Environ. Sci. Technol. **1997**, *31*, 860−865

Enhanced Accumulation of Pb in Indian Mustard by Soil-Applied Chelating Agents

MICHAEL J. BLAYLOCK,*,† DAVID E. SALT,‡ SLAVIK DUSHENKOV,† OLGA ZAKHAROVA,† CHRISTOPHER GUSSMAN,† YORAM KAPULNIK,† BURT D. ENSLEY,† AND ILYA RASKIN‡

Phytotech, Inc., One Deer Park Drive, Suite I, Monmouth Junction, New Jersey 08852, and AgBiotech Center, Rutgers University, Cook College, P.O. Box 231, New Brunswick, New Jersey 08903

Phytoremediation is emerging as a potential cost-effective solution for the remediation of contaminated soils. Because contaminants such as lead (Pb) have limited bioavailability in the soil, a means of solubilizing the Pb in the soil and facilitating its transport to the shoots of plants is vital to the success of phytoremediation. Indian mustard (*Brassica juncea*) was used to demonstrate the capability of plants to accumulate high tissue concentrations of Pb when grown in Pb-contaminated soil. Concentrations of 1.5% Pb in the shoots of *B. juncea* were obtained from soils containing 600 mg of Pb/kg amended with synthetic chelates such as EDTA. The accumulation of Pb in the tissue corresponded to the concentration of Pb in the soil and the concentration of EDTA added to the soil. The accumulation of Cd, Cu, Ni, and Zn from contaminated soil amended with EDTA and other synthetic chelators was also demonstrated. The research indicates that the accumulation of metal in the shoots of *B. juncea* can be enhanced through the application of synthetic chelates to the soil, facilitating high biomass accumulation as well as metal uptake.

Introduction

The use of plants to remove toxic metals from soils (phytoremediation) is emerging as a potential strategy for cost-effective and environmentally sound remediation of contaminated soils (*1*−*4*). Certain plants, known as metal hyperaccumulators, have been discovered that contain unusually high concentrations of heavy metals in their tissue. Hyperaccumulators of Ni and Zn, for example, contain as much as 5% of these metals on a dry weight basis (*5, 1*). Phytoremediation as a soil cleanup technology seeks to exploit the ability of metal-accumulating plants to extract metals from the soil with their roots and to concentrate these metals in above-ground plant parts. The metal-rich plant material can be safely harvested and removed from the site without extensive excavation, disposal costs, and loss of topsoil associated with traditional remediation practices.

The success of phytoremediation is dependent on several factors. Plants must produce sufficient biomass while accumulating high concentrations of metal. The metal-accumulating plants also need to be responsive to agricultural practices to allow repeated planting and harvesting of the metal-rich tissues. In addition, these plants should preferentially accumulate environmentally important toxic metals (e.g., Pb, Cd, Cr, etc.). Known metal accumulators do not meet these criteria. The ability to cultivate a high biomass plant with a high content of toxic metals on a contaminated soil will be a determining factor in the success of phytoremediation. Therefore, enhancing metal accumulation in existing high yielding crop plants without diminishing their yield is the most feasible strategy in the development of phytoremediation.

In addition, the availability of metal in the soil for plant uptake is another limitation for successful phytoremediation. For example, Pb, one of the most important environmental pollutants, has limited solubility in soils and availability for plant uptake due to complexation with organic matter, sorption on oxides and clays, and precipitation as carbonates, hydroxides and phosphates (*6*). For soils with pH between 5.5 and 7.5, where Pb solubility is controlled by phosphate or carbonate precipitates, the maximum activity of Pb^{2+} in the soil solution is approximately $10^{-8.5}$ M, or about 0.6 ppb (*7*). The Pb^{2+} activity in the soil solution should remain constant regardless of the magnitude of the soil Pb concentration because of the equilibrium between the solution and the solid phase in the soil. Extremes in soil pH above 7.5 or below 5.5 will either decrease or increase the solubility accordingly. In most soils capable of supporting plant growth, however, the soluble Pb levels will remain very low and will not allow substantial uptake by the plant even if it has the genetic capacity to accumulate this metal. Vegetation growing in heavily contaminated areas often has less than 50 μg/g Pb in the shoots (*8*). In addition, many plants retain Pb in their roots via sorption and precipitation with only minimal transport to the above-ground harvestable portions (*9, 4*). Nevertheless, some Pb hyperaccumulators have been reported in isolated instances in extremely contaminated soils. The most frequently cited example reports *Thlaspi rotundifolium* (L.) Gaud.-Beaup, with shoot Pb concentrations of 8500 μg/g dry weight (*10*), but very few other examples are found. Cultivars of *Brassica juncea* (L.) Czern. (Indian mustard, a high biomass forage and oil crop) have also demonstrated the ability to accumulate as high as 1.5% Pb in shoot tissues when grown in nutrient solution with high concentrations of soluble Pb (*11*). At lower Pb concentrations in solution, the shoot tissue accumulations were substantially less, although root concentrations were very high. In spite of the significant capacity of *B. juncea* plants to concentrate Pb and translocate it to the shoots in solution culture, little uptake into the shoots was observed in *B. juncea* plants growing in soils where Pb bioavailability is limited (*11*). In this paper, we report on the enhanced uptake of Pb accumulation in soil-grown *B. juncea* plants with synthetic chelates. The magnitude of this enhancement may be sufficient to make phytoextraction of Pb-contaminated soils a viable environmental technology.

Materials and Methods

Growth chamber studies were conducted to evaluate the effectiveness of soil-applied chelating agents at increasing metal uptake in a known metal accumulator, Indian mustard (*B. juncea*, cv. 426308) (*11*). A Sassafras Ap silt loam soil was collected from the Rutgers University Horticultural Farm and amended with $CdCO_3$, $CuCO_3$, $2NiCO_3{\cdot}3Ni(OH)_2{\cdot}4H_2O$, $PbCO_3$, and/or $ZnCO_3$ to achieve desired concentrations. The carbonate forms were selected in order to provide the metals in a form of limited solubility dependent on the reaction of the metals with the soil. The soil was treated with lime to pH 7.3 and fertilized with urea (150 mg of N/kg), potassium chloride (83 mg of K/kg), and gypsum (70 mg of $CaSO_4$/kg).

* Corresponding author fax: 908-438-1209; e-mail: soilrx@aol.com.
† Phytotech, Inc.
‡ AgBiotech Center.

S0013-936X(96)00552-4 CCC: $14.00

The effect of pH on metal uptake was also studied by adjusting the quantity of lime added to the soil to achieve soil pH values of 5.0, 6.0, 6.5, 7.0, and 7.5. The amended soils were allowed to equilibrate for a period of 2 weeks in the greenhouse undergoing three cycles of saturation with water and air-drying, before being remixed and planted. The soil was then placed in 8.75 cm diameter round pots (350 g of soil/pot) and planted with *B. juncea* seeds. Phosphate fertilizer was added as a spot placement of triple super phosphate 1 cm below the seeds at planting at the rate of 44 mg of P/kg. After seedling emergence, the pots were thinned to two plants per pot.

The plants were grown for 3 weeks in a growth chamber using a 16-h photoperiod and weekly fertilization treatments of 16 and 7 mg/kg N (urea) and K (KCl), respectively. Potassium salts of the synthetic chelators CDTA (*trans*-1,2-cyclohexylenedinitrilotetraacetic acid), DTPA (diethylenetrinitrilopentaacetic acid), EDTA (ethylenedinitrilotetraacetic acid), EGTA (ethylenebis[oxyethylenetrinitrilo]tetraacetic acid), and citric or malic acid were applied to the soil surface as solutions 3 weeks after seedling emergence using four replications of each treatment. The pots were placed in individual trays to prevent loss of amendments from leaching. Following the amendment applications, the soil was irrigated to field capacity on a daily basis. The plants were grown for 3 weeks after seedling emergence (at the onset of flowering) before applying chelates to the soil. The plants were harvested 1 week after the amendment treatment by cutting the stem 1 cm above the soil surface. The plant tissue was dried at 70 °C and then wet-ashed using nitric and perchloric acids. The resulting solution was analyzed for metal content by inductively coupled plasma spectrometry (ICP) (Fisons Accuris, Fisons Instruments, Inc., Beverly, MA). Soil samples were collected from the pots and analyzed for water-soluble metals by equilibrating 2.5 g of soil with 25 mL of 0.01 M KNO_3 for 2 h. The suspensions were centrifuged, and the supernatant solution was analyzed for soluble metals by ICP. Certified National Institute of Standards and Technology plant and soil standards were carried through the digestions, extractions, and analysis as part of the QA/QC protocol. Reagent blanks and spikes were used where appropriate to ensure accuracy and precision in the analysis.

A further investigation into this observed phenomenon of chelate-enhanced uptake was conducted using a hydroponic system in the growth chamber. *B. juncea* seedlings were grown for 3 weeks in a nutrient solution containing 28.7 mg/L $NH_4H_2PO_4$, 0.71 mg/L H_3BO_3, 164.1 mg/L $Ca(NO_3)_2$, 0.02 mg/L $CuSO_4$, 2.66 mg/L ferric tartrate, 60.19 mg/L $MgSO_4$, 0.45 mg/L $MnCl_2$, 0.004 mg/L MoO_3, 151.7 mg/L KNO_3, and 0.055 mg/L $ZnSO_4$. The 3-week-old plants were transferred to solutions of distilled water adjusted to either pH 3.5 or pH 5.5 using HNO_3 containing $PbNO_3$ and/or K_2EDTA at equimolar (0.2 mM) rates. The plants were harvested 1 week after the imposition of the Pb and EDTA treatments. The roots and shoots were rinsed with DI water and analyzed for metal content.

A field study was also conducted at a former cable manufacturing site in Bayonne, NJ, with Pb-contaminated soil (pH 8.3, 1200 mg of Pb/kg). The soil was fertilized with 150, 44, 83, and 70 mg/kg N, P, K, and $CaSO_4$, respectively, before rototilling to 15 cm depth. The surface soil (0−15 cm) was then excavated and placed in lysimeters (48-qt ice chests). Approximately 65 kg of soil was placed in each lysimeter and placed on the surface of the soil in the field. *B. juncea* seeds were planted and grown for 3 weeks before treatment application. Applications of EDTA and acetic acid were given as 1-L solutions applied to equal 5.0 mmol/kg EDTA and acetic acid. A light irrigation (0.25 cm) was provided following the application of the amendments. The plants were harvested 1 week after the amendment application. Root and shoot tissue was collected and washed with DI water to remove soil deposition before analysis.

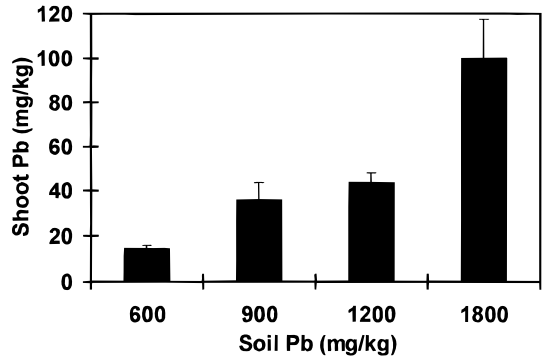

FIGURE 1. Shoot Pb concentrations of *B. juncea* grown in Sassafras Ap soil (limed to pH 7.3) with Pb added as lead carbonate.

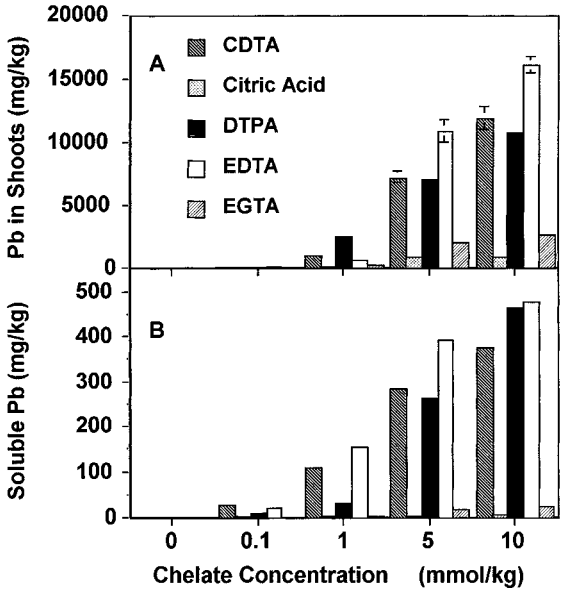

FIGURE 2. Shoot Pb concentrations of *B. juncea* (A) and water-soluble Pb (B) in Sassafras Ap soil (limed to pH 7.3) containing 600 mg of Pb/kg as lead carbonate as a result of soil-applied chelating agents.

Results

Metal concentration in the soil is expected to play a large role in determining the metal uptake by the plant and the metal content of the shoot material. In the case of Pb, however, a high total Pb concentration in the soil does not necessarily result in high Pb concentrations in the shoots due to its insolubility. In soils with Pb added as lead carbonate at the rates of 600, 900, 1200, and 1800 mg/kg, only very low levels of Pb (<100 mg/kg) were accumulated in the shoots of *B. juncea* (Figure 1). The accumulation was dependent on the soil concentration and increased from 45 to 100 mg/kg as the soil Pb increased from 1200 to 1800 mg/kg.

Through the addition of synthetic chelators to metal-contaminated soil, accumulation of Pb in *B. juncea* was enhanced. The initial study was conducted to evaluate the potential of soil-applied chelators and amendments at four different concentrations to enhance the uptake of Pb and other metals from contaminated soils. Subsequent studies were conducted using lower concentrations of EDTA to optimize chelate additions with respect to potential field applications. The application of EDTA, DTPA, CDTA, EGTA, and citric acid to the soil solubilized Pb in the soil and also increased Pb uptake and translocation to the shoots (Figure 2). The concentration of Pb in the shoots increased with the concentration of chelator applied to the soil. A dramatic increase in shoot Pb concentrations occurred between the 1 and 5 mmol/kg EDTA treatments. Both EDTA and DTPA

TABLE 1. Dry Matter Yield of *B. juncea* Grown in Soil Treated with Chelating Agents[a]

chelating agent	concentration (mmol/kg)				
	0 (g/pot DW)	0.1 (g/pot DW)	1.0 (g/pot DW)	5.0 (g/pot DW)	10 (g/pot DW)
CDTA	2.05 ± 0.16	2.07 ± 0.22	1.95 ± 0.38	0.99 ± 0.09	1.13 ± 0.04
DTPA	2.05 ± 0.16	2.53 ± 0.19	1.05 ± 0.09	1.11 ± 0.15	1.31 ± 0.03
EDTA	2.05 ± 0.16	2.37 ± 0.05	1.38 ± 0.17	0.93 ± 0.08	1.02 ± 0.13
EGTA	2.05 ± 0.16	2.33 ± 0.22	1.88 ± 0.25	0.88 ± 0.09	1.17 ± 0.15
citric acid	2.05 ± 0.16	2.08 ± 0.13	1.54 ± 0.12	0.95 ± 0.10	0.89 ± 0.18

[a] Plants were grown for 3 weeks before treatment applications. Values are means ±1 SE.

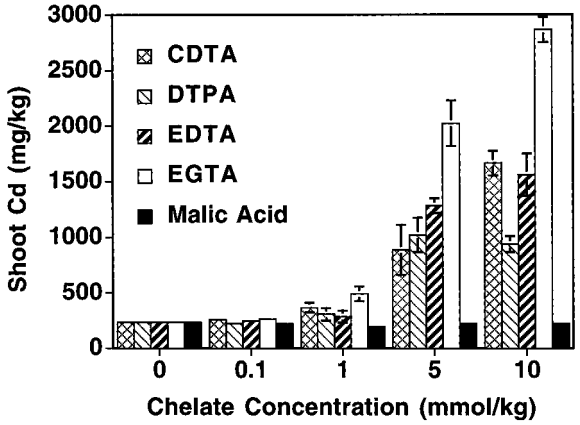

FIGURE 3. Effect of soil-applied chelating agents on shoot Cd concentrations in *B. juncea* grown in a Sassafras Ap soil amended with cadmium carbonate (100 mg of Cd/kg) and limed to pH 7.3.

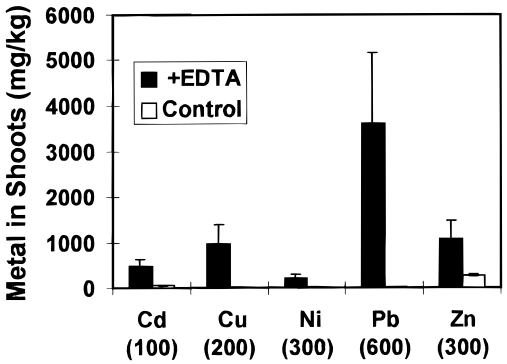

FIGURE 4. Enhancement of shoot metal concentrations in *B. juncea* grown in Sassafras Ap soil containing added Cd, Cu, Ni, Pb, and Zn through soil-applied EDTA (2.5 mmol/kg). Soil metal concentrations are given (in mg/kg) in parentheses.

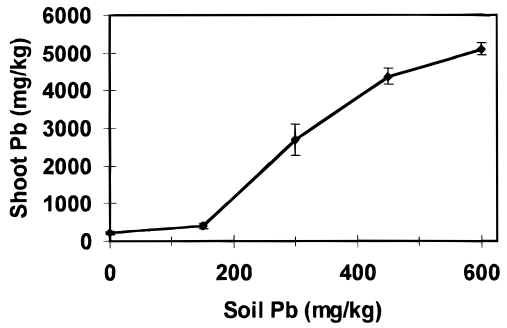

FIGURE 5. Accumulation of Pb in shoots of *B. juncea* grown in a Sassafras Ap soil (limed to pH 7.3) with Pb added as lead carbonate and amended with 0.5 mmol/kg EDTA.

solubilized approximately 470 mg of Pb/kg (2.2 mmol of Pb/kg) in the soil at the 10 mmol/kg rate; however, the EDTA application produced plants with 1.6% Pb in the shoots as compared to only 1.0% Pb in the shoots of DTPA-treated plants (Figure 2A). The Pb solubilizing capacity of CDTA in the soil was similar to DTPA at the 5 mmol/kg rate and resulted in greater Pb uptake as compared to DTPA in the plant tissue at the 5 and 10 mmol/kg rates. EGTA was much less effective at solubilizing Pb in the soil and also at increasing Pb uptake by the plants. Citric acid produced only a small increase in Pb uptake by the plants. In the absence of an applied chelating agent, Pb uptake in the shoots was minimal.

Plant dry matter yield was also significantly affected by the application of the treatments. Plants grown in untreated or the 0.1 mmol/kg treated soil produced nearly twice the biomass of the plants receiving the 10 mmol/kg chelate application (Table 1). However, the magnitude of the metal accumulation at the 10 mmol/kg treatment was several thousand to over ten thousand times greater than the control treatments. The untreated plants nearly doubled their biomass during the time period between the treatments and harvest.

The uptake of Cd was also enhanced through the addition of chelating agents to the soil (Figure 3). The addition of EGTA to the soil increased Cd shoot concentrations to approximately 2800 mg/kg in the 10 mmol/kg treatment as compared to only 220 mg/kg in the control treatments. CDTA, DTPA, and EDTA were less effective than EGTA but still produced Cd accumulations of approximately 1000 mg/kg at the 5 mmol/kg treatment and 1500 mg/kg at the 10 mmol/kg treatment of CDTA and EDTA. Increasing the DTPA rate from 5 to 10 mmol/kg did not increase Cd uptake into the shoots. Malic acid amendments did not increase Cd uptake above the control treatment.

An additional study using a soil containing multiple metal contaminants showed that the ability of a soil-applied chelator such as EDTA to increase metal uptake is not limited to Pb and Cd. A 2.5 mmol/kg EDTA treatment to soil containing Cd, Cu, Ni, Pb, and Zn substantially increased the uptake of those metals to the shoots of *B. juncea* (Figure 4). Shoot concentrations of Cu, Pb, and Zn were at or above 1000 mg/kg, with Pb showing the highest concentration at almost 3600 mg/kg. Converting the shoot metal concentrations to a molar basis, however, showed Cu, Pb, and Zn with similar concentrations of 15.3, 17.4, and 16.3 μmol/g, respectively. Shoot content of Cd and Ni were significantly less with respective concentrations of 4.3 and 3.5 μmol/g.

Because Pb is conceivably the most important metallic soil contaminant, we concentrated our subsequent research efforts on the phytoremediation of Pb and on defining the role of synthetic chelates in this process. As shown in Figure 2, increasing the solubility of Pb through the addition of a chelator can produce much higher concentrations of Pb in the plant than the increase in the total Pb concentration in the soil (Figure 1). This effect can be observed even at low total soil Pb concentrations. Soil containing total Pb concentrations added as lead carbonate (150, 300, 450, and 600 mg/kg) and amended with 0.5 mmol/kg EDTA produced plants with as much as 5000 mg of Pb/kg at the 600 mg/kg level, with a sharp increase in Pb content of the shoots as the soil Pb increased from 150 to 300 mg/kg (Figure 5). Other mechanisms of increasing metal solubility, such as soil acidification, can also increase metal uptake somewhat, but

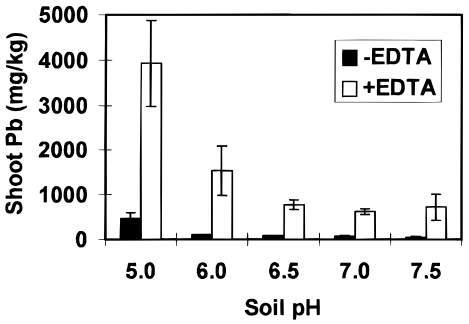

FIGURE 6. Shoot Pb concentrations of *B. juncea* grown in Sassafras Ap soil limed to different pH values with 600 mg of Pb/kg added as lead carbonate and amended with 2.5 mmol/kg EDTA.

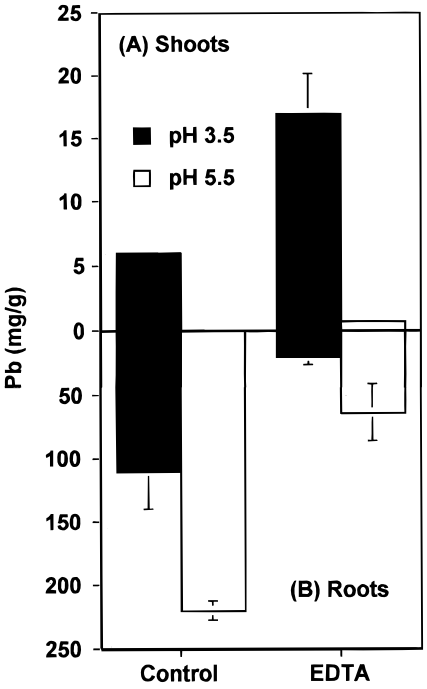

FIGURE 7. EDTA effects on Pb distribution between roots and shoots of *B. juncea* grown in solution culture at pH 3.5 and pH 5.5.

concentrations greater than 1000 mg of Pb/kg in the shoot material were only observed through the addition of a chelator. Figure 6 shows the effect of soil pH in combination with EDTA amendments on Pb accumulation in *B. juncea*. Lead uptake increased slightly to 445 mg/kg as the pH decreased to 5.0 in the absence of EDTA, but increased dramatically to almost 4000 mg/kg when EDTA was added to the pH 5 soil. The uptake of Pb from hydroponic solutions was also strongly related to solution pH in the presence of EDTA in the solution (Figure 7). At pH 5.5 without EDTA addition, only trace levels of Pb were found in the shoot tissue. The addition of 0.2 mM EDTA increased the shoot tissue Pb to approximately 0.8 mg/g. At pH 3.5 shoot Pb uptake was substantially higher. In the absence of EDTA, the shoots accumulated 6 mg of Pb/g. EDTA increased the Pb uptake in this treatment to 17 mg/g. Root Pb concentrations decreased at the lower pH and with the addition of EDTA. Roots in the control treatment at pH 5.5 contained 225 mg of Pb/g. Lowering the solution pH to 3.5 decreased the root Pb to 110 mg/g, and the addition of EDTA decreased the root Pb to 65 and 20 mg/g at pH 5.5 and pH 3.5, respectively.

A field study was conducted to assess the effectiveness and feasibility of using a soil-applied chelator to facilitate metal removal as part of phytoremediation activities. Addition of EDTA to soils in the field lysimeter studies confirmed the laboratory studies showing the effectiveness of a soil-applied chelator at increasing Pb uptake. The addition of EDTA increased Pb uptake in the shoots from 28 mg/kg in the control treatments to 785 mg/kg. Acetic acid was applied in conjunction with EDTA to determine if a reduction in soil pH would also enhance Pb uptake in the field. Application of the acetic acid with EDTA resulted in a further increase in Pb accumulation to 1475 mg/kg Pb in the shoots (Table 2) even though the soil pH decreased only slightly from 8.3 to 7.8. The accumulation of Pb in the shoots was also greater than root Pb concentrations with the addition of the EDTA and acetic acid, indicating decreased binding of Pb by the root tissue as a result of the EDTA application.

TABLE 2. Pb Uptake in Root and Shoots of *B. juncea* in Field Lysimeter Studies

treatment	shoot Pb (mg/kg)	root Pb (mg/kg)
EDTA	785	152
EDTA + acetic acid	1471	466
control	28	101

Discussion

Successful phytoremediation is a combination of several steps and processes. Because total metal removal is a function of the metal concentration in the shoots and the total harvestable biomass, the first step in phytoremediation is to produce high rates of plant biomass at the contaminated site. This can be accomplished through intensive cultivation, which facilitates rapid plant establishment and growth. *B. juncea* and other oil-seed Brassicas can produce 18 t/ha of biomass (*12*) in approximately 2.5 months of cultivation. Growth rates in excess of 200 kg ha of dry matter^{-1} day^{-1} are normally achieved by these plants under agricultural conditions (*13*). Biomass production will likely be lower for the plants cultivated on metal-contaminated sites, which are not ideal for agriculture.

The second step vital to the success of phytoremediation is the induction of Pb accumulation in the shoots. Even *B. juncea*, which has the genetic potential to accumulate Pb and Cd (*11, 14*), cannot efficiently remove these metals from the soil matrix unless they are solubilized. Therefore, successful phytoremediation must involve mobilization of toxic metals into the soil solution that is in direct contact with plant roots. We have shown that this can be achieved with the use of synthetic chelates (Figure 2).

In soil, the applied chelate acts first to complex the soluble metals in the soil solution. As the free metal activity decreases, dissolution of bound metal ions begins to compensate for the shift in equilibrium. This process continues until the chelate is saturated, the supply of metal from solid phases is exhausted, and/or equilibrium is achieved and the insolubility of the solid phase restricts the activity of the free metal. In the case of EDTA, the formation of Pb−EDTA is expected to be the dominant metal−EDTA complex in most soils between pH 5.2 and pH 7.7, providing that the total Pb concentration and the solubility of the specific Pb solid phase(s) is not limiting (*15*). As a result, if EDTA is added in sufficient amounts, nearly all of the soluble Pb will be complexed as Pb−EDTA with only very low activities of Pb^{2+}. For example, adding 10 mmol of EDTA/kg of soil (Figure 2), which exceeds the quantity of Pb in the soil (approximately 3 mmol/kg), resulted in extraction of 2.2 mmol/kg of soluble Pb (73% of the total soil Pb). The remaining Pb was not available for complexation with the EDTA, presumably due to its speciation as highly stable complexes. The EDTA addition rates of 5, 1, and 0.1 mmol/kg resulted in a lower proportion of the total soil Pb being solubilized (64, 22, and 3%, respectively). The amount of Pb in the shoots was always directly proportional to the amount of EDTA added to the soil (Figure 2). The amount of soluble Pb in the soil appears to be a key factor

to the enhancement of Pb uptake. When total soil Pb is limiting, the soluble Pb pool may also be limiting, even in the presence of a chelator such as EDTA, with respect to the uptake of Pb by the root and translocation to the shoots (Figure 5).

The greater ability of EDTA to enhance Pb uptake above that of other chelators also appears to be related to the binding capacity of EDTA for Pb relative to the other chelators. Norvell (*16*) ranked chelating agents for Pb^{2+} at pH 7, under slightly reduced conditions, in the order of EDTA ≈ DTPA > CDTA > EGTA. In our studies, EGTA was the most effective chelator for enhancing Cd accumulation in *B. juncea*. This observation corresponds to predictions that, above pH 5.7, Cd−EGTA is expected to be the dominant metal−EGTA species (*15*) as compared to the less favorable Cd binding affinities of the other chelates tested. The extent of competition from other cations in the soil solution will vary between soil systems and therefore the effectiveness of the chelate, but it appears that the selection of chelators with the highest binding affinities based on existing thermodynamic data provides a good estimate of potential successful amendments for enhancing metal uptake.

Soil pH is an important parameter in determining the effectiveness of applied EDTA in enhancing metal uptake (Figure 6). Corresponding results showing an increase in EDTA-extractable Pb as a function of decreasing pH have been noted in soil washing studies (*17*). The addition of soil-acidifying agents, such as acetic acid, had an additive effect on EDTA-mediated shoot metal uptake. Field lysimeter studies showed that amending an alkaline soil with acetic acid in the presence of EDTA almost doubled Pb accumulation in the shoots (Table 2). More detailed hydroponic experiments (Figure 7) confirmed that acidification stimulates Pb movement from roots to shoots. Lowering the pH of a hydroponic solution from 5.5 to 3.5 in the presence of EDTA dramatically increased shoot Pb accumulation and decreased Pb retention in the roots.

Lead retention in the roots is based on Pb binding to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of lead carbonates deposited in the cell wall (*9*). EDTA, in combination with low pH, effectively prevents cell wall retention of Pb, thereby making it available for translocation to the shoot. This effect is observed in both the soil (Figure 6) and hydroponic systems (Figure 7). Adding ^{14}C-labeled EDTA−Pb to the hydroponic medium resulted in the accumulation of ^{14}C-labeled compounds in the shoots (data not shown). Since the HPLC retention time of the ^{14}C-labeled compound was identical to an authentic EDTA standard, we assumed that shoots of experimental plants contained [^{14}C]EDTA. The amount of EDTA detected in the shoots was sufficient to chelate most of the Pb accumulated in the tissue. Therefore, it is likely that Pb enters the plant and is transported to the shoot as an EDTA complex.

The enhanced uptake of chelated Pb relative to ionic Pb contradicts the common understanding of chelate behavior in chelate-buffered nutrient solutions. It is generally believed that the chelated forms of metals are less available for uptake as compared to the ionic forms (*18, 19*). Some evidence suggests that chelated metals may be absorbed by the root. Root-exuded chelating agents (phytosiderophores) complexed with Fe are readily and preferentially absorbed by plants (*20*). The transport of synthetic chelate−metal complexes across the membrane is not widely accepted. Bell et al. (*21*) reported that barley leaf concentrations of Cu, Zn, and Mn were generally greater when total Cu, Zn, and Mn concentrations were greater despite maintaining constant activities of free Cu^{2+}, Zn^{2+}, and Mn^{2+} in solution through the use of chelators. The uptake of chelated metal was related to possible breaks in the root endodermis and Casparian strip and/or exchange between the chelated metal and the free metal in the rhizosphere and not uptake of the chelate metal complex (*21*). One important consideration is that much of the work conducted with chelates in nutrient solutions involved concentrations much lower than were applied in this study. The magnitude of the metal accumulation observed in this study (>1% shoot Pb concentrations) is also much greater than the metal accumulations observed in studies with much lower chelate concentrations, indicating that perhaps a different mechanism is involved.

Crop plants pump large amounts of water from the soils. The transpiration ratio of crop plants ranges between 850 and 300 kg of water used/kg of dry matter produced (*22*). We already reported that blocking transpiration blocks Cd uptake by *B. juncea* plants (*14*). We have also observed that placing soil-grown *B. juncea* plants close to a fan increased EDTA-mediated Pb uptake by 30%, while placing a plastic bag over a plant will reduce it by 35% (data not shown). These treatments should increase and reduce transpiration, respectively. Thus, we propose that transpiration is a major force that drives Pb accumulation in the shoots. Mass flow of water from the soil and into the plant delivers solubilized Pb to the shoots, where water evaporates and Pb remains. We also suggest that most of the mobile Pb in the plant is chelated by EDTA, which prevents its precipitation in the roots and vascular system. In addition, EDTA may directly facilitate Pb movement through the root cell membranes and extracellular spaces. This intriguing possibility requires further study.

Our data demonstrate that accumulation of five toxic metals by *B. juncea* plants can be enhanced with synthetic chelates (Figure 4). EDTA-enhanced accumulation of Pb was further enhanced by lowering the pH of the substrate. These amendments were applied several days before harvest when plants were already established. Applying chelates and other soil amendments in the field will certainly require further study and optimization to obtain the maximum metal uptake and removal. However, laboratory results show that plants can be treated to accumulate up to 1.5% of their dry weight in Pb from soils containing 600 ppm Pb. Assuming 6 t/ha biomass, 6 weeks of cultivation, and three crops per season, *B. juncea* plants can remove 180 kg of Pb/ha in a growing season. Ashing of the harvested biomass would further concentrate the extracted Pb by an additional 10-fold (assuming plant dry matter is 10% ash) resulting in a 250-fold concentration of the extracted Pb over the soil level. The duration of this extraction efficiency as the total soil Pb concentration decreases is unclear but will most probably be a function of the magnitude of the EDTA-extractable Pb pool.

We have estimated that the addition of chelators to the soil will result in an increased cost of $7.50/t of treated soil, which would not severely impact the overall cost of phytoremediation as an alternative. Additional concerns regarding the potential mobility of chelated metals are being addressed through modeling of hydrological dynamics of the soil profile and monitoring of metal migration. However, the integration of intensive cropping practices and development of extensive root systems along with careful management of irrigation should result in a net water loss from the soil during the growing season (*23*). Phytoremediation offers an attractive alternative for remediation of metal-contaminated soils. The results presented in this paper suggest that, with integration of this technology with the proper agronomical and engineering skills, phytoremediation of Pb-contaminated soils will soon become a competitive remediation tool.

Acknowledgments

This work was supported in part through a Small Business Innovation Research Grant (DE-FG02-95ER82053) awarded to Phytotech, Inc. by the U.S. Department of Energy. The

authors gratefully acknowledge the valuable technical assistance of Tracey Ledder.

Literature Cited

(1) Baker, A. J. M.; McGrath, S. P.; Sidoli, C. M. D.; Reeves, R. D. *Resour. Conserv. Recycl.* **1994**, *11*, 41.
(2) Chaney, R. L. *Land Treatment of Hazardous Wastes*; Noyes Data Corp.: Park Ridge, NJ, 1983; pp 50−76.
(3) Raskin, I.; Kumar, N. P. B. A.; Dushenkov, S.; Salt, D. E. *Curr. Opin. Biotechnol.* **1994**, *5*, 285.
(4) Salt, D. E.; Blaylock, M. J.; Kumar, N. P. B. A.; Dushenkov, V.; Ensley, B. D.; Chet, I.; Raskin, I. *Biotechnology* **1995**, *13*, 468.
(5) Brown, S. L.; Chaney, R. L.; Angle, J. S.; Baker, A. J. M. *Soil Sci. Soc. Am. J.* **1995**, *59*, 125.
(6) McBride, M. B. *Environmental Chemistry of Soils*; Oxford University Press: New York, 1994.
(7) Lindsay, W. L. *Chemical Equilibria in Soils*; Wiley: New York, 1979.
(8) Cunningham, S. D.; Berti, W. R.; Huang, J. W. In *Bioremediation of Inorganics*; Hinchee, R. E., Means, J. L., Burris, D. R., Eds.; Batelle Press: Columbus-Richland, 1995; pp 33−54.
(9) Dushenkov, V.; Kumar, N. P. B. A.; Motto, H.; Raskin, I. *Environ. Sci. Technol.* **1995**, *29*, 1239.
(10) Reeves, R. D.; Brooks, R. R. *Environ. Pollut. (Ser. A)* **1983**, *31*, 277.
(11) Kumar, N. P. B. A.; Dushenkov, V.; Motto, H.; Raskin, I. *Environ. Sci. Technol.* **1995**, *29*, 1232.
(12) Bhargawa, S. C. In *Oilseed Brassicas in Indian Agriculture*; Chopra, V. L., Prakash, S., Eds.; Vikas: New Delhi, 1991; pp 161−197.
(13) Downey, R. K.; Robbelen, G. Brassica species. In *Oil Crops of the World*; Robbelen, G., Downey, R. K., Ashri, A., Eds.; McGraw-Hill: New York, 1989; pp 339−362.
(14) Salt, D. E.; Prince, R. C.; Pickering, I. J.; Raskin, I. *Plant Physiol.* **1995**, *109*, 1427.
(15) Sommers, L. E.; Lindsay, W. L. *Soil Sci. Soc. Am. J.* **1979**, *43*, 39.
(16) Norvell, W. A. In *Micronutrients in Agriculture*, 2nd ed.; SSSA Book Series, No. 4; Mortvedt, J. J., et al., Eds.; Soil Science Society of America: Madison, WI, 1991; p 187.
(17) Peters, R. W.; Shem, L. In *Metal Speciation and Contamination of Soil*; Allen, H. E., et al., Eds.; Lewis Publishers: Ann Arbor, MI, 1995; pp 55−274.
(18) Brown, J. C.; Tiffin, L. O.; Holmes, R. S. *Plant Physiol.* **1960**, *35*, 878−886.
(19) Chaney, R. L. *J. Plant Nutr.* **1988**, *11*, 1033.
(20) Romheld, V.; Marschner, H. *Plant Physiol.* **1986**, *80*, 175.
(21) Bell, P. F.; Chaney, R. L.; Angle, J. S. *Plant Soil* **1991**, *130*, 51.
(22) Chrispeels, M. J.; Sadava D. E. In *Plants, Genes, and Agriculture*; Chrispeels, M. J., Sadava, D. E., Eds.; Jones and Bartlett Publishers: Boston, London: 1994; p 190.
(23) Allison, G. B.; Gee, G. W.; Tyler, S. W. *Soil Sci. Soc. Am. J.* **1994**, *58*, 6.

Received for review June 25, 1996. Revised manuscript received October 22, 1996. Accepted October 28, 1996.[⊗]

ES960552A

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.