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Chelate-Assisted Pb Phytoextraction: Pb Availability, Uptake, and Translocation Constraints

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Chelates have been shown to enhance phytoextraction of Pb from contaminated soil. Mechanisms behind this phenomenon, however, remain largely unexplored. In this paper we examine chelate effect on Pb dissolution, plant Pb uptake, and internal plant Pb translocation. EDTA was found to be the most efficient in increasing water-soluble Pb concentration in our test soil. Unfortunately, Pb−EDTA is highly water-soluble and poses potential risks to groundwater in its application. In addition, it would not appear to be ideally suited for plant uptake and translocation based upon the relative water solubility of Pb-EDTA. We demonstrated that *N,N*′-di(2-hydroxybenzyl)ethylenediamine *N,N*′-diacetic acid (HBED) resulted in *Zea mays* root Pb content significantly higher than did EDTA, indicating that a chelate better than EDTA might be designed. Fortuitously, EDTA appears to increase overall plant transpiration, the driving force in phytoextraction of the Pb−chelate complex from soil. We also found that there was a significant increase in Pb uptake and translocation for corn transplanted into soil, then treated with EDTA, as compared to plants germinated and grown in Pb-contaminated soil to which EDTA was subsequently applied. These results demonstrate that significant improvement over current chelate-assisted phytoextraction of Pb may be possible.

Introduction

The potential for success in phytoextraction varies widely and is dependent on the nature of the contaminants, media, plants, and agronomic handling (*1*). Among the major soil-contaminating metals, Pb appears to be one of the most technically challenging. One measure of this difficulty is reflected in the paucity of naturally evolved plant species which hyperaccumulate Pb (*2*). Field samples of plants with greater than 500 μg/g Pb on a dry weight basis are extremely rare.

Pb exists in several forms in soil, and the specific chemical forms of Pb greatly affect the Pb availability to plant (*3*, *4*). Even though the total Pb concentration in many contaminated soils may be high, the phytoavailable Pb fraction is often exceedingly low. A serendipitous breakthrough occurred when chelate amendments applied to soil to increase solution Pb levels in soil also resulted increased Pb content in plants (*5*, *6*, *7*). This chelate-induced hyperaccumulation normally proves fatal to the plant; however, the dying plant can still be harvested and processed for Pb recovery or disposal. When effective chelates have been added (e.g., with a log of the binding constant higher than 18), Pb^{2+} is primarily in the complex form (*8*). Evidence appears increasingly clear that the form of Pb taken into the plant and translocated up the stem after soil amendment with a chelate is the Pb−chelate complex [e.g., EDTA−Pb (*6*) and HEDTA−Pb (Berti and Lamble, personal communication)]. The mechanisms for transport of these complex structures are unknown.

Our initial assumption was that an ideal system for chelate-enhanced phytoextraction begins with a robust plant growing in contaminated soil. The chelate is designed to bind Pb preferentially and tightly, despite high background levels of other divalent ions. The chelate itself is highly water soluble to facilitate use, but once complexed with Pb should become more lipophilic to facilitate uptake and translocation within the plant as well as decrease the risk of off-site migration of a very soluble Pb complex. Once internal to the plant, the Pb complex should stay intact to mitigate potential toxic effects on the plant and should have no effect on the ability of a plant to continue to actively transpire, and if possible, continue growing. To these authors' knowledge, few of these basic parameters have been measured, compared, or optimized.

The literature to date reports a number of chelates that have been used for chelate-induced hyperaccumulation (*5*−*7*). These include EDTA, CDTA, DTPA, EGTA, EDDHA, and malic acid. These chelates, however, are not specific to Pb binding and are subject to numerous interferences with other soil cations present at much higher concentrations. Ideally, a metal-specific chelate should be used to maximize Pb content in soil solution with minimum soil chelate application. Over the past decade, studies in search of Pb-specific binding ligands have pointed to some molecular attributes for Pb specificity, including thiohydroxamato and macrocyclic structures (*9*, *10*). Some compounds with those structures were selected for this study.

Although little is known about the specific pathway Pb−chelate complexes take to enter the plant root and eventually plant xylem, much is known about the process, and by inference it can be used to design phytoextraction strategies in plants. Solute transport from the external parts of the root to the central root xylem where the material is carried to the shoots takes place through two major pathways: the apoplastic (cell wall space between cell membranes) and the symplastic (crossing many cell membranes along the path). In the apoplastic pathway, the presence of the lipophilic Casparian strip at the root endodermis disrupts the apoplastic water flow and directs it to cross cell plasma membranes at least twice, where selective transport as well as passive permeation of solutes occur (*11*). Under normal plant physiological conditions, the Casparian strip guarded pathway accounts for over 99% of water flow through the roots (*12*). Under chelate-induced phytoextraction this is uncertain. Hydrophilic compounds favor the apoplastic pathway, whereas lipophilic compounds favor the symplastic pathway. The root uptake rate and xylem steady-state concentration of a compound depend on the compound's lipophilicity. This is often measured as an octanol−water partition coefficient (log K_{ow}). Translocation efficiency of many compounds from root periphery to xylem has been measured, and this information is most likely applicable to phytoextraction (*13*, *14*). The translocation efficiency follows a biphasic distribution curve between the compound's log K_{ow} and its steady-state concentration in the transpiration stream.

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Materials that are very hydrophilic, as in the case of EDTA, are not favored for maximal plant transport; neither are compounds that are too hydrophobic (e.g., the gasoline additive tetraethyl lead).

In addition to the relative lipophilicity and molecular size of a compound, accumulation in plants is dependent on the total flux of solute movement up into the shoots, i.e., the plant transpiration rate. The effect of soil chelate amendment on plant transpiration rates, which in turn affects plant Pb accumulation, is also critical. Additionally, a reduced xylem flow might also help explain some of the increases in xylem Pb concentration observed after soil EDTA treatment (*5*).

This study is an attempt to examine a number of these fundamental limiting factors so that a better phytoextraction system may be designed. This includes choice of chelate, effect on plant uptake, Pb and chelate relative phytotoxicity, and effect of Pb chelate on plant transport pathways.

Materials and Methods

Plant Material and Pb Treatment. *Hydroponic Experiments.* Corn seeds (*Zea mays*, Pioneer 3379) were germinated in potting soil (Metro-mix 360, Scotts-Sierra Horticultural Products Company, Marysville, OH), and three-day old uniform seedlings were gently uprooted, cleaned with deionized (DI) water, and transferred to 8-L hydroponic culture containers with aeration and continuous nutrient solution flow through. Plants were grown for 6 days before treatments, as detailed in Huang and Cunningham (*7*). To test corn uptake of chelate−Pb complex, plants were treated with different chelate−Pb complexes (in hydroponic solution), as detailed in the figure legends. Chelate−Pb complexes were made by adding a precise amount of $Pb(NO_3)_2$ to chelate solution with slightly higher molar excesses of chelates. Plant samples were taken every 24 h for 4 days, with three replicates for each treatment.

Soil Experiments. Selected chelates were applied to Pb-contaminated soil to evaluate the effect of Pb on plant growth and Pb phytoextraction efficiency. Pb-contaminated soil was obtained from an industrially contaminated site and mixed in an inverted cone blender (Crossley Economy, East Palestine, OH). This soil has been previously used in experiments reported from this lab (*5*). The average soil Pb content of this batch was 2500 μg/g. Soil was supplemented with N and K in the form of $(NH_4)_2SO_4$ and K_2SO_4, each 150 mg/kg soil. Plants were germinated directly in the Pb-contaminated soil, then EDTA and HBED were added to soil at a rate of 1.5 mmol/kg soil 10 days after seed germination. In the transplanting experiment, plants were germinated in potting soil. Plants were carefully washed free of soil 10 days after germination, then transplanted to the Pb-contaminated soil. The soil was then treated with chelates EDTA and HBED at the same rate. For the soil chelate treatment, appropriate amounts of 0.5 mM EDTA or HBED solution were added in a single application to the soil, according to the weight of soil in the pot, to make up the amount of chelate to 1.5 mmol/kg soil. Plants were grown in chelate-treated Pb-contaminated soil for 10 days and were watered daily to keep the soil water to field capacity.

All experiments were conducted in the greenhouse under natural light supplemented with high-pressure sodium lamps (Metalarc Lamp M/MS Metal Halide, Sylvania, Manchester, NH) to maintain a daily cycle of 16 h light to 8 h dark and a light flux density of 600−800 mol photons m^{-2} s^{-1} during that period. Air temperature ranged from 18 to 25 °C, the natural variations in the greenhouse.

Soil and Plant Tissue Ion Composition Measurement. Plant materials were harvested, shoot fresh weights taken, and then washed with deionized water. Roots were washed with DI water and blotted dry prior to weighing. Plant samples were dried at 60 °C in a drying oven to a constant weight for dry weight measurements. Subsamples of ground shoot samples (200 mg) and root samples (100 mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ (10:7, by volume) in an automatic microwave digester (Prolabo A 300, Questron Co., Mercerville, NJ).

Metal ion composition of the digestion solution was analyzed with inductively coupled plasma spectroscopy (ICP) (Spectro Analytical Instruments, Inc., Fitchburg, MA).

For soil Pb dissolution experiments, Pb-contaminated soil (2.0 g aliquots) was shaken in extraction solution containing 0.5 mM testing chelates for 6 h, 48 h, and 120 h. After centrifugation at 2200 g for 20 min, the supernatant was filtered through Whatman #1 filter paper (Whatman, Hillsboro, OR), and directly analyzed for metal ion concentrations by ICP.

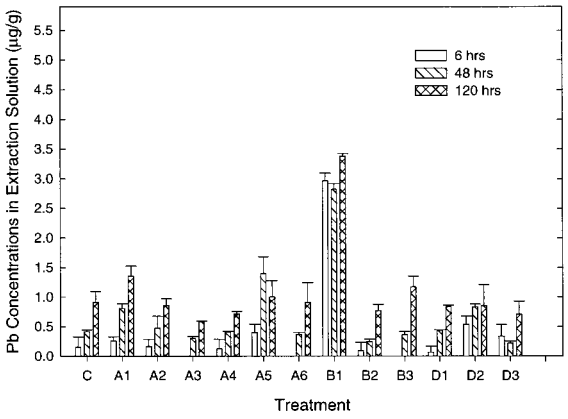
Plant Transpiration Measurement. A sap flow measurement system FLOW32 (Dynamax, Inc., Houston, TX) was used to determine transpiration rate and observe the effect of applied synthetic chelates on transpiration of corn grown in Pb-contaminated soil. Baker and Van Bavel (*15*) described in detail the instrumentation and its working theories. In this study, a 5-mm diameter stem flow gauge (model SGA 5, Dynamax, Inc., Houston, TX) was installed on each plant 2 days before taking measurements so as to allow for the gauges to conform well to the shape of the stem and to achieve stable heat transfer between stem and gauge. An electrical insulating compound, Dow-Corning compound 4 (Dow Corning Corporation, Midland, MI), was evenly applied to the plant stem surface area where the sap flow gauge was attached. Sap flow measurements were taken every 5 min and were averaged and recorded every 15 min. Dynamax Inc. supplied FLOW32 software for downloading control programs of gauge parameters, flow data logging, and data retrieval. The acquired sap flow data were calibrated every day against the predawn K_{sh} value (a parameter defined as the thermal conductance constant) obtained from the gauge for each individual plant. Total sap flow during a day was calculated from each calibrated data point using a numerical integration method, the Composite Simpson's rule (*16*):

$$\int_a^b f(x)\,dx = h/3[f(a) + 2\sum_{j=1}^{(n/2)-1} f(x_{2j}) + 4\sum_{j=1}^{n/2} f(x_{2j-1}) + f(b)]$$

where, $f(a)$ is the flow rate of the first measurement, $f(b)$ is the flow rate of the last measurement, j is the individual measurements between a and b ($j = 1$ to 54), n is the number of measurements from 5:00 a.m. to 7:00 p.m. ($n = 56$), h is the interval between measurements (0.25 h).

Ligand−Pb log K_{ow} Measurement. A shake-flask method was used to determine the log K_{ow} value of the chelate−Pb complexes. Octanol saturated deionized water (1 mL) was mixed with 1 mL of 0.5 mM chelate solution, to which 4 mL of deionized water saturated octanol was added, and the mixture was shaken in a 25 mL capped glass tube on a wrist-shaker (Burrell Corporation, Pittsburgh, PA) at room temperature near neutral pH overnight. Assuming no free Pb in the presence of excess ligand, the aqueous phase chelate−Pb concentration was measured as Pb with ICP and the octanol phase chelate−Pb concentration was calculated by subtracting the aqueous phase chelate−Pb concentration from the total concentration.

Chemicals and Data Analysis. The following chemicals were obtained from Aldrich (Milwaukee, WI): 3-(2-thienyl)-DL-alanine; 2,2′,2″,2‴-[1,2-ethanediildenetetrakis(thio)tetrakisacetic acid; 8-hydroxy-7(4-sulfo-1-naphthlazo)5-quinolinesulfonic acid; *meso*-2,3-dimercaptosuccinic acid; 2,5-dihydroxy-1,4-benzendisulfonic acid; L-penicillamine; and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraactic acid (TETA). Imniodi(methylphosphonic acid) was from Fluka



C: Control
A1: 8-HYDROXY-7-(4-SULFO-1-NAPHTHLAZO) 5-QUINOLINESULFONIC ACID
A2: MESO-2,3-DIMERCAPTOSUCCINIC ACID
A3: L-PENICILLAMINE
A4: 2,2′,2″,2‴-[1,2-ETHANEDILIDENETETRAKIS(THIO)TETRAKISACETIC ACID
A5: 2,5-DIHYDROXY-1,4-BENZENDISULFONIC ACID
A6: 3-(2-THIENYL)-DL-ALANINE
B1: 4,7,13,16,21,24-HEXAOXO-1,10-DIAZABICYCLO[8.8.8]HEXACOSANE
B2: 1,4,7,10-TETRACYCLODODECANE
B3: 1,4,7,10,13,16-HEXAOXACYCLO OCTADECANE
D1: IMINODI(METHYLPHOSPHONIC ACID)
D2: IMINODIACETIC ACID
D3: CITRIC ACID

FIGURE 1. Pb solubilization from Pb-contaminated soil using bidentate and multidentate ligands with potential Pb binding functional groups. Data are means ± SE of three replicate samples.

(Ronkonkoma, NY). All other chemicals were obtained from Sigma (St. Louis, MO).

Data calculation and statistical analysis including Tukey's test and ANOVA were conducted using SYSTAT software (SYSTAT, Inc., Chicago, IL). Sample replications were indicated in the figure legend.

Results

Soil-Bound Pb Dissolution − Chelate Testing. The physical and chemical characteristics of the Pb-contaminated soil were previously reported (*5*).

The effect of natural and synthetic chelates on solublizing soil-bound Pb in our test soil is shown in Figures 1 and 2. Figure 1 contains selective ligands from three types of structural groups, including sulfur-containing structures (group A: meso-2,3-dimercaptosuccinic acid, 3-(2-thienyl)-DL-alanine, 2,5-dihydroxy-1,4-benzendisulfonic acid, L-penicillamine, 2,2′,2″,2‴-[1,2-ethanedilidenetetrakis(thio)tetrakisacetic acid, and 8-hydroxy-7(4-sulfo-1-naphthlazo)5-quinolinesulfonic acid); macrocyclic structures (group B: 4,7,13,16,21,24-hexaoxo-1,10-diazabicyclo[8.8.8]hexacosane, 1,4,7,10,13,16-hexaoxacyclo octadecane, and 1,4,7,10-tetracyclododecan); and small acid molecules (group D: iminodiacetic acid, iminodi(methylphosphonic acid) and citric acid). The selected compounds tested here did not increase the soluble Pb concentration over that of control, except for 4,7,13,16,21,24-hexaoxo-1,10-diazabicyclo[8.8.8]-hexacosane.

Figure 2 shows the soil extraction results for a group of synthetic chelates containing at least two carboxymethyl groups. All of these structures significantly increased soluble Pb concentration. Among all the chelates tested, EDTA appears to be the best in solublizing soil-bound Pb and maintaining a high soluble Pb concentration over the experimental period (5 days). HBED, a more lipophilic Pb complex, significantly increased the soluble Pb concentration over that of the control in 6 h, but this soluble fraction decreased over time.

Plant Pb Uptake of Ligand−Pb Complex. Four synthetic chelates in Figure 2 were used in further testing of plant Pb uptake. Plants grown in hydroponic culture were supplied with chelate−Pb complex to determine the effect of the Pb complex structure on plant Pb accumulation.

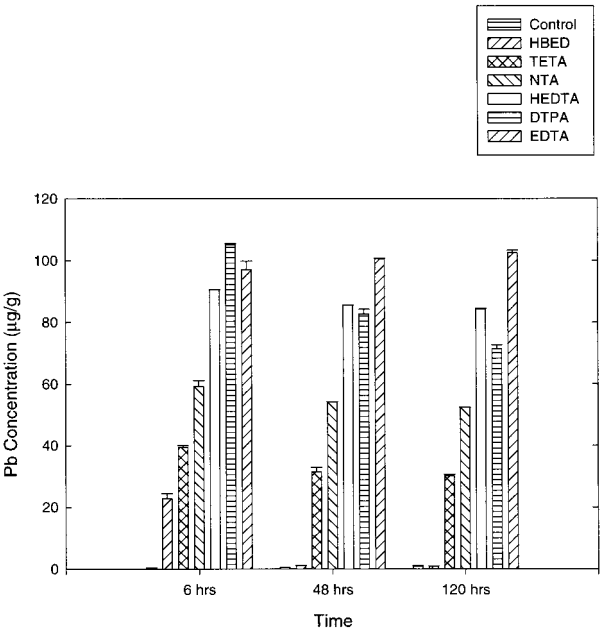


FIGURE 2. Effect of synthetic chelates with varying numbers of carboxymethyl groups on solubilization of Pb from contaminated soil. Data are means ± SE of three replicate samples. Abbreviations: HBED: *N,N′*-di(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid; TETA: 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid; NTA: nitrilotriacetic acid; HEDTA: *N*-(2-hydroxyethyl)ethylenedinitrilotriacetic acid; DTPA: diethylenetrinitrilopentaacetic acid; EDTA: ethylenedinitrilotetraacetic acid.

TABLE 1. Comparison of Chelate Pb Binding and Water Partitioning Properties[a]

	EDTA	DTPA	HEDTA	HBED
log *K*	18.1	18.7	15.6	18.2
ClogP	−3.86	−4.91	−4.09	−1.91
L-Pb log K_{ow}	−1.65	−1.18	−1.18	−0.39

[a] log *K*, chelate binding constant with Pb (ref 23); ClogP, calculated chelate log K_{ow} values (ref 17, 18); L-Pb log K_{ow}, estimated log K_{ow} of chelate−Pb complex, as determined with the methods detailed in Materials and Methods.

The four chelates all have high binding constants to Pb, but their octanol/water partitioning values are different (Table 1). Free chelate (or ligand) log K_{ow} (ClogP) (for the neutral form of each chelate) was calculated with the SRC log K_{ow} program (*17*, *18*), which showed that EDTA, DTPA, and HEDTA were similar in the log K_{ow}, and were all much lower than HBED. The L-Pb log K_{ow} was measured in this study, as detailed in Materials and Methods. The resolution of ICP for Pb determination is 0.100 μg/g (0.48 μM). This limits the theoretical measurement of a compound's log K_{ow} to the range of −3.0 to 3.0 (at a starting aqueous phase compound concentration of 500 μM). The measured ligand−Pb complex log K_{ow} values are higher than free ligands' ClogPs, and HBED−Pb had a much higher log K_{ow} than those of EDTA, DTPA, or HEDTA.

Figure 3 shows that there was no significant difference in shoot or root Pb accumulation among EDTA−Pb, HEDTA−Pb, and DTPA−Pb treatments. Interestingly, there was a significant increase in root Pb content in the HBED treatment compared with the other three treatments. However, the root to shoot translocation efficiency of HBED−Pb was apparently lower than that of EDTA−Pb, HEDTA−Pb, and DTPA−Pb

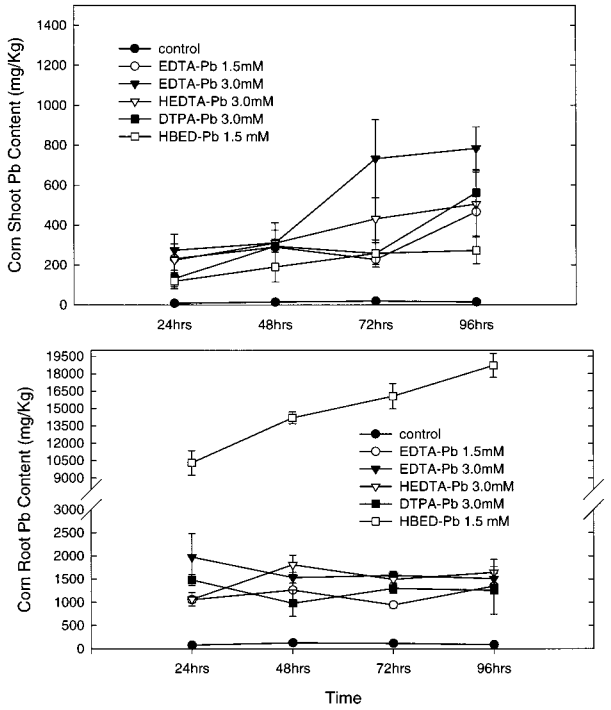


FIGURE 3. Chelate effect on corn Pb uptake. Plants were grown in hydroponics supplemented with Pb-ligand complexes, as indicated in the figure. Plant samples were taken every 24 h after the Pb complex treatment. Data are means ± SE from three replicate samples in each treatment.

such that there were no differences in the shoot Pb contents.

Soil Chelate Amendment. Corn grown in Pb-contaminated soil treated with EDTA had significantly increased shoot and root Pb uptake compared to those without EDTA treatment (Figure 4 a, b). However, the increased Pb uptake into roots was not observed in soil treated with HBED. Instead, an enhanced root uptake of Cu and Mn and shoot accumulation of Mn were observed with HBED treatment.

Compared to EDTA, HBED does not bring as much soil-bound Pb into solution; rather, it reacts with Fe, Mn, Ca, and Cu more strongly, as indicated in Figure 5. HBED initially solublized a significant amount of Pb and Zn (Figure 5, 6 h data point), but this could not be maintained in the presence of other cations, as the binding constants would suggest (Table 2). Significantly more Fe, Cu, and Mn were solublized from this soil by HBED than EDTA (Figure 5). This effect probably led to higher root Cu and Mn contents in corn grown in HBED-amended Pb-contaminated soil (Figure 4). Therefore, for the particular elemental matrix of the soil used in this study, HBED is not as efficient as EDTA to increase Pb phytoavailability.

During soil chelate treatment, plant transpiration rate was monitored by measuring xylem sap flow. Figure 6 shows the total daily sap flow of corn integrated from flow rate data collected every fifteen minutes each day. At the chelate application rate of 1.5 mmol/kg soil, there was no significant effect on plant transpiration in the HBED treatment, whereas in the EDTA treatment, a significant increase in transpiration rate was observed.

The total shoot Pb accumulation in corn grown in chelate-amended Pb-contaminated soil was also affected by the time of corn planting and chelate addition. Transplanting corn to a Pb-contaminated soil, which then was treated with EDTA, resulted in significantly higher shoot Pb content than when similar plants were grown in identical Pb-contaminated soil and then treated with EDTA (Figure 7). The transplanted plants also had higher shoot accumulations of Cu and Mn,

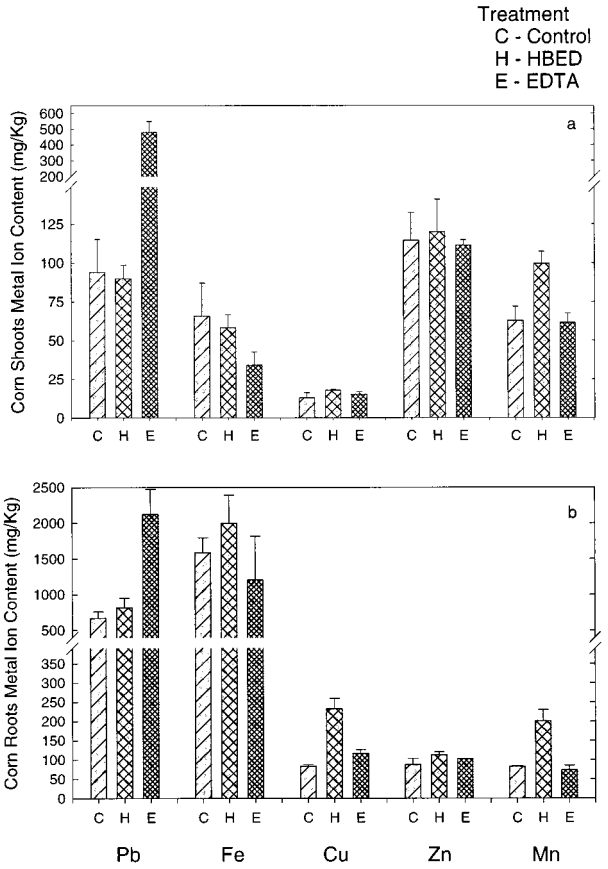


FIGURE 4. Heavy metal uptake by corn in response to EDTA and HBED treatments in Pb-contaminated soil. Data are means ± SE from three replicate samples in each treatment. a: shoot ion content; b: root ion content.

whereas other elements, such as Mg^{2+} and K^{+}, were not affected (data not shown). Another way to express this increase in metal translocation efficiency, was by a larger shoot to root ion concentration ratio. For Pb, transplanted plants had a ratio of 1.57, whereas untransplanted plants had a ratio of 0.23. This same ratio differential was also observed in Cu and Mn, e.g., 0.60 versus 0.13 for Cu, and 2.18 versus 0.83 for Mn.

Discussion

The selection of compounds in this study is based upon their potential as Pb-specific chelates, as reported in the literature. The compounds used here do not represent an exhaustive selection of all the potential chelates, but rather representatives of structural classes intended to explore possibilities in efficient Pb chelates. Chen et al. (*19*) examined the feasibility of a large number of chelates for heavy metal removal from contaminated soil, as EDTA treatment resulted in metals being strongly retained in soil matrix. Pyridine-2,6-dicarboxylic acid, *N*-(2-acetamido) iminodiacetic acid and *S*-carboxymethyl-L-cysteine prove to be more effective chelates in recovering Pb after treatment (*20,21*).

No chelate has ever been designed for optimal phytoextraction efficiency, and it is likely that further technical refinements can be made. Sulfur is generally considered more optimal in binding Pb than O or N because of the larger electron field of the donor that can better match the large electron diffusion field of Pb. The thiohydroxamato structure was explored for synthesis of chelating agents specific to Pb (*9*). From the soil extraction results, however, the selected compounds with a sulfur-containing subgroup, including *meso*-2,3-dimercaptosuccinic acid, a chelating agent in the

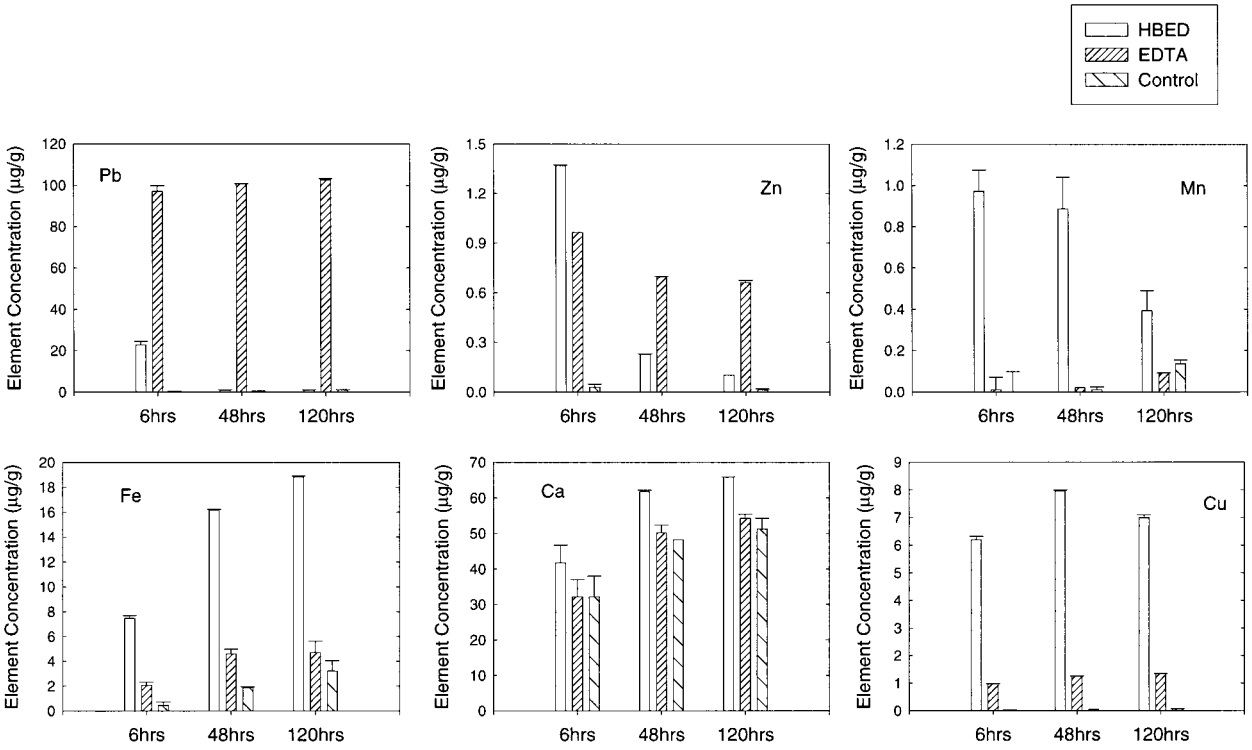


FIGURE 5. Comparison of the effect of EDTA and HDEB on heavy metal dissolution from Pb-contaminated soil. Data are means ± SE of three replicate samples.

TABLE 2. Metal Binding Constants[a] for EDTA and HBED

metals	EDTA	HBED
Mg	8.9	10.5
Ca	10.7	9.3
Mn	13.9	14.8
Zn	16.5	18.4
Fe^{2+}/Fe^{3+}	14.3/25.1	18.2/39.7
Cu	18.8	23.7
Pb	18.1	18.2

[a] reference 23.

therapy of lead poisoning in humans and animals, did not result in significant increase in Pb concentrations in solution. Also, L-penicillamine, a metal-chelating agent, did not solublize Pb well from the contaminated soil. The macrocyclic compounds examined here were reported to be employed in constructing metal-specific ligands based upon ring size and donor selectivity toward metal ions (*10*). The ether ligands complexed with Pb, and 1,4,7,10,13,16-hexaozacyclo octadecane and 1,4,7,10-tetracyclododecan were reported to selectively transport Pb^{2+} over Ag^{2+} in a three-phase transport system (*22*). Yet none of these compounds yielded significantly higher soluble soil Pb concentration than the water control in extraction experiments (Figure 1).

Interestingly, EDTA, a nonspecific synthetic chelate, appears to be the most efficient in solublizing soil-bound Pb relative to the other chelates that have similar binding constants to Pb in the particular Pb-contaminated soil tested here (Figure 2). There is no simple correlation between the amount of soluble Pb extracted and the ligand's binding constant with Pb, as in this study the Pb dissolution capability of the chelates tested, in ascending order at 120 h, is HBED, TETA, NTA, DTPA, HEDTA, and EDTA (Figure 2), while the binding constant log *K* is 18.24, 14.55, 11.4, 15.6, 18.66, and 18.1, respectively (*23*). This may perhaps be explained by the presence of other cations with higher binding constants, in particular Fe^{3+}. However, in soil system phytoextraction,

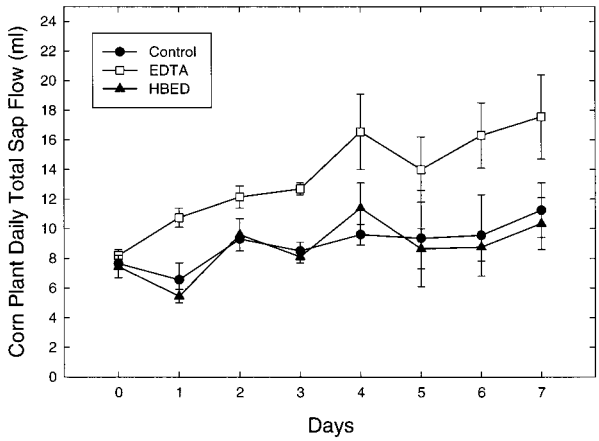


FIGURE 6. Effects of chelate application on corn transpiration. Plants were germinated and grown in Pb-contaminated soil for 10 days prior to soil chelate treatments with EDTA and HBED at 1.5 mmol chelate per kilogram soil. Water was used in the control treatment. Sap flow gages were installed to plants 3 days before chelate treatments, and the flow measurements were detailed in Materials and Methods. Total daily sap flow was integrated from the transpiration rate measured at 15-min intervals from 5:00 a.m. to 7:00 p.m. Data are mean ± SE from two sensors on two individual plants for each treatment.

chelate binding to various metal ions is most likely not in equilibrium, especially considering the constant removal of phytoavailable metal ions. Plant uptake of Pb may be limited by the forms of soluble Pb, as well as the amount of soluble Pb content in soil.

The application of strong Pb chelates (with log of binding constant greater than 18), to Pb-contaminated soil results in soluble Pb in the water phase within the soil in the form of L−Pb complexes. It is increasingly clear from other reports that this complex form was directly taken up by plants (*6*). The accumulation of Pb in plants is therefore dependent on the physical and chemical properties of the L−Pb complex

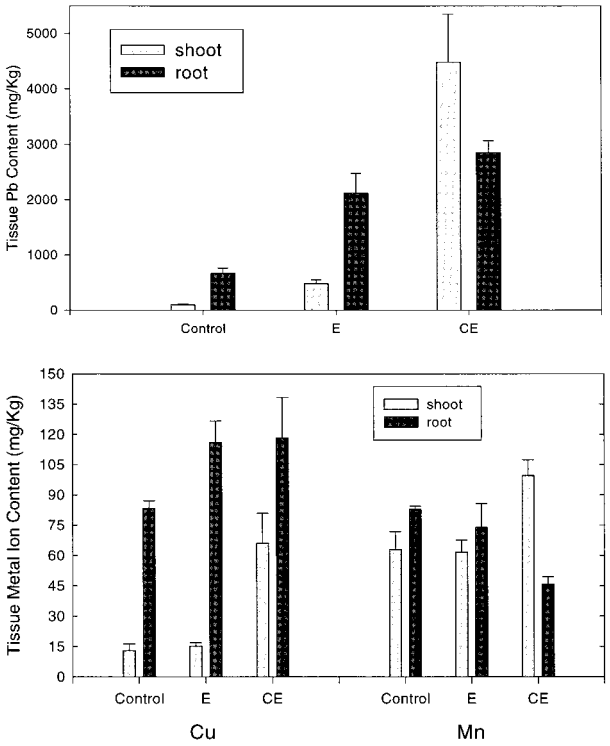


FIGURE 7. Effect of EDTA treatment and transplantation on corn Pb uptake and translocation. Control: directly seeded plants with water added to soil. E: directly seeded plants with EDTA added to soil. CE: transplanted plants followed by soil EDTA treatment.

which directly impact absorption, interactions with other solution ions, plant uptake, plant transport, and translocation. The uptake of a compound into roots, as measured by the root concentration factor (RCF), is correlated to a compound's log K_{ow}, in that the more lipophilic, the higher the RCF (*13*, *14*). Compared to EDTA−Pb, DTPA−Pb, and HEDTA−Pb, HBED−Pb is about 10 times more lipophilic, as indicated by the complex's log K_{ow} (Table 1). This higher lipophilicity was reflected in higher root Pb content in the HBED−Pb treatment (Figure 3). Even though EDTA is the best among all of the ligands tested here in solublizing soil-bound Pb, clearly, it is not the best from the perspective of plant root uptake, because of the high hydrophilicity of EDTA−Pb. Substitution of two carboxymethyl groups in EDTA with two hydroxylbenzyl groups generates HBED, which is much more lipophilic. This higher lipophilicity should give HBED−Pb improved root uptake and translocation over EDTA−Pb. Unfortunately, HBED also has a much higher affinity for Fe (log *K* 37.9) than for Pb (log *K* 18.2). This may be the cause of the higher root uptake, and yet little translocation up into the shoots as the strong affinity for Fe internal to the plant root displaces the Pb. In addition, HBED would prove a poor choice in a Pb-contaminated soil because of the high Fe content in all soils. Indeed, HBED, although transiently good at solublizing Pb into soil solution, eventually forms a stable complex with Fe over time (Figure 5). Despite the failure of this particular compound, the change in Pb−chelate lipophilicity toward higher RCF, if coupled to a more specific Pb−chelate, bodes well for the design of a better chelating agent. For maximum shoot accumulation of a compound, as measured by the transpiration stream concentration factor (TSCF), the optimal log K_{ow} should be higher than that of HBED−Pb, perhaps as high as many of the soil-applied herbicides which act similarly (e.g., log K_{ow} of 2.5−3) (*14*). Nevertheless, the results here point in the direction of designing a Pb-binding ligand for phytoremediation of Pb-contaminated soil by optimizing the Pb−chelate's lipophilicity and increasing Pb specificity.

Evidence in this study also suggests that changes in plant root transport pathways may lead to increased phytoextraction efficiency. Plant manipulation during growth will affect root transport pathways (*24*). Using trisodium 3-hydroxy-5,8,10-pyrenetrisulfonate (PTS), a fluorescent tracer for apoplastic transport pathways, Moon et al. (*24*) showed that transplanting *Avicennia marina* from sand to sand (repotting) resulted in xylem sap PTS concentrations two magnitudes higher in transplanted plants as compared with undisturbed plants and that plants lost the K^+: Na^+ selectivity. The data presented in this research indicate that transplanting plants significantly affected the total shoot Pb accumulation in EDTA amended soil, as a result of increased root uptake and increased root to shoot translocation (Figure 7).

Under normal plant growth conditions, the pathway not guarded by the endodermal Casparian strip constitutes less than 1% of the total water movement; however, under stress conditions, such as anaerobic conditions (*12*), salinity stress (*25*), root disturbance (*24*), and high phosphorus nutrition (*26*), the percentage of this pathway significantly increases. Therefore, it is likely that the stress caused by the transplantation enhances shoot Pb accumulation by increasing water flux through this pathway. EDTA−Pb is a very soluble complex as indicated by its low log K_{ow} (Table 1); therefore, Pb flux to shoot in the transport pathway unguarded by Casparian strip may well explain the increased shoot Pb content. It is further reasonable to assume that other treatments might also alter plant transport pathways that favor soluble Pb fluxes.

Our finding on the enhanced plant transpiration rate in EDTA treatments (Figure 6) merits consideration as an element for maximum Pb accumulation in shoots, even thought the reason for this change in the plant transpiration rate is unclear.

In summary, we have demonstrated in this study that the chemical and physical nature of the Pb−chelate in soil aqueous phase interacts with uptake mechanisms in the plant to contribute to the total phytoextraction efficiency. We conclude that the best soil amendment treatment so far for Pb phytoextraction is EDTA although it is not the most optimal for Pb uptake by plants. Future research using chelate amendments to increase Pb phytoextraction efficiency should be directed to (1) optimizing chelate structure for solublizing soil-bound Pb and increasing Pb binding specificity, (2) enhancing plant transport and translocation of Pb, and (3) manipulating plant transport pathways that favor the mass translocation of soluble Pb.

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