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Phytoremediation of Lead-Contaminated Soils: Role of Synthetic Chelates in Lead Phytoextraction

JIANWEI W. HUANG,* JIANJUN CHEN, WILLIAM R. BERTI, AND SCOTT D. CUNNINGHAM

DuPont Central Research and Development, Environmental Biotechnology, GBC 301, Newark, Delaware 19713

Lead phytoextraction, using plants to extract Pb from contaminated soils, is an emerging technology. Calculations of soil Pb mass balance suggest that this technology will be economically feasible only if systems can be developed to employ high biomass plants that can accumulate greater than 1% Pb in their shoots. In this study, we investigated the potential of adding chelates to Pb-contaminated soils to increase Pb accumulation in plants. The addition of chelates to a Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}) increased shoot Pb concentrations of corn (*Zea mays* L. cv. Fiesta) and pea (*Pisum sativum* L. cv. Sparkle) from less than 500 mg kg^{-1} to more than 10000 mg kg^{-1}. The surge of Pb accumulation in these plants was associated with the surge of Pb level in the soil solution due to the addition of chelates to the soil. For the chelates tested, the order of the effectiveness in increasing Pb desorption from the soil was EDTA > HEDTA > DTPA > EGTA >EDDHA. We also found that EDTA significantly increased Pb translocation from roots to shoots. Within 24 h after applying EDTA solution [1.0 g of EDTA (kg soil)$^{-1}$] to the contaminated soil, Pb concentration in the corn xylem sap increased 140-fold, and net Pb translocation from roots to shoots increased 120-fold as compared to the control (no EDTA). These results indicate that chelates enhanced Pb desorption from soil to soil solution, facilitated Pb transport into the xylem, and increased Pb translocation from roots to shoots. Results from this study suggest that with careful management, chelate-assisted Pb phytoextraction may provide a cost-effective soil decontamination strategy.

Introduction

Heavy metal contamination of surface soils is prevalent at many industrial and mining sites throughout the world. Lead is considered one of the most frequently encountered heavy metals of environmental concern and is the subject of much remediation research (*1*). Severe Pb contamination in soils may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and Pb toxicity in plants, animals, and humans (*2*−*4*). In addition to industrial and mining activities, elevated soil Pb levels have also occurred due to the use of Pb in paints, gasoline, explosives, and anti-spark linings as well as from the disposal of municipal sewage sludges enriched in Pb (*5*−*7*). The remediation of Pb-contaminated soils represents a significant expense to many industries and governmental agencies (*8*, *9*).

Over the last 10 years there has been increasing interest in developing a plant-based technology (phytoremediation) to remediate heavy metal-contaminated soils (*1*, *10*−*13*). Phytoremediation of Pb-contaminated soils encompasses two different strategies: phytostabilization and phytoextraction (*8*, *9*, *14*). Phytostabilization is the use of plants and soil amendments to reduce the intrinsic hazard of Pb-contaminated soil by reducing Pb bioavailability in the soil (*8*). Phytoextraction is the use of plants to remove Pb from contaminated soils. Through the continued cultivation of selected plant species on Pb-contaminated sites, the soils could eventually be decontaminated. Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation, phytoextraction may provide an attractive alternative for the cleanup of Pb-contaminated soils.

The goal of Pb phytoextraction is to reduce Pb levels in a soil to acceptable levels within a reasonable time frame (3−20 years). The timing is site specific and depends on initial and final soil−Pb concentrations and species, future land use, and the degree of risk that the site might pose to human health and the environment. Our previous calculations of soil Pb mass balance indicate that, to achieve this goal, we need to use plant species/cultivars that are able to accumulate greater than 1% Pb in shoots and produce more than 20 t of shoot biomass ha^{-1} $year^{-1}$ (*15*). Originally we, as well as most others in the field, believed that we would need to develop plants with Pb accumulation characteristics similar to reported Pb hyperaccumulating plants (*16*, *17*). An example of such plants is *Thlaspi rotundifolium*, which is reported to be able to accumulate shoot Pb concentrations of 130−8200 mg kg^{-1} with a mean of 1100 mg kg^{-1} (*16*). However, this plant species, like other Pb-hyperaccumulating species reported in the literature, is not suited for phytoextraction of Pb from contaminated soils due to its slow growth and small biomass. Since there are no reports of Pb-hyperaccumulating plants of high biomass, we conducted an extensive screening program to examine physiological aspects of Pb accumulation in more than 50 plant species/cultivars when cultivated on Pb-contaminated soils. We have found that some agronomic crops, such as corn and pea, can accumulate a shoot Pb concentration greater than that of *T. rotundifolium* (*15*, *18*). Despite all our efforts at managing agronomic parameters (soil pH, nutrient status, irrigation, and plant density), these crops could rarely accumulate shoot Pb concentrations greater than 1000 mg kg^{-1} when grown in untreated Pb-contaminated soils (*15*). This is far short of our 10 000 mg kg^{-1} target for Pb phytoextraction.

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 (*15*, *19*, *20*). For most Pb-contaminated soils that we have studied, Pb in soil solution is usually less than 0.1% of total soil Pb. Thus, the Pb availability to plants is limited. Furthermore, for plants grown in these soils, Pb translocation from roots to shoots was less than 30% for the best Pb translocating plants (*15*). Two major limitations to the Pb phytoextraction are the low Pb bioavailability in soil and the poor Pb translocation from roots to shoots. A key to Pb phytoextraction is to increase and maintain Pb concentrations in the soil solution. Chelates have been used in soils and nutrient solutions to increase the solubility of metal cations in plant growth media and are reported to have significant effects on metal accumulation in plants (*21*−*24*).

* Address correspondence to this author at his present address: Phytotech Inc., 1 Deer Park Drive, Suite I, Monmouth Junction, NJ 08852. Fax: 908-438-1209, e-mail: JHPHYTO@AOL.COM.

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TABLE 1. Physical and Chemical Characteristics of Pb-Contaminated Soil Used in This Study

soil properties	Pb-contaminated soil
sand (%)	70.0 ± 5.0
silt (%)	17.5 ± 0.7
clay (%)	12.5 ± 0.6
soil texture	sandy loam
organic mater content (%)	3.5 ± 0.2
Soil pH (1:1 soil/water ratio)	5.5 ± 0.1
CEC (mequiv/100g)	40.0 ± 6.0
total soil Pb (mg kg^{-1})	2450 ± 20

Chelates have been used to remove Pb from contaminated soils in soil washing remediation techniques (*25*). It has also been documented that chelates could significantly increase Pb accumulation in plants. For example, Marten and Hammond (*26*) investigated the effects of applying EDTA to a Pb-contaminated soil on Pb accumulation in bromegrass and found that added EDTA increased shoot Pb concentration from 5 to 35 mg kg^{-1} in bromegrass. Hale and Wallace (*27*) reported that the addition of DTPA to a loam soil increased ^{210}Pb radioactivity by 20-fold in shoots of bush bean. Recently, we explored various potential methods to enhance Pb accumulation in plants, including experiments demonstrating that the addition of a synthetic chelate (HEDTA) to Pb-contaminated soils could rapidly and dramatically increase Pb desorption from soil to soil solution (*15*). This increase of Pb concentration in soil solution triggered a surge of Pb accumulation in corn plants grown on the Pb-contaminated soil (*15*).

The objectives of this study were (i) to compare the relative efficiency of selected synthetic chelates in enhancing Pb phytoextraction, (ii) to identify the best synergistic combinations between chelates and plants to maximize the total Pb removal and to minimize total chelate usage, and (iii) to investigate the chemical and physiological mechanisms involved in chelate triggered Pb hyperaccumulation in plants.

Materials and Methods

Soil Characterization. Lead-contaminated soil was collected from an industrial site in northern New Jersey. The soil was contaminated with Pb during the manufacture of explosives over a 50-year period. At the time of collecting, the soil was screened to pass through a 1.0-cm sieve. The soil was then air-dried to approximately 8% water content and mixed in an inverted cone blender with a counter-rotating central beater bar. The following procedures were used to characterize the soil. Soil pH was measured using 1:1 soil/water ratio; cation exchange capacity (CEC) was determined by exchangeable base (*28*); organic matter content was measured by the Walkley Black method (*29*); and particle size was measured by the hydrometer method (*30*). Total soil Pb was determined by digesting 500 mg of soil in a mixture of concentrated HNO_3/$HClO_4$ (v:v, 10:7), and the digested samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). The selected physical and chemical properties of the Pb-contaminated soil are presented in Table 1.

Seedling Preparation. Seeds of corn (*Zea mays* L. cv. Fiesta), pea (*Pisum sativum* L. cv. Sparkle), goldenrod (*Solidago bicolor* L.), and sunflower (*Helianthus annuus* L. cv. Avante) were soaked in water for 5 h and then sown in commercial potting mixture (Metro mix 360, Scotts Sierra Horticultural Products Company, Marysville, OH). Seeds of ragweed (*Ambrosia artemisiifolia* L.) were soaked in concentrated sulfuric acid for 30 min, washed with deionized water, and soaked in water for 10 h. The acid-treated ragweed seeds were then sown in the potting mixture. The seedlings were watered daily with a nutrient solution for a period ranging from 2 weeks for corn, pea, and sunflower to 4 weeks for ragweed and goldenrod so that all seedlings at the time of transplanting had similar biomass. Uniform seedlings were selected from each species and transplanted to the Pb-contaminated soil.

Chelate Treatments. The Pb-contaminated soil was fertilized with (in mg kg^{-1}) N, 150 [as $(NH_4)_2SO_4$], and K, 150 (as K_2SO_4). The chelate treatments were imposed after fertilization with N and K. In experiments designed to determine the effects of chelate levels on the Pb desorption from soil to soil solution and Pb accumulation in plants, HEDTA was added to the Pb-contaminated soil at the rates of 0, 0.5, 1.0, and 2.0 g kg^{-1} with three replicates for each treatment. In experiments designed to compare the relative efficiency of different chelates in Pb phytoextraction from contaminated soils, the fertilized soil was treated with the synthetic chelates (0 or 0.5 g kg^{-1}) of DTPA, as free acid; EDDHA, as free acid; EDTA, as trisodium salt; EGTA, as free acid; or HEDTA, as trisodium salt. The chelates were obtained from Sigma Chemical Company. After transplanting seedlings to the Pb-contaminated soil treated with or without chelates, all plants were grown in a growth chamber with a 16-h, 22 °C/8 h, 18 °C day−night regime. The soil moisture content was maintained at field capacity by adding water as needed. Unless otherwise specified, the plants were harvested 1 week after transplanting to the Pb-contaminated soils. During the harvest, plants were cut 1 cm above the soil and the shoots were washed with deionized water. The roots were washed in tap water until free of soil and then washed with deionized water. The plant samples were then dried in an oven at 70 °C and ground in a stainless steel Wiley mill to pass through a 0.85-mm diameter screen.

Soil Solution Extraction. The Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}) and a non-contaminated soil (total soil Pb 25 mg kg^{-1}) from a local farm were treated with N, K, and chelates as described earlier. The soils were watered to field capacity and kept at room temperature for 24 h before extracting soil solution. The soil solution was extracted using a centrifugation technique (*31*). During centrifugation, the soil solution was passed through a 0.45-μm filter. The metal composition in the soil solution was directly analyzed by ICP-AES.

Xylem Sap Collection. When corn plants grown in the Pb-contaminated soil reached a leaf stage of 5−6, EDTA treatments were initiated by adding an appropriate concentration of EDTA solution to the soil for each pot to yield an EDTA level of 0, 0.5, or 1.0 g (kg of soil)$^{-1}$. Corn shoots were cut 1 cm above the root−shoot junction 24 h after the EDTA application. Immediately following the cutting, xylem sap was collected every 15−30 min for a period of 8 h. The xylem sap collected from each pot (three plants/pot) during this period was combined to give a composite sample for each pot for the analysis of Pb concentration in the sap by ICP-AES.

Plant Analysis. Subsamples of ground plant material (400 mg) were digested in a mixture of concentrated HNO_3/$HClO_4$ (10:7, vol:vol), using a two-step digestion on an open vessel automatic microwave digester (Prolabo A 300, Questron Co., Mercerville, NJ). The digested samples were brought to a constant volume with deionized water, and the digests were analyzed for Pb by ICP-AES. Data reported in this paper were analyzed using Statistical Analysis System (*32*). A probability of 0.05 or less was considered to be statistically significant.

Results

Chelate Effects on Soil−Pb Desorption. To test the effects of chelates on Pb desorption from soil to soil solution, we examined Pb concentration in soil solution in response to HEDTA added to the Pb-contaminated soil and a non-contaminated soil. Lead in soil solution increased linearly with increasing levels of HEDTA added to the Pb-contam-

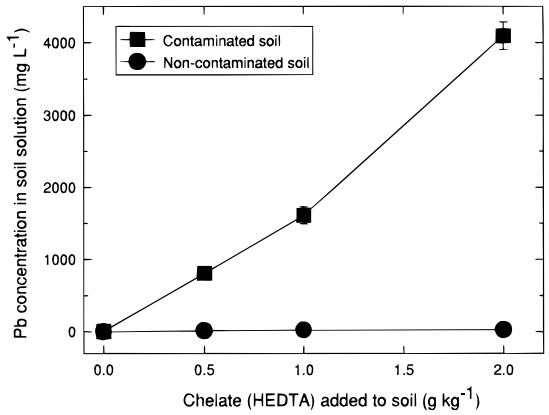

FIGURE 1. Effects of adding HEDTA to a Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}) on Pb concentrations in soil solution. After mixing with the synthetic chelate (HEDTA), both contaminated and non-contaminated soils were watered to field capacity and set in the room temperature for 24 h before extracting soil solution by centrifugation. The soil solution was passed through a 0.45-μm filter during the centrifugation. Error bars represent ± SE (n = 4).

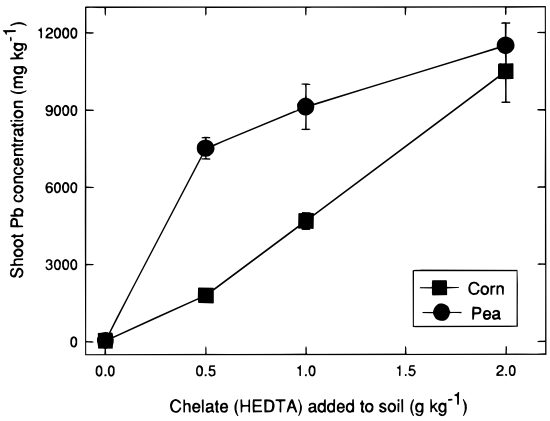

FIGURE 2. Effects of adding chelate (HEDTA) on Pb accumulation in shoots of corn and pea grown on a Pb-contaminated soil with a total soil Pb of 2500 mg kg^{-1}. Error bars represent ± SE (n = 3).

inated soil (Figure 1). For non-contaminated soil, the added HEDTA did not significantly affect the Pb concentration in soil solution (Figure 1). When no chelate was added, the Pb in the soil solution of the contaminated soil was less than 4 mg L^{-1}; at the highest chelate level of HEDTA (2.0 g kg^{-1}), the soil solution Pb increased to 4000 mg L^{-1}, a 1000-fold increase in soil solution Pb concentration.

Chelate Effects on Pb Accumulation in Plants. Within 1 week after transplanting corn and pea plants to the Pb contaminated soil treated with HEDTA, shoot Pb concentrations in both species increased significantly. Shoot Pb concentrations of corn increased linearly with increasing levels of HEDTA in the soil, while the rate of increase in shoot Pb concentration of pea varied with the chelate level (Figure 2). When HEDTA applied was equal or less than 1.0 g kg^{-1}, Pb concentration in pea shoots was significantly higher than that in corn shoots. At an HEDTA level of 2.0 g kg^{-1}, however, shoot Pb concentrations in the two species were not significantly different (Figure 2).

When no HEDTA was added to the contaminated soil, the percentage of absorbed Pb translocated from roots to the shoots was less than 30% for both corn and pea (Figure 3). With 0.5 g of HEDTA kg^{-1} added to the soil, Pb translocation increased to 72% for corn and 88% for pea. Increasing the level of HEDTA to 2.0 g kg^{-1} further increased Pb translocation in corn to 84%; however, Pb translocation in pea had reached a steady state (Figure 3).

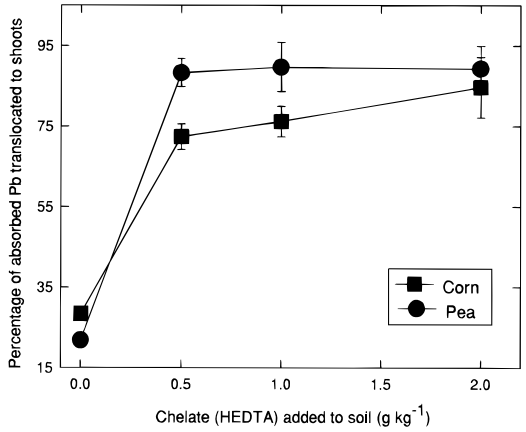

FIGURE 3. Effects of adding chelate (HEDTA) on percentage of plant absorbed Pb translocated from roots to the shoots of corn and pea grown on a Pb-contaminated soil with a total Pb of 2500 mg kg^{-1}. The plant absorbed Pb denotes total Pb accumulated in shoots and roots. Error bars represent ± SE (n = 3).

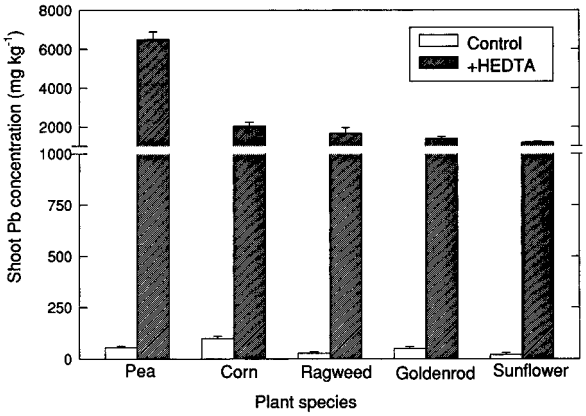

FIGURE 4. Lead accumulation in shoots of five plant species grown on a Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}) in response to the addition of HEDTA [0.5 g (kg of soil)$^{-1}$]. Error bars represent ± SE (n = 3).

Response of Plant Species to Chelate Treatment. In our previous studies, we found that plant species vary significantly in Pb accumulation (*14*, *15*, *18*). In this study, we tested the effects of applied chelate (HEDTA) on Pb accumulation in five plant species growing on a Pb-contaminated soil. In every species tested, there was a surge in shoot Pb concentrations in response to the HEDTA (0.5 g kg^{-1}) treatment. Among the species tested, the stimulation in shoot Pb concentration was highest in pea. Shoot Pb concentration in pea increased 120-fold within 1 week after applying HEDTA (Figure 4). Compared to the control (no chelate applied), the shoot Pb concentrations in other plant species increased from 30-fold in sunflower to 50-fold in corn (Figure 4).

Effects of Chelates on Pb Desorption in Soil and Pb Accumulation in Plants. To search for an ideal chelate to enhance Pb phytoextraction from contaminated soils, we compared the relative efficiency of five synthetic chelates in increasing Pb desorption from soil to soil solution, with their relative ability to stimulate Pb accumulation in shoots of pea and corn grown on a Pb-contaminated soil. Treatments consisted of the five chelates (DTPA, EDTA, EDDHA, EGTA, and HEDTA) at 0.5 g (kg of soil)$^{-1}$. The control represents plants grown on the Pb-contaminated soil without added chelate. Among the chelates tested, EDTA was the most efficient in increasing shoot Pb concentrations in both pea and corn (Figure 5 A,B). Within 1 week following the application of chelates, shoot Pb concentration of pea increased from 50 mg kg^{-1} for the control to 11 000 mg kg^{-1} for the EDTA-treated soil. The order of the effectiveness in

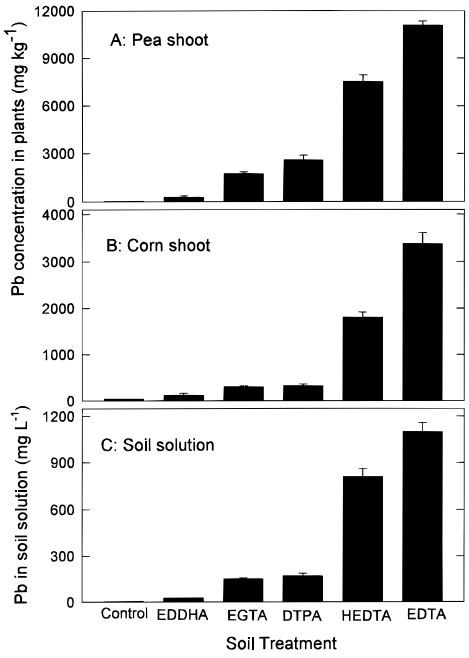

FIGURE 5. Relative efficiency of five synthetic chelates in enhancing Pb accumulation in shoots of pea (A) and corn (B) grown on the chelates treated Pb-contaminated soil and Pb desorption from soil to soil solution (C). Control denotes the Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}) without added chelates. The rate of each chelate used was 0.5 g (kg of soil)$^{-1}$. Error bars represent ± SE (n = 3).

TABLE 2. Relative Efficiency of Five Synthetic Chelates[a] in Enhancing Pb Accumulation in Shoots of Corn and Pea Plants Grown in Pb-Contaminated Soil with a Total Soil Pb of 2500 mg kg^{-1}

	total Pb accumulation in shoots[2]	
chelate used	corn (μg plant^{-1})	pea (μg plant^{-1})
control[c]	42 ± 7	81 ± 4
EDDHA	112 ± 13	276 ± 62
DTPA	300 ± 70	1930 ± 205
EGTA	909 ± 53	2080 ± 207
HEDTA	1110 ± 52	5670 ± 280
EDTA	2410 ± 140	8960 ± 620
ANOVA P>F	0.001	0.001

[a] Each chelate was used at a rate of 0.5 g (kg of soil)$^{-1}$ and was mixed with the Pb-contaminated soil before transplanting corn and pea plants (14-day-old). The plants were harvested 1 week after transplanting to the contaminated soil. [b] Values are mean ± SE (n = 3). [c] Control denotes the plants grown in the Pb-contaminated soil without added chelate.

increasing Pb accumulation in both pea and corn was the following: EDTA > HEDTA > DTPA > EGTA > EDDHA. It is important to note that the effectiveness of these chelates on Pb desorption from soil to soil solution paralleled the Pb accumulation in plants (Figure 5A–C). For example, Pb concentration in soil solution varied from 1100 mg L^{-1} for EDTA treatment to 27 mg L^{-1} for EDDHA treatment. When no chelate was added, the soil solution Pb was 3.5 mg L^{-1} (Figure 5C).

Similar to the effects of chelates on shoot Pb concentration, chelates also significantly increased total Pb accumulation in shoots for both corn and pea (Table 2). For each of the five chelates tested, total Pb accumulation in pea shoots was significantly higher (P < 0.001) than that in corn shoots. Among the chelates tested for each plant species, there were significant differences in their ability to stimulate Pb accumulation in the shoots. Once again, for the chelates tested, the rank of the efficiency in enhancing total Pb accumulation

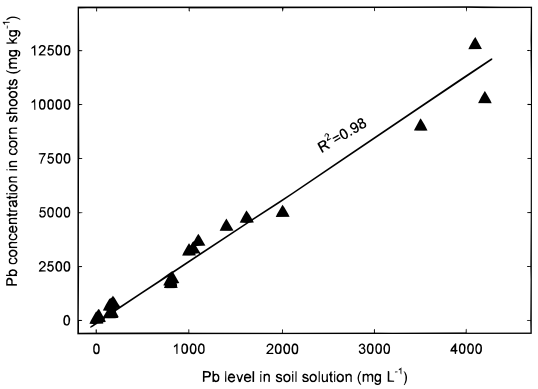

FIGURE 6. Correlation between Pb levels in soil solution and shoot Pb concentrations of corn grown on chelates treated Pb-contaminated soil. The different soil solution Pb levels were obtained by applying EDTA, EDDHA, EGTA, and DTPA at 0.5 g kg^{-1} and HEDTA at 0.5, 1.0, and 2.0 g kg^{-1} to the Pb-contaminated soil (total soil Pb 2500 mg kg^{-1}).

TABLE 3. Effects of Adding EDTA to Pb-Contaminated Soil[a] with Total Soil Pb of 2500 mg kg^{-1} on Pb Concentration in Xylem Sap and Pb Accumulation in shoots[b] of 21-Day-Old Corn Grown in Contaminated Soil

EDTA added to soil (g kg^{-1})	Pb concentration in xylem sap (mg L^{-1})	Pb translocation to shoots (μg plant^{-1} day^{-1})
0.0	0.15 ± 0.02	0.83 ± 0.11
0.5	6.93 ± 0.08	28.00 ± 0.42
1.0	21.30 ± 2.05	99.80 ± 5.52
ANOVA P>F	0.001	0.001

[a] EDTA treatment was initiated by adding the appropriate EDTA solution to the soil surface for each pot. Shoots were cut 1 cm above root–shoot junction 24 h after applying the EDTA. Immediately following the harvest, xylem sap was collected for 8 h for each treatment. [b] Values are mean ± SE (n = 3).

in shoots was the same as that found in enhancing soil Pb desorption (Table 2 and Figure 5).

We also examined the correlation between Pb concentrations in corn shoots and Pb levels in soil solution (Figure 6). The different Pb levels in soil solutions in this experiment were obtained when DTPA, EDTA, EDDHA, and EGTA were applied at 0.5 g kg^{-1} and when HEDTA was applied at 0.5, 1.0, and 2.0 g kg^{-1} to the Pb-contaminated soil. Lead concentrations in the corn shoots increased linearly with increasing Pb levels in the soil solution. The value of R^2 indicates that 98% of the increase in Pb concentration of corn shoots was associated with the increase in soil solution Pb resulting from the application of chelate to the Pb-contaminated soil.

Chelate Effect on Pb Translocation. In order to understand the mechanisms involved in chelate-triggered Pb hyperaccumulation in plants, we studied the effect of EDTA on Pb concentration in xylem sap of corn shoots. Applying EDTA to Pb-contaminated soils significantly increased Pb transport into the xylem of corn plants. For example, 24 h following application of EDTA solution to the corn plants grown in the contaminated soil, Pb concentration in shoot xylem sap increased 46-fold for the treatment of 0.5 g of EDTA (kg of soil)$^{-1}$ and 140-fold for 1.0 g of EDTA (kg of soil)$^{-1}$ compared to Pb concentration in xylem sap for the control (Table 3). The addition of EDTA also resulted in a huge increase in net Pb translocation to the shoots. Compared to the control, the addition of EDTA [1.0 g (kg of soil)$^{-1}$] increased Pb translocation 120-fold within 24 h (Table 3).

Discussion

Lead in soils is classified as a soft Lewis acid, which implies a strong covalent character to many of the ionic bonds it

forms in soils and plants. The strength of these bonding interactions has significant impact on Pb phytoextraction. These include the observation that, in every contaminated soil we have examined, soil solution Pb concentrations represent a very small fraction of the total soil Pb. Furthermore, the majority of Pb absorbed by plant roots is sequestered there. Therefore, a key to the Pb phytoextraction is to increase Pb solubility in contaminated soils and Pb translocation from roots to shoots. Results from this study demonstrate that some chelates can rapidly and dramatically increase Pb concentrations in soil solution as well as Pb translocation from roots to shoots for a number of plant species (Figures 1−5). A major factor for the chelate-induced surge of Pb concentration in soil solution could be the chelation between Pb and the chelates in the soil solution that drives Pb desorption from soil to soil solution. For the five chelates tested, the efficiency in enhancing soil Pb desorption from this soil is significantly different (Figure 5). The differences may reflect the different ability of the chelates to form Pb−chelate complex or the selectivity of the chelate for Pb than for other major cations in the soil. It should be noted that the molar concentrations of the chelates applied were varied because of the different molecular weights. The final molar concentrations of the chelates applied were (in mmol kg^{-1}): DTPA, 1.27; EDTA, 1.39; EDDHA, 1.39; EGTA, 1.31; and HEDTA, 1.45. Since the chelate-enhanced Pb accumulation in plants differed by more than 1000-fold (Figure 5), the relative small variation in molar concentration did not affect the order of the efficiency in enhancing Pb accumulation for the chelates tested. The significantly different efficiency among the five chelates in enhancing soil Pb desorption deserves further attention. The results indicate that, with further screening, a more efficient and Pb-specific chelate may be found. The more efficient a chelate is in soil Pb desorption, the less chelate would be needed to add to a Pb-contaminated soil. Another factor contributed to the chelate-induced increase in Pb desorption could be due to the addition of cations that are counterions of the chelates used.

The chelate-induced increase in Pb concentration of soil solution appears directly associated with the increase in Pb concentration in the xylem, Pb translocation from roots to shoots, and Pb accumulation in plants (Tables 2 and 3, Figures 3 and 5). These results indicate that chelates may eliminate two major limiting factors in Pb phytoextraction from contaminated soils: the low bioavailability of Pb in the soils and the low Pb translocation from roots to shoots. The mechanisms involved in this phenomena are not yet understood. There may be several mechanisms involved in this chelate-triggered Pb hyperaccumulation in plants. First, the increase in Pb level in soil solution is a major one. This is clearly demonstrated by the results presented in Figure 6. In this experiment, five chelates are used, and the increase of Pb solution by these chelates varied significantly. However, when shoot Pb concentrations were regressed against Pb levels in soil solution, all data from the five chelates tested fell on the same regression line (Figure 6). The regression analysis also indicates that more than 98% of the increase in shoot Pb accumulation could be accounted by the increase of Pb concentration in soil solution. Second, chelates could buffer Pb activity near the root surface and thus maintain a constant supply of free Pb to the Pb uptake sites of roots. Third, Pb−chelate complex may be directly absorbed by roots and translocated to the shoots. This interpretation seems to contradict the general belief in plant nutrition research. It is generally believed that plants do not absorb or translocate synthetic chelates or the complex of ion and synthetic chelate (*33*, *34*). This belief is based on the conditions that the concentration of synthetic chelates used is low, usually in the micromolar range. However, under the experimental condition of this study, the chelate concentration in root zone was in the millimolar range. In the case of EDDHA used, we observed a purple color (EDDHA solution color) in leaves of corn and pea plants within 12 h after applying the EDDHA solution to the contaminated soil. This observation suggests that the chelate was rapidly absorbed by roots and translocated from roots to shoots. It has been reported that Pb−EDTA was transported into roots and translocated from roots to shoots for a number of plant species (*35*). It may also be possible that synthetic chelates at high concentrations alter plant ion transport systems such that Pb uptake and translocation are facilitated. The exact mechanism of this chelate-induced surge of Pb translocation and accumulation is not clear. Further research is needed to understand the mechanism involved.

Environmental concerns will require that the chelate addition be minimized. This may be done by engineering controls or by the discovery and the use of more Pb-specific chelates. However, our data show that, with the soil used in this study, there are significant plant species differences in the ability to accumulate Pb in the plants from chelate treated soils (Figures 2 and 4). Despite the overall large increase of shoot Pb concentration by the application of chelates, some plant species are significantly more efficient in shoot Pb accumulation at equivalent chelate addition to the contaminated soil (Figure 4). These results suggest that further improvements in the chelate selection and application process should be made in parallel with the selection of plant species. The results from this study indicate that phytoextraction of Pb from contaminated soils may be accomplished by growing a high biomass crop that is very sensitive to the chelate-induced surge of Pb accumulation in plants. A possible scenario for field application could be that, after the crop had reached a substantial size, a selected chelate would be applied to facilitate rapid Pb accumulation in plants. In this form of Pb phytoextraction, unlike the original hyperaccumulator concept, the plant would contain relatively low Pb concentration for most of its life. After the application of chelates, Pb accumulation in plant shoots would be increased rapidly, and the plants would be harvested shortly after the chelate addition. This strategy may have advantages in reducing the risk that might have been present by having plants with high Pb levels in the field for long periods of time.

The addition of chelates to Pb-contaminated soils as a part of Pb phytoextraction may bring a new dimension to the field application and additional health, safety, and environmental concerns. The application of novel irrigation techniques, which can quantitatively control water flux in plant root zone, may be of use here. Other possibilities are inherent in using an *in situ* soil washing or a leaching model with engineering controls. The time of chelate application is important to avoid possible chelate-induced metal movement into the groundwater. Since chelate-triggered Pb hyperaccumulation in plants is rapid, the chelate can be applied to the root zone when the vegetation is well-established on the contaminated sites. It is also important to avoid rain days for the chelate application. Further research is needed before the chelate-assisted phytoextraction technique can be widely used at contaminated sites. To our knowledge, the results reported here represent a significant step forward in the development of Pb phytoextraction using high biomass plants that can accumulate greater than 1.0% Pb in shoots. Additional research is needed concerning the most appropriate plant−chelate−Pb combination to be employed at an individual site. Furthermore, engineering and field development will also be required to determine the best field application methods, agronomics, and site monitoring. Based on currently available data, the calculated costs of Pb phytoextraction (costs of chemicals, plant cultivation, and biomass treatment) is only a fraction of other alternative process such as excavation of the contaminated soil for landfill. We conclude that, at many sites with careful management, chelate-assisted

Pb phytoextraction may provide a cost-effective soil decontamination strategy.

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