Bull. Environ. Contam. Toxicol. (2002) 69:624–631

DOI: 10.1007/s00128-002-0106-6

Bulletin of Environmental Contamination and Toxicology

Chelate-Enhanced Phytoextraction of Lead-Contaminated Soils Using Coffeeweed (*Sesbania exaltata Raf.*)

G. B. Begonia, G. S. Miller, M. F. T. Begonia, C. Burks

Department of Biology, Jackson State University, Jackson, MS 39217, USA

Received: 2 May 2002/Accepted: 7 August 2002

Toxic heavy metal contamination of soils poses a major environmental and health problem, and as such, still needs an economical and environmentally safe technological remedy. In spite of the ever-growing number of toxic metal-contaminated sites, the most commonly used methods dealing with heavy metal pollution are either the extremely costly process of excavation and burial or simply isolation of the contaminated sites. Such cleanup is practical only for small areas, often a hectare or less, and cleaning a hectare to a depth of one meter costs between $600,000 and $3,000,000 depending on the type and intensity of pollution (Moffat 1995).

Recently, heavy metal phytoextraction has emerged as a promising, cost-effective alternative to the conventional engineering-based remediation (Salt et al. 1995). The objective of phytoextraction is to reduce heavy metal levels below regulatory limits within a reasonable time frame. To achieve this objective, plants must accumulate high levels of heavy metals and produce high amounts of biomass. Early phytoextraction research dealt with hyperaccumulating plants, which have the ability to concentrate high amounts of heavy metals in their tissues. However, hyperaccumulators often accumulate only a specific element and are slow-growing, low-biomass-producing plants with little known agronomic attributes. Moreover, there is no known hyperaccumulating plant for lead (Pb), one of the most widespread and toxic metal pollutants in soils.

Previous hydroponic studies revealed that uptake and translocation of heavy metals in plants are enhanced by increasing heavy metal concentration in the nutrient solution (Ghosh and Rhyne 1999). The bioavailability of heavy metals in the soil is therefore, of paramount importance for successful phytoextraction. Lead has limited solubility in soils, and its availability for plant uptake is minimal due to complexation with organic and inorganic soil colloids, sorption on oxides and clays, and precipitation as carbonates, hydroxides, and phosphates (McBride 1994). Therefore, successful phytoextraction must include mobilization of heavy metals into the soil solution that is in direct contact with the roots. In most soils

Correspondence to: G. B. Begonia

capable of supporting plant growth, the readily available levels of heavy metals, especially Pb, are low and do not allow substantial plant uptake if chelates are not applied. Chelates have been shown to desorb heavy metals from the soil matrix into soil solution (Jorgensen 1993), facilitate Pb transport into the xylem, and increase Pb translocation from roots to shoots of several fast-growing, high-biomass-producing plants (Blaylock et al. 1997; Huang et al. 1997; Vassil et al. 1998; Wu et al. 1999).

Using a modified hydroponic system (Ghosh and Rhyne 1999) and Pb-amended sand (Begonia et al. 2000), coffeeweed*(Sesbania exaltata Raf.)* was identified as a potential phytoextraction species because of its high biomass yield under elevated Pb levels and its ability to translocate high amounts (e.g., 15,770 mg/kg) of Pb into its shoots. The main objective of this study was to further evaluate the effectiveness of coffeeweed as a phytoextraction species. Specifically, this experiment was conducted to determine whether pre-plant or pre-harvest amendments of ethylenediaminetetraacetic acid (EDTA) alone or in combination with acetic acid can further enhance the shoot accumulation of Pb by coffeeweed grown on a Pb-contaminated soil.

MATERIALS AND METHODS

Plants were maintained under a naturally-lit greenhouse with 31°C/20°C day/night temperatures. Supplemental light for 12 hrs were provided by high intensity super halide lamps (1000 W H.Y. Lites Horizontal System, High Yield, Inc., Camas, WA). The photosynthetically active radiation (PAR; 400-700 nm) measured at the canopy level was no less than 1600 μmol photons m^{-2} sec^{-1} as measured with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Coffeeweed seeds were obtained from Azlin Seeds, Leland, MS. Unless otherwise specified, six seeds were sown in each 1.9L plastic pot containing a growth medium composed of sieved silty clay loam soil (pH 8.2; 1.5% organic matter), peat, and sand mixed in 4:2:1 volumetric proportions. Emerged seedlings were thinned out to 2 plants per pot at 5 d after planting. Using a hand throwel, three concentrations (0, 1000, 2000 mg Pb/kg dry growth medium) of Pb (supplied as lead nitrate) were thoroughly mixed with the growth medium before planting. These Pb levels are within previously reported Pb concentration ranges found in various contaminated sites (Blaylock et al. 1997; Huang et al. 1997; Salt et al. 1998). Plants were watered every 2 to 3 d, depending on the evaporative demand, with full strength nutrient solution (Begonia et al. 1998). In some treatments, EDTA (0, or 5 mmol/kg dry growth medium) was either incorporated with the growth medium before planting (pre-plant) or applied as a 100 mL aqueous solution one wk before harvest (pre-harvest). Moreover, a 100 mL aqueous solution of acetic acid (5 mmol/kg dry growth medium) was also added to some of the treatments one wk before harvest. On average, 100 mL of nutrient solution were added to each pot to ensure that soil moisture content was

maintained at field capacity and that no excess soil moisture drained from perforations at the bottom of each pot. A 7-in. plastic saucer was placed beneath each pot to prevent cross contamination between treatments.

Any symptoms of metal toxicity (e.g., discoloration, pigmentation, yellowing, stunting) exhibited by plants were visually noted during the experimental period. All plants were harvested at six wk after planting. During harvest, shoots and roots were separated, and roots were washed with distilled water to remove any adhering debris, then oven-dried at 70°C for 48 hr. Dried samples were weighed and ground in a Wiley mill equipped with a 425 μm (40-mesh) screen. Lead contents of each 200 mg dry, ground plant tissue were extracted using modified nitric acid-hydrogen peroxide procedures (Begonia et al. 1998). Lead concentrations were quantified using atomic absorption spectrometry (Thermo Jarrell Ash Model AA Scan 4) and expressed as μg Pb/g dry wt of plant tissue. This analytical system had a 98% recovery efficiency and detection limit of 5 ppb Pb.

In this experiment, each treatment replicate consisted of one pot containing 2 plants. Treatments were arranged in a completely randomized design (CRD) with four replications. Data were analyzed using Statistical Analysis System (SAS). Treatment comparisons were done using Fisher's Protected Least Significant Difference (LSD) test. In this study, a probability $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Regardless of Pb treatment, the timing of EDTA amendments, whether before planting (pre-plant) or a wk before harvest (pre-harvest), did not significantly affect root biomass (Table 1). However, there was a significant reduction in root biomass of plants grown at 2000 mg Pb/kg with pre-plant EDTA addition as compared to the untreated control. The biomass of coffeeweed shoots differed significantly and were generally lower with increasing concentrations of Pb treatment (Table 1). At lower Pb treatment (1000 mg Pb/kg), shoot biomass was significantly higher in plants grown in media with pre-harvest EDTA amendments than with plants treated with EDTA prior to planting. Most of the plants grown in Pb- and EDTA-amended soil appeared to be stunted with signs of mild chlorosis. Generally, the plants survived at all levels of applied Pb, however plants grown in media with EDTA only but no Pb exhibited the least shoot biomass (i.e., greatest stunting).

Coffeeweed exhibited reduced shoot biomass when grown at 5 mmol EDTA/kg and 0 mg Pb/kg, indicating the phytotoxic effects of the chelate at a relatively high concentration (Table 1). It is known that phytotoxicity causes stress to the plant

resulting in a reduction in biomass and eventual death (in some cases). Cunningham and Ow (1996) described one of the metal-resistance mechanisms in plants as a specific high-affinity ligand. These ligands, which are natural metal-binding peptides known as phytochelatins and metallothioneins, make the metal less toxic to the plant, and at a certain EDTA threshold, these ligands may be activated. We are not certain whether this resistance mechanism also exists in coffeeweed hence, further study is warranted. Vassil et al. (1998) also demonstrated that free protonated EDTA (H-EDTA) was more phytotoxic to *Brassica juncea* than a Pb-EDTA complex. This previous finding supports our present observation that at 0 mg Pb/kg, a 5 mmol/kg EDTA amendment was phytotoxic to coffeeweed. Although we did not observe any phytotoxic effect of 5 mmol EDTA/kg on wheat (Begonia et al. 2002), an amendment formulation combining lower EDTA doses and biosurfactants may be an attractive alternative to higher rates of soil EDTA application as suggested by Elless and Blaylock (2000).

Table 1. Effects of various concentrations of Pb and EDTA on coffeeweed root and shoot biomass.

Treatment		Biomass (mg/plant)	
Lead (mg Pb/kg)	EDTA (mmol/kg)	Root	Shoot
0	0	32.9 a	153.0 bc
0	5 Pre-plant	23.5 ab	49.4 e
1000	0	25.8 a	136.0 cd
1000	5 Pre-plant	19.4 ab	94.6 de
1000	5 Pre-harvest	25.3 ab	259.3 a
1000	5*Pre-harvest	26.5 a	209.4 ab
2000	0	16.8 ab	118.9 cd
2000	5 Pre-plant	7.9 b	62.9 e
2000	5 Pre-harvest	21.3 ab	85.2 de
2000	5*Pre-harvest	15.4 ab	169.3 bc

* indicates that an aqueous solution of acetic acid (5 mmol/kg growth medium) was added at the same time as the aqueous solution of EDTA. Means with a similar letter do not differ significantly using Fisher's Protected LSD test ($P \leq 0.05$).

Table 2 shows the root Pb concentrations of coffeeweed grown in different Pb and EDTA treatments. Root Pb accumulation was significantly greater with pre-plant than with pre-harvest application of EDTA, only at the highest Pb treatment (2000 mg Pb/kg). However, root Pb accumulation in plants exposed to both pre-harvest EDTA and acetic acid application were similar to Pb accumulation of

plants exposed to pre-plant EDTA amendments. It was also noted that at 1000 mg Pb/kg, majority of the absorbed Pb remained in the roots when no chelate was applied. This could be due to Pb binding to ion exchangeable sites on the cell wall and extracellular deposition in the form of Pb carbonates deposited on the cell walls as previously demonstrated (Dushenkov et al. 1995).

Lead accumulation by the roots was significantly higher with pre-plant application as compared to pre-harvest amendment of EDTA. It is suspected that the low root Pb accumulation with pre-harvest EDTA application may have been caused by the EDTA-Pb complex leaching downward into the planting pot, away from the root. Root depth and density, therefore, are important factors in phytoextraction. It was observed that during the duration of the study, the root system of coffeeweed was not extensive as compared to the fibrous root systems of most monocots including wheat in our recent study (Begonia et al. 2002). Roots provide a large surface-to-volume ratio to maximize the total uptake of various elements and compounds from the soil (Kumar et al. 1995). Using hydroponics systems, Dushenkov et al. (1995) concluded that root Pb absorption is a rapid process and may be the fastest component of metal removal by plants. But, the ability of plants to translocate Pb to shoots varies much more than their ability to accumulate metals in roots.

One of the requisites to the success of phytoextraction is the enhancement of Pb accumulation in the harvestable biomass (e.g., shoots). Vassil et al. (1998) demonstrated that coordination of Pb transport by EDTA enhances the mobility within the plants of this otherwise insoluble metal ion, allowing plants to accumulate high concentrations of Pb in shoots. In this study, shoot Pb accumulation increased with increasing concentrations of Pb applied to the growth medium. This increase was especially discernible in plants grown at 2000 mg Pb/kg growth medium with pre-plant EDTA amendments (Table 2). Also at 2000 mg Pb/kg growth medium, shoot Pb accumulation was significantly lower with pre-harvest EDTA and acetic acid addition, as compared to pre-plant amendments of 5 mmol EDTA. Shoot Pb accumulation was also lower in plants exposed to pre-harvest EDTA amendment as compared to plants treated with EDTA before planting, especially at the highest Pb treatment.

Lead, being a soft Lewis acid, forms a strong covalent bond not only with soil components but with plant tissue components as well (Huang et al. 1997). It is believed that since the xylem cell walls have a high cation exchange capacity, the upward movement of metal cations are severely retarded (Salt et al. 1998). Bringing the Pb into solution with a chelating agent, not only makes more Pb bioavailable for root uptake, but also moves the Pb that is sequestered in the xylem cell wall upwards and into the shoots. In an earlier study, Blaylock et al. (1997) demonstrated with Indian mustard (*Brassica juncea)* that induced phyto-

Table 2. Root and shoot Pb concentrations of coffeeweed grown in different concentrations of Pb and EDTA.

Treatment		Lead Conc. (μg Pb/kg dry wt)	
Lead (mg Pb/kg)	EDTA (mmol/kg)	Root	Shoot
0	0	0.0 c	0.0 d
0	5 Pre-plant	0.0 c	0.0 d
1000	0	4201.6 b	376.5 cd
1000	5 Pre-plant	2832.7 bc	2595.8 bc
1000	5 Pre-harvest	2700.8 bc	302.7 cd
1000	5*Pre-harvest	2801.0 bc	623.8 cd
2000	0	4106.2 b	506.0 cd
2000	5 Pre-plant	9322.7 a	8469.4 a
2000	5 Pre-harvest	3671.4 b	3512.0 b
2000	5*Pre-harvest	8181.5 a	615.7 cd

* indicates that an aqueous solution of acetic acid (5 mmol/kg growth medium) was added at the same time as the aqueous solution of EDTA. Means with a similar letter do not differ significantly using Fisher's Protected LSD test ($P \leq 0.05$).

extraction (i.e., equivalent to pre-harvest chelate amendment in our study) brings more of the Pb ions into solution and decreases the binding of Pb by the root tissue, thereby facilitating some of the desirable characteristics of a hyperaccumulator, such as high metal uptake by the roots, and translocation of the metal from the root to the above ground shoots. We believe that EDTA enhanced Pb desorption from soil to soil solution and facilitated transport from roots to shoots as previously demonstrated in EDTA-mediated phytoextraction studies using corn, peas and *Brassica juncea* (Huang et al. 1977; Blaylock et al. 1977; Vassil et al. 1998). Corollary studies relating the available Pb levels in soils and chelate-mediated shoot Pb uptake are currently being undertaken in our laboratory. Our preliminary results indicate that with EDTA application, there is a positive correlation between bioavailable Pb levels in soil and shoot Pb accumulation.

In a previous study by Blaylock et al. (1997) using EDTA and acetic acid, the pH of the soil was decreased only slightly from 8.3 to 7.8 . Similarly, in our experiment, the pH of the soil before planting was 8.2, and decreased to 7.4 at harvest. Soil pH not only represents an easily determined feature of soil but is an easily managed agronomic parameter as well. Several plant nutrients become less available to plants at the extremes of pH values and other elements become available in toxic amounts (Bridges 1970). Likewise, the bioavailability and plant

uptake for Pb (free lead) can be accomplished by lowering soil pH. In this study, it was observed that root Pb uptake increased as the soil pH was decreased (data not shown).

The results of this study indicated that coffeeweed can be an efficient Pb-accumulating plant when coupled with other phytoextraction strategies such as lower pH, and the use of a chelate. Chelates, however, may pose environmental risks and possible contamination of the groundwater if allowed to stay long in a polluted soil, a serious concern raised in many phytoextraction studies reviewed by Lasat (2002). It is therefore likely that further technical refinements are needed on chelate-assisted phytoextraction particularly the EDTA threshold requirements for efficiency of Pb uptake. Other engineering control measures will have to be provided to prevent leaching of soluble Pb into the ground water, thereby preventing a secondary source of Pb contamination (Huang et al. 1997).

Acknowledgments. Research support, graduate, and undergraduate fellowships were provided by a U.S. Department of Defense (Army)-funded Bioremediation Education, Science, and Technology (BEST) grant. We also thank Dr. Elgenaid Hamadain, Assistant Professor of Biology (JSU) and Dr. Yiming Liu, Assistant Professor of Chemistry (JSU) for their expert advise regarding statistical analyses and Pb determinations, respectively.

REFERENCES

Begonia GB, Davis CD, Begonia MFT, Gray CN (1998) Growth responses of Indian mustard [*Brassica juncea* (L.) Czern.] and its phytoextraction of lead from a contaminated soil. Bull Environ Contam Toxicol 61: 38-43

Begonia GB, Begonia MFT, Miller GS, Kambhampati MS (2000) Phytoremediation of metal-contaminated soils: Jackson State University research initiatives In: Centeno JA, Collery P, Vernet G, Finkelman RB, Gibb H, Etienne JC (eds). Proc. Sixth International Conf Metal Ions in Biology and Medicine 6: 682-684

Begonia MFT, Begonia GB, Butler A, Burrell M, Ighoavodha O, Crudup B (2002) Chelate-assisted phytoextraction of lead from a contaminated soil using wheat *(Triticum aestivum L.)*. Bull Environ Contam Toxicol 68: 705-711

Bridges EM (1970) World soils. University Press, Cambridge

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31: 860-865

Cunningham SD, Ow WD (1996) Promises and prospects of phyto-remediation. Plant Physiol 110: 715-719

Dushenkov V, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Environ Sci Technol

29: 1239 -1245

Elless MP, Blaylock MJ (2000) Amendment optimization to enhance lead extractability from contaminated soils for phytoremediation. Int J Phytol 2: 75-89

Ghosh S, Rhyne C (1999) Influence of EDTA on Pb uptake in two weed species, *Sesbania* and *Ipomoea*, in hydroponic culture. J Mississippi Acad Sci 44 :11

Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead contaminated soils: Role of synthetic chelates in lead phytoextraction. Environ Sci Technol 31: 800-805

Jorgensen SE (1993) Removal of heavy metals from compost and soil by ecotechnological methods. Ecol Eng 2: 89-100

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: The use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232-1238

Lasat MM (2002) Phytoextraction of toxic metals: A review of biological mechanisms. J Environ Qual 31: 109-120

McBride MB (1994) Environmental chemistry of soils. Oxford University Press, New York

Moffat AS (1995) Plants proving their worth in toxic metal cleanup. Science 269: 302-303

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of metals from the environment using plants. Biotechnology 13: 468-474

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Ann Rev Plant Physiol Plant Mol Biol 49: 643-668

Vassil AD, Kapulnik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol 117: 447-453

Wu J, Hsu FC, Cunningham SD (1999) Chelate-assisted Pb phytoextraction : Pb availability, uptake, and translocation constraints. Environ Sci Technol 33: 1898- 1905