Bull. Environ. Contam. Toxicol. (2003) 71:379–386

DOI: 10.1007/s00128-003-0175-1

Bulletin of Environmental Contamination and Toxicology

Phytoremediation of a Lead-Contaminated Soil Using Morning Glory (*Ipomoea lacunosa* L.): Effects of a Synthetic Chelate

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Received: 3 December 2002/Accepted: 16 April 2003

Lead (Pb) is one of the most persistent metals with a soil retention time of about 150-5000 years in the environment (Friedland 1990). Because of its history as an air emission pollutant, Pb has been fairly mobile and is particularly soluble in acid environments (Singer and Munns 1996). Also Pb is one of the most toxic metals in the environment with a limited solubility in soils and availability to plants due to complexation with organic matter and precipitates as carbonates, hydroxides, and phosphates (McBride 1994). Pb solubility is controlled by phosphates or carbonate precipitates in soils with pH 5.5 – 7.5 (Lindsay 1997). Extremes of soil pH above 7.5 and below 5.5 will either decrease or increase Pb solubility (Blaylock et al. 1997).

Phytoremediation, the use of plants to remove toxic metals such as Pb from soils, is one of the emerging and environmentally sound strategies for the clean up of contaminated soils. The success of phytoremediation depends on the selection of suitable plant species that produce large biomass, accumulate and translocate metal from the widespread root system to the above-ground biomass, and tolerate toxic concentrations of metals. Another requisite to successful phytoremediation is the availability of metals in a non-residual form for absorption by the roots and subsequent translocation to the shoot. Chelating agents such as ethylenedinitrilotetraacetic acid (EDTA), ammonium nitrite, citric acid, trans-1, 2-cyclohexylenedinitrilotetraacetic acid (CDTA), diethylenetrinitrilopentaacetic acid (DTPA), ethylenebis[oxyethlylenetrinitrilo]tetraacetic acid (EGTA), and malic acid (Blaylock et al. 1997; Huang et al. 1997; Begonia et al. 2002) were widely used in phytoremediation studies to increase the availability of Pb for uptake by plants. Phytoremediation has several advantages such as: (a) clean-up of contaminated soils, (b) easy to recycle the biomass and extract the heavy metals in an economic way, (c) reduce the costs associated with soil dredging, (d) reduce water pollution by avoiding dumping of dredged soils into coastal and inland waters, and (e) reduce lead concentrations in the air (Huang et al. 1997). Identification of hyperaccumulators of heavy metals is highly challenging. The significance of hyperaccumulators of metals was discussed in the clean up of contaminated sites using *Thlaspi caerulescens* (Brown 1995). Uptake of metals by plants may depend upon various factors such as type and concentration of metal, type of plant and its species, plant age, growth conditions, growth rate, type

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of soil and its physical and chemical conditions such as pH and organic matter content. Many plants transport only a small amount of Pb to the shoots and most of the absorbed Pb remain in the roots (Cunningham et al. 1995; Dushenkov et al. 1995). However, a hyperaccumulator such as *Thlaspi rotundifolium* (L.) accumulated 8500 μgPb/g dry weight (Reeves and Brooks 1983). Certain cultivars of *Brassica juncea* (L.) Czern accumulated about 1.5% Pb in shoot tissues in hydroponic cultures with high concentrations of Pb in solution (Nanda Kumar et al. 1995).

MATERIALS AND METHODS

Seeds of morning glory (*Ipomoea lacunosa* L.) were obtained from Azlin Seeds, Leland, MS. Plants were grown in the greenhouse at Jackson State University. Several bags (~18.5 kg/bag) of potting mix and delta top soil were purchased from Hutto's Garden, Jackson, MS and air-dried for 3-4 days under greenhouse conditions. Germination and growth of plants were highly responsive to a growth medium consisting of 2/3 potting mix and 1/3 delta top soil. Plastic pots (1.9L) were filled with approximately two kg of growth medium. A 7-inch plastic saucer was placed beneath each pot to prevent cross contamination between treatments. Any leachate collected in each saucer was poured back into its corresponding pot. Periodically, these saucers were also rinsed with deionized distilled water and were poured back into the respective pots.

Using standard procedures, soil samples were analyzed at the Mississippi State University Soil Laboratory to determine various physico-chemical parameters. Five EDTA concentrations (0, 0.1, 0.5, 1.0, 5mM) and four Pb (0, 500, 1000, 2000mg/L) treatments were arranged in factorial in a Randomized Complete Block (RCB) design with five replications. Lead (supplemented as lead nitrate) and EDTA were added as aqueous solutions to appropriate pots (250mL/pot) before seeding. Five germinated (radicle~5mm) seeds of morning glory were planted in each pot and were thinned out to two plants per pot nine days later. The plants were periodically watered with 100mL of deionized distilled water for the first two wks. Beginning at 3 wks after emergence, all plants were watered twice a wk with 100mL of modified Hoagland's nutrient solution (Begonia 1997). When the plants were approximately 4-5 wks old, 250mL of nutrient solution were added to each pot 3-4 times a wk and continued the same until the time of harvest. All the plants were harvested 7 wks after planting when they reached the flowering stage. Harvested roots were cleaned thoroughly with distilled water. Shoot, root, and soil samples were dried in an oven at 80°C for 36-48 hours to obtain a constant weight. Dried tissue samples were powdered using an Osterizer 14 Speed blender. Shoot tissue powders were stored in labeled plastic containers for subsequent Pb extractions. Lead contents of each 200mg plant tissue and 100mg soil samples were extracted using nitric acid-hydrogen peroxide and nitric acid-hydrofluoric acid, respectively (USEPA 1983). Lead contents of digestates were quantified using inductively coupled plasma (ICP) spectroscopy (Perkin Elmer Optima 3000) and expressed as μg Pb/g dry wt. The detection limit of the instrument was 1 ppb Pb.

RESULTS AND DISCUSSION

The physico-chemical characteristics of the growth medium are summarized in Table 1. Metals in the environment are considered as stress factors in that they cause physiological reaction changes (strain), which may result in total inhibition of plant growth, or resistance which can be achieved by tolerance by allowing a plant to survive the effects of internal stress (Levitt 1980). Tolerance therefore, is conferred by the possession of special physiological mechanisms, which collectively enable a plant to function normally even in the presence of high concentrations of potentially toxic elements (Baker 1987). Morning glory is one

Table 1. Physico-chemical properties of sandy clay loam (SCL) soil (n=4).

Soil parameter	Mean±SE
% Clay	4.38±0.36
%Silt	22.63±1.95
%Sand	73.00±1.72
pH	7.95±0.03
% Organic matter	6.24±0.60
Potassium (meq/100g)	9.59±2.82
Calcium (meq/100g)	17.30±7.78
Magnesium (meq/100g)	33.40±1.78
Total (meq/100g)	33.40±1.78
%Base saturation of potassium	19.34±0.32
%Base saturation of calcium	60.96±0.23
%Base saturation of magnesium	19.71±0.11
Extractable phosphorus levels (kg/hectare)	385.00±0.46
Extractable potassium levels (kg/hectare)	5669.00±389.41
Extractable calcium levels (kg/hectare)	9137.59±455.26
Extractable magnesium levels (kg/hectare)	1772.50±87.12
Extractable zinc levels (kg/hectare)	3.70±0.08
Extractable sulfur levels (kg/hectare)	992.77±104.63

of these metal-tolerant plant species, which survived and continued its normal growth and development even at 2000mg/L Pb and 5 mM EDTA amendments. There were no significant differences in shoot and root dry biomass of plants grown in different Pb concentrations (Figure 1). However, EDTA exerted a significant effect on shoot and root dry biomass. Within each Pb concentration, there was a discernible decrease in root biomass, especially at 5mM EDTA concentration. The decrease in biomass was more pronounced in roots exposed to higher concentration of EDTA. One of the keys to a successful phytoremediation of metal-contaminated soils is to identify the hyperaccumulators, which can produce greater quantities of root and shoot biomass even at toxic levels of heavy metals. In the present study, morning glory showed no morphological disorders during the entire growth period, and produced greater root and shoot biomass, which indicated that this is an excellent plant species for phytoremediation studies. Our observations are in direct contrast to previous studies indicating

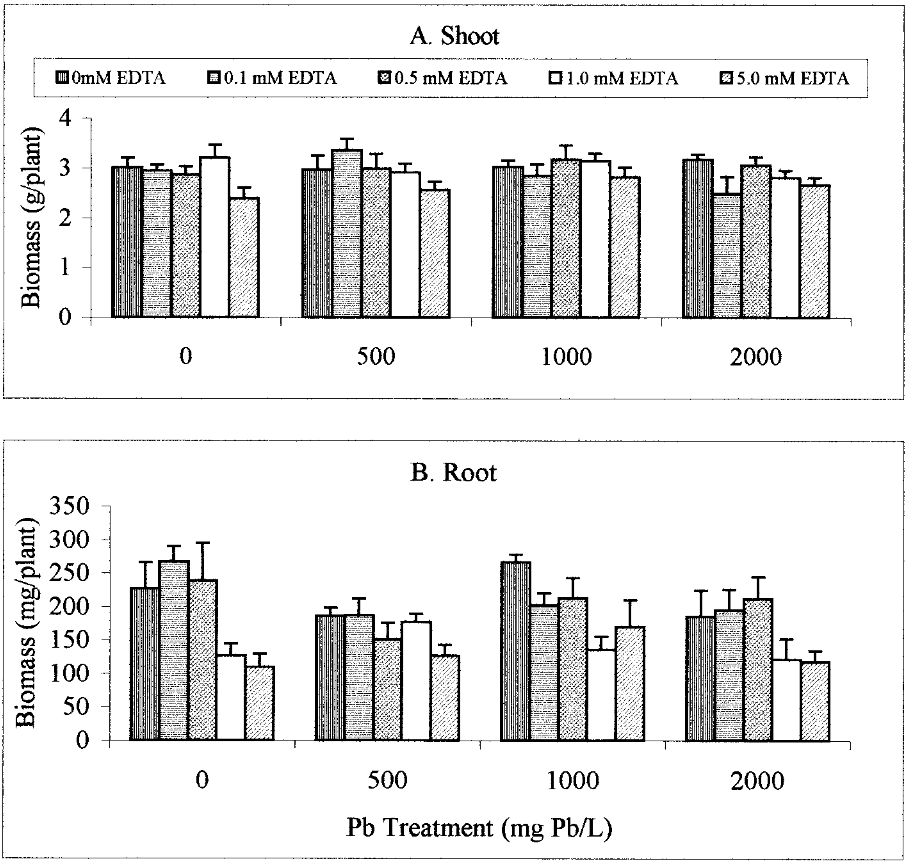

Figure 1. Effects of various concentrations of Pb and EDTA on shoot (A) and root (B) dry biomass of *Ipomoea lacunosa* (L). Vertical bars indicate standard error of the mean of 4 replications.

biomass reductions due to different doses of Pb (Ebbs and Kochian 1997; Zaman and Zereen 1998).

The elements are absorbed only to a limited extent, so this represents avoidance rather than tolerance. In other cases, the elements accumulate in roots with little translocation to shoots (Taylor 1987). In still others, both roots and shoots contain much higher amounts of metals, which represent true tolerance (Salisbury and Ross 1992). Recently, an important and phylogenetically widespread mechanism of tolerance was discovered. Metals are detoxified by chelation with phytochelatins, which are produced in plants when excess amounts of Zn and Cu are present. Plants with excess tolerance due to phytochelatins is a true adaptive response to an environmental stress (Rauser 1990). Morning glory may have the ability to synthesize phytochelatins to withstand against the toxic levels of Pb and well adapted to the high toxic levels of this metal (stress) in its growth medium (environment). This could be a subject for future research to further elucidate the mechanism of tolerance of morning glory to toxic Pb levels.

Metal concentration in the soil is not necessarily a major factor that governs its accumulation in the tissues of a plant (Blaylock et al. 1997; Huang et al. 1997). Concentrations of Pb in the shoot were relatively low in comparison to the root tissue due to the residual nature of Pb in the absence of chelating agents such as EDTA (Figures 2A and 2B). Availability of metal to the plant in a non-residual form plays a critical negative role in phytoremediation. In view of these constraints regarding bioavailability of metals, scientists have started testing different soils by amending them with different chelating agents such as EDTA, CDTA, malic acid, EGTA. (Blaylock et al. 1997; Nanda Kumar et al. 1995; Begonia et al. 2002).

Pb concentrations in root tissues were consistent with Pb levels in the growth medium. This means that plants exposed to higher soil Pb also had higher concentrations of root Pb (Figure 2A). Results on soil analyses (Figure 2C) indicated that there was no Pb in control (0mg/L) in all the EDTA-amended soils. Increasing levels of EDTA in the growth medium showed a decreasing trend in the remaining Pb in soil, which indicated that the availability of Pb to the plant was enhanced from 500mg/L Pb and 0mM EDTA to 2000mg/L Pb and 5mM EDTA (Figure 2C).

The surge in translocation of Pb from roots to the shoots was increased from 12.76±1.93 to 53.32±7.5% in soils that were amended with 0.1 and 5.0mM EDTA, respectively (Figure 2B). *Ipomoea lacunosa* L. exhibited an accumulation of Pb in its shoot tissues with increasing levels of EDTA. Results also indicated that shoot Pb increased as concentration of Pb in the growth medium was increased. Two way ANOVA indicated that the impact of EDTA, Pb concentrations, and their interaction in accumulation of Pb in shoot tissues was highly significant at 1% level. Statistical results based on Duncan's multiple comparison test confirmed that mean differences between all different treatments were significant ($P<0.05$), except for the treatments between 500 and 1000mg/L Pb concentrations.

The chelating agent, EDTA, had a great impact on the bioavailability of Pb to the roots of morning glory. In addition to the enhancement of bioavailability of Pb to the roots, it also enhanced the translocation of Pb from roots to the shoot tissues. Two way ANOVA confirmed that the impact of EDTA, Pb treatments, and their interactions were highly significant at 1% level on the remaining amount of Pb in the growth medium. Duncan's multiple comparison test showed that mean differences between different groups of Pb treatments were significantly different from their critical range values at $P<0.05$. Results of the present study also suggested that shoots of *Ipomoea lacunosa* L. have high ability to accumulate Pb (Figure 2B). In addition, availability and accumulation of Pb in shoot and root tissues showed significant relationships and interaction with EDTA in all

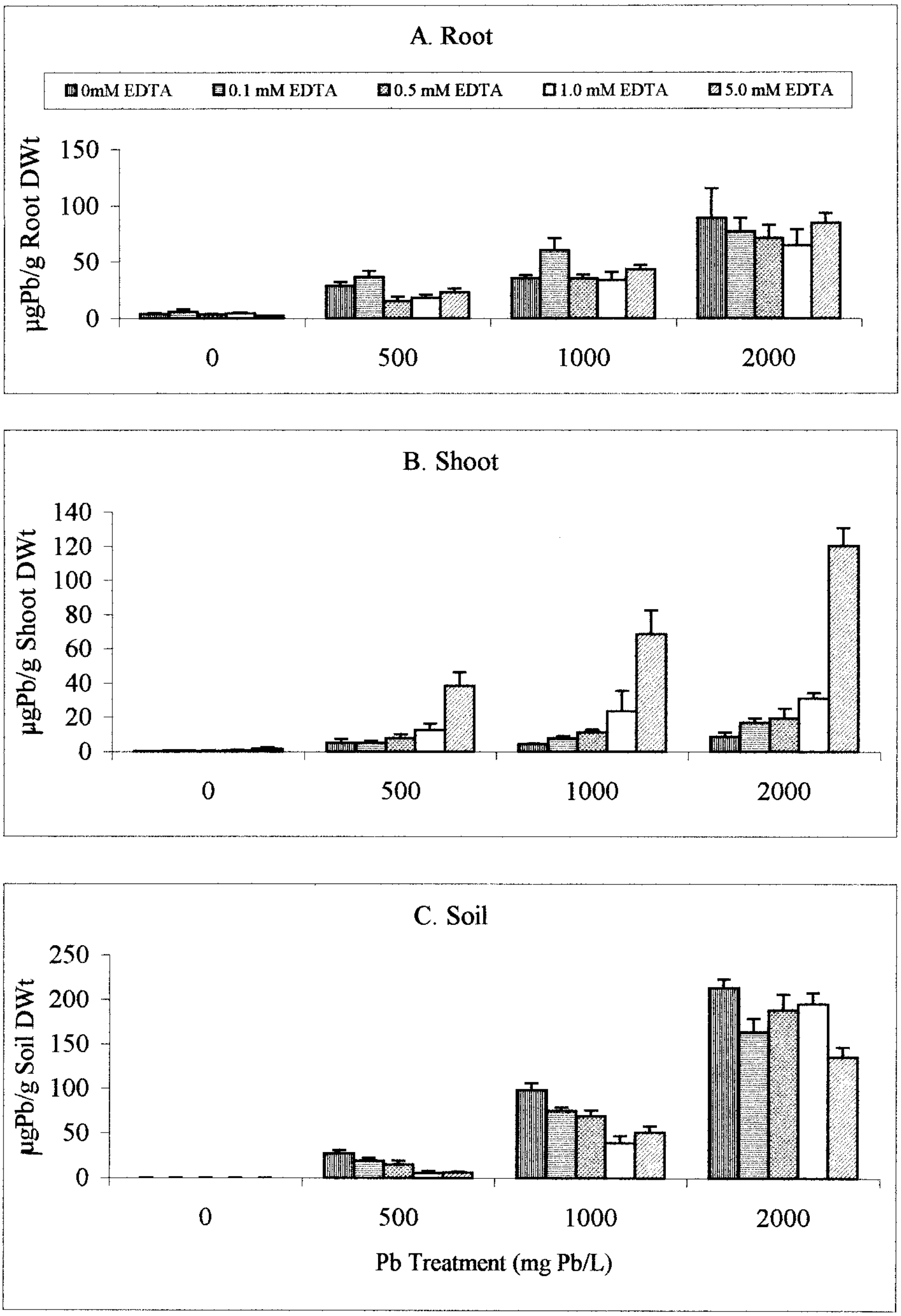

Figure 2. Effects of different levels of Pb and EDTA on Root (A), shoot (B), and soil (C) Pb concentrations after harvest. Vertical bars indicate standard error of the mean of 4 replicates.

treatments. Similar studies reflecting the enhancement of Pb accumulation in the above ground parts were reported in several species of *Brassica*, sunflower, corn, and beans by the addition of chelating agents such as EDTA, CDTA, DTPA, EGTA (Blaylock et al. 1997; Huang et al, 1997; Dushenkov et al. 1995).

Results of this study suggest that EDTA has eliminated two major constraints in the phytoremediation of lead-contaminated soils. They are: (a) low bioavailability of Pb, and (b) slow and/or no translocation of lead from roots to the shoot tissues. Amendments of soils with 5 mM EDTA showed a 50-fold increase in the percentage translocation of Pb from roots to the shoot tissues. This was one of the great attributes of morning glory that makes it a potential species for phytoextraction of Pb-contaminated soils.

Acknowledgments. The senior author expresses his gratitude to Jackson State University and Title III program at Southern University at New Orleans for their partial financial support to carry out this research at Jackson State University as a part of his dissertation.

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