leaf tissue higher than 30 ppm (DW) are considered excessive or toxic to plants. However, in our study, the Pb concentrations in the shoots and roots of vetiver were up to 243 and 2278 mg kg^{-1} DW, respectively. These values are the highest shoot and root Pb concentrations reported in the literature for vetiver. However, the vetiver grown in highly contaminated soils had no perceived phyto-toxicity symptoms, indicating that they could tolerate the high Pb in soils and in their tissues. Furthermore, vetiver is also able to tolerate a variety of pollutants in soil and water (Pinthong et al., 1998; Truong, 2000). The threshold of toxic metals in soils (mg/kg) to vetiver are As (100 - 250), Cd (20 - 60), Cr (200 - 600), Ni (347), Cu (50 - 100), Hg(> 0.12), Se (> 74) and Zn (> 750). The threshold of toxic metals in vetiver shoot (mg/kg) are As (21 - 72), Cd (45 - 48), Cr (5 - 18), Ni (> 100), Cu (13 - 15), Hg(> 6), Se (> 11) and Zn (880) (Truong, 2000). Therefore, this specific ecotype would be useful specially for remedying quite highly contaminated soils， such as mining, tailing as well as quarry where most other plants can not survival due to the hostile growing conditions, including toxic levels of heavy metals. In Australia, vetiver is highly successful in the rehabilitation of old quarries and mines and is able to stabilize the erodible surface first so other species can colonize the areas later (Truong, 2000).

In the EDTA-assisted phytoextraction experiments, the total amount of heavy metals absorbed by shoots and roots only accounted for a very small proportion of the labile metal pool in soils (Chen et al., 2002; Wenzel et al., 2002). The wet weather conditions in some areas will facilitate possible seepage, and potential heavy metal migration along the soil profiles would be of concern. The leached heavy metals from the upper soil layers were partially presented in metal-EDTA complexes, which can be validated by geochemical speciation of the leachates using MINTEQA2 (Allison et al., 1991), and by the batch and column leaching test (Sun et al., 2001). Because EDTA is a nonselective complexing agent, the competition for EDTA by other elements, such as Fe, Mn, Ca, and Mg in the soils, might influence the Pb-, Cu-, Zn-, and Cd-EDTA complexes. The formation constant (log K) of EDTA for Fe(III), Cu, Pb, Zn, Cd, Mn, Ca are 26.5, 19.7, 19.0, 17.5, 17.4, 14.8, and 11.6, respectively (Norvell, 1991). The Pb, Cu, Zn and Cd may be released from the complexes with EDTA, and subsequently re-adsorbed into the soil compartment.

Soil matrix is an excellent adsorbent for both organic and inorganic chemical compounds. The physical and chemical properties of soils determine the extent to which the soil retards downward migration of contaminants. The major adsorption surfaces for heavy metals in soils are clay particles and organic matter (Elzahabi and Yong, 2001). The retention of leached heavy metals in soils is often well correlated with how much organic matter is contained in the soil. The organic component of the soil constituents has a high affinity for heavy metals because of the presence of ligands or carboxyl, phenolic, alcoholic and carbonyl compounds which can form chelates with metals (Yong et al., 1992). Heavy metal retention has also been found to generally increase with increases in pH, CEC, clay content, and the metal oxide content of the soil (Cline and Reed, 1995). The soil's redox potential and salinity, and the iron and manganese oxides contained in it should also be taken into consideration (Tam and Wong, 1996).

In our heavy metal retention study (Experiment 3), the total Pb, Cu, Zn and Cd retained by the soil columns (with planted vetiver) were 120, 72, 101 and 3.08 mg, respectively, according to the amounts of heavy metals added initially and the amount of metals in the leachate solutions (see Table 6). The quantities of Pb, Cu, Zn and Cd in the vetiver shoots were approximately 0.16, 0.53, 2.63 and 0.05 mg, respectively, calculated from the total shoot biomass of 45 g DW. The concentrations of Pb, Cu, Zn and Cd in vetiver roots were 5.28, 133, 59.5, and 0.20 mg/kg, respectively. Based on the data, the possible quantities of heavy metals absorbed by vetiver roots were estimated to be 0.16 mg for Pb, 3.96 mg for Cu, 1.77 mg for Zn and 0.006 mg for Cd (according to a root/shoot biomass ratio of about 0.66 in the pot experiments). In comparison with the metals retained in the soil columns, the amounts of heavy metals adsorbed by the vetiver roots and shoots were relatively small. Therefore, a large amount of heavy metals (approximately 99.7% of Pb, 93.8% of Cu, 95.6% of Zn and 98.2% of

Cd) in the leachate solutions were absorbed by the soil matrixes under the influence of the vetiver growth in the soil columns.

The decrease of the soil moisture content is also very important for the adsorption of heavy metals in soils because heavy metals are usually transported in solution. This was supported by the results from the soil leaching column experiment of the present study. The average evaporation of the soil columns with vetiver growth was about 100 ml per day compared with 28 ml for the columns without vetiver, based on the estimation from the additional rainwater volume and the leachate solution collected (see Table 5). Vetiver could absorb heavy metals leached from the upper soil layers in its roots and shoots. More importantly, it was able to significantly decrease the water content of the soil by its high transpiration rate. This process may play an important role in immobilizing the heavy metals in soil matrixes in our soil column experiment. In the soil leaching columns planted with vetiver, the highest Pb concentrations in the leachate solution collected at the bottom (56 cm) was less than 3.0 mg L^{-1}; this is below the TCLP regulatory limitation (5 mg Pb L^{-1}) for stabilised hazardous waste. As for Cu, Zn and Cd, if the net vertical percolation of rainwater was lower than 300 mm (see Fig. 1 - 4), then very little metals were leached out from the soil columns.

Vetiver grass is a high-biomass plant with a high C4 photosynthetic efficiency (Mucciarelli et al., 1998), the average dry matter yield is 99 t/ha/year in southeast of China (Zhang, 1998). Owing to its' high tolerance to heavy metals, there is a great potential to use this plant in phytoextraction strategy although vetiver is not a hyper-accumulator. Chen et al. (2000) found that the total above ground uptake of Cd by vetiver was even greater than that of the hyperaccumulator *T. caerulescens* owing to the former's high biomass. Moreover, vetiver grows very fast with a fine, deep and penetrating root system, which is capable to reach down to 3 - 4 m in the first year (Truong, 2000), and the root yield is about from 1500 to 2400 kg/ha after 16 or 20 months transplant (Dethier et al., 1997). The cultivar of vetiver, widely utilized in bio-engineering purposes, is generally sterile (will not become a weed) and easily asexually reproduced using simple agricultural practices (by root subdivision, each slip normally consists of 2-3 tillers). If other agricultural practices can be adopted, such as intercropping with other high biomass plants (e.g., mustard, corn and sunflower) or genetic engineering approaches (transfering the genes of a hyperaccumulator to vetiver), the vetiver grass may become a very competitive plant in phytoremediation in the near future.

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Table 1

The physicochemical properties of the soils used in the study

Physicochemical Properties	Soils used in the pot experiments	Soils used in soil columns
pH ($CaCl_2$)	7.02	6.04
Electrical conductivity at 25°C ($\mu S\ cm^{-1}$)	292	282
Sand (%) >0.05 mm	34.5	54.3
Silt (%) 0.05-0.001 mm	45.1	31.1
Clay (%) <0.001 mm	20.4	14.6
N_{TOTAL} (%)	0.11	0.10
Organic matter (%)	2.68	1.67
Cation Exchange Capacity (cmol kg^{-1})	9.26	3.29
Field water capacity (%)	35.6	27.4
Background total metal content (mg kg^{-1})		
Pb	86.7	44.2
Cu	91.1	26.7
Zn	185	131
Cd	1.79	0.45

Table 2

Lead concentrations in the shoots and roots of vetiver and the translocation ratios (TR*) of Pb from roots to shoots at the 14th day after EDTA application in the pot experiment.

	Pb amendment soils (mg kg^{-1})	The EDTA application concentration (mmol kg^{-1} soil)			
		0	0.5	2.5	5.0
shoot	0	0.81±0.06	----	----	3.59±0.42
	500	0.82±0.12 a A	6.06±0.37 b A	25.7±3.23 c B	42.2±4.09 d C
	2500	6.52±0.17 a A	21.3±3.63 a A	86.3±13.5 b B	160±28.1 c C
	5000	43.0±0.71 a A	68.6±4.76 b B	127±7.7 c C	243±13.5 d D
Root	0	4.16±0.42	----	----	15.6±4.13
	500	60.3±5.08 a A	83.5±7.75 a A	200±22.5 b B	266±43.0 c B
	2500	205.8±21.8 a A	242±29.6 a A	464±50.3 b B	951±126 c C
	5000	556±28.4 a A	871±82.8 a AB	1440±303 b B	2280±462 c C
TR*	0	19.7±3.0	----	----	23.4±2.5
	500	1.35±0.06 a A	7.37±1.30 ab AB	13.9±5.3 bc B	17.2±2.0 c B
	2500	3.18±0.29 a A	8.76±0.91 b B	18.9±0.2 c C	18.0±1.0 c C
	5000	7.73±0.37 a A	7.88±0.14 a A	9.48±2.98 a A	11.4±3.8 a A

Note: Mean and standard deviation (n = 3~4); *TR: defined as the percent of shoot Pb concentration versus root Pb concentration; The different capital and small letters stand for significance at 0.01 and 0.05 levels, respectively,

Table 3
Heavy metal concentrations in the leachate solutions from the soil columns

Heavy metals solution		Heavy metals concentrations (mg/L)			
Code	Volume (ml)	Pb	Cu	Zn	Cd
Solution 1	800 (31.4)*	285	271	533	7.48
Solution 2	800 (31.4)	262	294	518	7.62
Solution 3	800 (31.4)	48.9	78.9	138	1.82
Solution 4	800 (31.4)	15.9	25.0	45.8	0.61

*: Figures in the brackets are the estimated amounts of rainwater precipitation (mm). The evaporation of soil columns was not considered because the leaching was carried out indoors for a short period of time (no more than two days). The leachate solution volume was nearly equal to the added rainwater volume. The precipitation (mm) was estimated according to the formula ($V=\pi r^2 h$).

Table 4

EDTA effects on the leachability of heavy metals in the soil columns after the percolation of artificial rainwater

Heavy metals	Initial heavy metal content in the soil*(mg)	Heavy metal content in the leachate (mg)#	Percentage leaching (%)
Pb	13100	490	3.73
Cu	3440	535	15.6
Zn	6900	988	14.3
Cd	63.2	14.0	22.2

*The total initial amount (mg) of heavy metal in the soils of the four soil columns before the application of EDTA.

#The total amount (mg) of heavy metals leaching from the four soil columns after the application of EDTA and 800 ml of rainwater.

Table 5

The leachate solution volume collected under the five soil columns (ml)

Treatment times	Added solution volume (ml)	Leachate Solution Volume (ml)				
		No.1*	No.2	No.3	No.4	No.5
Day 1	200 (31.4)#	0	0	0	23	38
Day 3	200 (31.4)	0	0	0	164	171
Day 5	200 (31.4)	0	0	0	153	159
Day 7	200 (31.4)	0	0	0	168	169
Day 9	350 (55.0)	0	27	24	304	310
Day 10	350 (55.0)	197	174	202	318	321
Day 11	350 (55.0)	219	268	244	339	346
Day 12	350 (55.0)	212	282	216	317	321
Day 13	350 (55.0)	222	294	266	325	331
Day 14	350 (55.0)	158	207	165	303	312
Day 15	350 (55.0)	299	284	288	333	327
Day 16	350 (55.0)	224	246	245	308	328
Day 17	350 (55.0)	283	279	304	322	335
Day 18	350 (55.0)	282	255	241	312	320
Day 19	350 (55.0)	256	275	269	329	338
Sum	4650 (731)	2350	2590	2470	4020	4130

*.The different treatment of soil columns (No.1 and No.2: planted vetiver grass and applied a heavy metals leachate solution; No.3: planted vetiver grass without heavy metal leachate solution application; No.4 and No.5 without vetiver plant and with heavy metal leachate solution application).

#. Figures in the brackets stand for the estimated rainwater precipitation (mm).

Table 6

The retention of heavy metals in the five long soil columns

Heavy metals	The amount of metal (mg)	The amount of leached metals (mg)				
		No.1*	No.2	No.3	No.4	No.5
Pb	122	2.19 (1.79%)#	2.41 (1.97%)	0.01	44.8 (36.6%)	49.3 (40.3%)
Cu	134	59.8 (44.6%)	63.3 (47.3%)	0.05	99.4 (74.3%)	109 (81.3%)
Zn	247	138 (55.7%)	155 (62.6%)	0.11	191 (77.2%)	208 (84.3%)
Cd	3.51	0.35 (9.9%)	0.52 (14.9%)	0.01	2.15 (61.3%)	2.12 (60.5%)

*.The different treatment of soil columns (No.1 and No.2: planted vetiver grass and applied a heavy metals leachate solution; No.3: planted vetiver grass without heavy metal leachate solution application; No.4 and No.5 without vetiver plant and with heavy metal leachate solution application).

#. Figures in the brackets stand for the estimated rainwater precipitation (mm).

Table 7

Heavy metal concentrations in the vetiver shoots grown in the long soil columns (mg kg^{-1} DW)

Heavy metals	The day after leaching began	Heavy metals concentrations		
		No.1*	No.2	No.3
Pb	0	2.25	4.16	3.49
	9	3.76	2.95	3.01
	16	3.10	3.77	3.16
Cu	0	5.91	5.86	6.94
	9	9.54	11.5	8.44
	16	12.7	10.7	7.35
Zn	0	28.7	32.0	33.9
	9	48.5	57.8	43.7
	16	54.4	62.6	49.9
Cd	0	0.31	0.34	0.43
	9	0.72	0.92	0.38
	16	1.11	1.16	0.33

*.The different treatment of soil columns (No.1 and No.2: planted vetiver grass and applied a heavy metals leachate solution; No.3: planted vetiver grass without heavy metal leachate solution application).

Table 8

The ratio of shoot Pb concentration to total soil Pb concentration in some plant species

Plant species	Pb-contaminated soils	Total soil Pb concentration (mg /kg)	Shoot Pb concentration (mg/kg)	The ratio of shoot Pb to total soil Pb	Time period of treatment	Reference
Thlaspi caerulescens	Metalliferous sites	90300	662	0.007	Plant sample from field	Baker et al., 1994
		11860	203	0.017		
		51600	166	0.003		
		6920	57	0.008		
		15500	222	0.014		
Zea mays L. cv. Fiesta	Pb-contaminated soil from an industrial site	2500	225	0.09	28 days	Huang and Cunning-ham, 1996
Brassica juncea (211000)			129	0.052		
Thlaspi rotundifolium			79	0.032		
Triticum aestivum (cv. Scout 66)			120	0.048		
Thlaspi caerulescens			58	0.023		
Brassica juncea (L.) Czern.			45	0.018		
Zea mays L.			40	0.016	7 days	
Brassica juncea	Artificially contaminated $PbCO_3$	600	15	0.025	7 days	Blaylock et al., 1997
		900	38	0.042		
		1200	45	0.037		
		1800	100	0.055		
Pisum sativum L	Pb-contaminated soil from an industrial site	2500	50	0.02	7 days	Huang et al., 1997
Vetiver grass (*Vetiveria zizanioides*)	Soil supplied with $Pb(NO_3)_2$	86.7	0.81	0.009	14 days	This Study
		439	0.82	0.002		
		2030	6.52	0.003		
		5090	42.3	0.008		

Figure Captions

Fig. 1. The leaching patterns of Pb from the soil columns.

Fig. 2. The leaching patterns of Cu from the soil columns.

Fig. 3. The leaching patterns of Zn from the soil columns.

Fig. 4. The leaching patterns of Cd from the soil columns.