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Chelate-Assisted Phytoextraction of Lead from a Contaminated Soil Using Wheat (*Triticum aestivum* L.)

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There has been an increasing interest in phytoextraction as a plant-based alternative for cost-effective and environmentally sound clean up of heavy metal-contaminated soils (Cunningham et al. 1995; Raskin et al. 1994; Salt et al. 1995). In phytoextraction, an efficient plant species must be able to absorb a substantial amount of the toxic metal into its roots and preferentially translocate the metal into the harvestable above-ground biomass for easier harvesting (Kumar et al. 1995). Through a cropping scheme, suitable species can be planted in succession ultimately leading to the reduction of soil metal concentrations to environmentally acceptable levels.

In addition to a plant's high biomass yield and tolerance to toxic metal levels, the success of phytoextraction is also dependent upon the availability of the toxic metal in the soil for plant uptake. For instance, lead (Pb), one of the most important environmental contaminants, has limited solubility in soils and its availability for plant uptake is minimal due to complexation with organic matter, sorption on oxides and clays, and precipitation as carbonates, hydroxides and phosphates (McBride 1994).

Another requisite to Pb phytoextraction is to increase and maintain Pb concentrations in the soil solution. Chelates have been used to increase the solubility of metal cations in soils (Means and Crerar 1978) and nutrient solutions and are reported to have significant effects on metal accumulation in plants (Jorgensen 1993; Ghosh and Rhyne 1998; Vassil et al. 1998). Recent studies (Blaylock et al. 1997; Huang et al. 1997) also demonstrated that addition of synthetic chelates to Pb-contaminated soils rapidly and dramatically increased Pb concentration in soil solution which consequently triggered a surge of Pb accumulation in shoots of corn, peas and Indian mustard.

Using a modified hydroponics system (Ghosh and Rhyne 1998) and Pb-amended sand (Begonia et al. 2000), wheat (*Triticum aestivum* L.cv. TAM-109) was identified as a suitable phytoextraction species because

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of its high biomass yield under elevated Pb levels, and its ability to accumulate high amounts of Pb into its shoots. Also, wheat can be grown during the colder months of a year-round cropping scheme thus ensuring ground cover of an otherwise barren, contaminated soil. The main objective of this study was to further evaluate the effectiveness of *T. aestivum* as a phytoextraction species. Specifically, this experiment was conducted to determine whether amendments of ethylene-diaminetetraacetic acid (EDTA) alone or in combination with acetic acid can further enhance the shoot uptake of Pb by wheat when grown on a Pb-contaminated soil.

MATERIALS AND METHODS

Plants were maintained on a laboratory bench at Jackson State University (JSU) during the summer 2000 season. Supplemental lights for 12 hr 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 1400 μmol photons m^{-2} s^{-1} as measured with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Wheat (*Triticum aestivum* L. cv. TAM-109) seeds were obtained from Arrowhead Mills, Hereford, TX through a local store. Unless otherwise specified, four seeds were sown in each 150 mL elongated plastic subsample tube or supercell (Stuewe and Sons, Inc., Corvallis, OR) containing sieved silty clay loam soil (pH 8.2; 1.5% organic matter) and peat mixed in 2:1 volumetric proportions. Emerged seedlings were thinned out to a desired population density (2 plants per tube) at 5 d after planting. Using a hand throwel, four concentrations (0, 500, 1000, 2000 mg Pb/kg dry soil) 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 modified nutrient solution (Begonia 1997). In some treatments, EDTA (0, or 5 mmol/kg dry soil) was either incorporated with the growth medium before planting or applied as a 100 mL aqueous solution one wk before harvest. Moreover, 100 mL aqueous solutions of acetic acid (5 mmol/kg dry soil) were also added to some of the treatments one wk before harvest. On average, 5 mL of nutrient solution were added to each tube 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 tube.

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. Pb contents of each 200 mg dry, ground plant tissue were extracted using modified nitric acid-hydrogen peroxide procedures (Begonia 1997; Begonia et al. 1998). Pb 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 consisted of a group of 4 subsample tubes (2 plants per tube) giving a total of 8 plants per treatment. Treatments (i.e., groups of 4 subsample tubes) were arranged in a Completely Randomized Design (CRD) with four replications. Data were analyzed using Statistical Analysis System (software version 8). Treatment separations were done using Least Significant Difference (LSD) test. In this study, a probability of 0.05 or less was considered to be statistically significant.

RESULTS AND DISCUSSION

Since total metal removal is a function of the metal concentration in the harvestable biomass (e.g., shoots), the first requisite in phytoextraction is the production of high plant biomass yield at the contaminated site. Generally, *T. aestivum* not only produced high biomass but was able to tolerate elevated levels of Pb/EDTA from the soil (Figs. 1A and 1B). In fact, plants grown at the two highest Pb treatments with pre-plant or pre-harvest EDTA amendments even had slightly higher shoot biomass than the untreated plants. There were no discernible toxic effects (e.g., discoloration, anthocyanin pigmentation, yellowing, stunting) of Pb/EDTA on wheat (Fig. 1A). However, there was a slightly significant reduction in root biomass at 2000 mg Pb/kg in combination with EDTA and acetic acid amendments one wk before harvest (Fig. 1B). These observations confirm our previous findings regarding the relative tolerance of this wheat variety to various levels of Pb and EDTA (Begonia et al. 2000).

Root Pb uptake increased with increasing levels of soil Pb treatments (Fig. 2B). The addition of EDTA before planting or a wk before harvest did not improve Pb uptake by the roots. On the contrary, root Pb uptake was significantly reduced in wheat grown at 2000 mg Pb/kg in conjunction with an EDTA amendment one wk before harvest. This observation was in direct contrast to a previous study (Huang et al. 1997) which showed increased Pb accumulation in xylem sap of Pb-

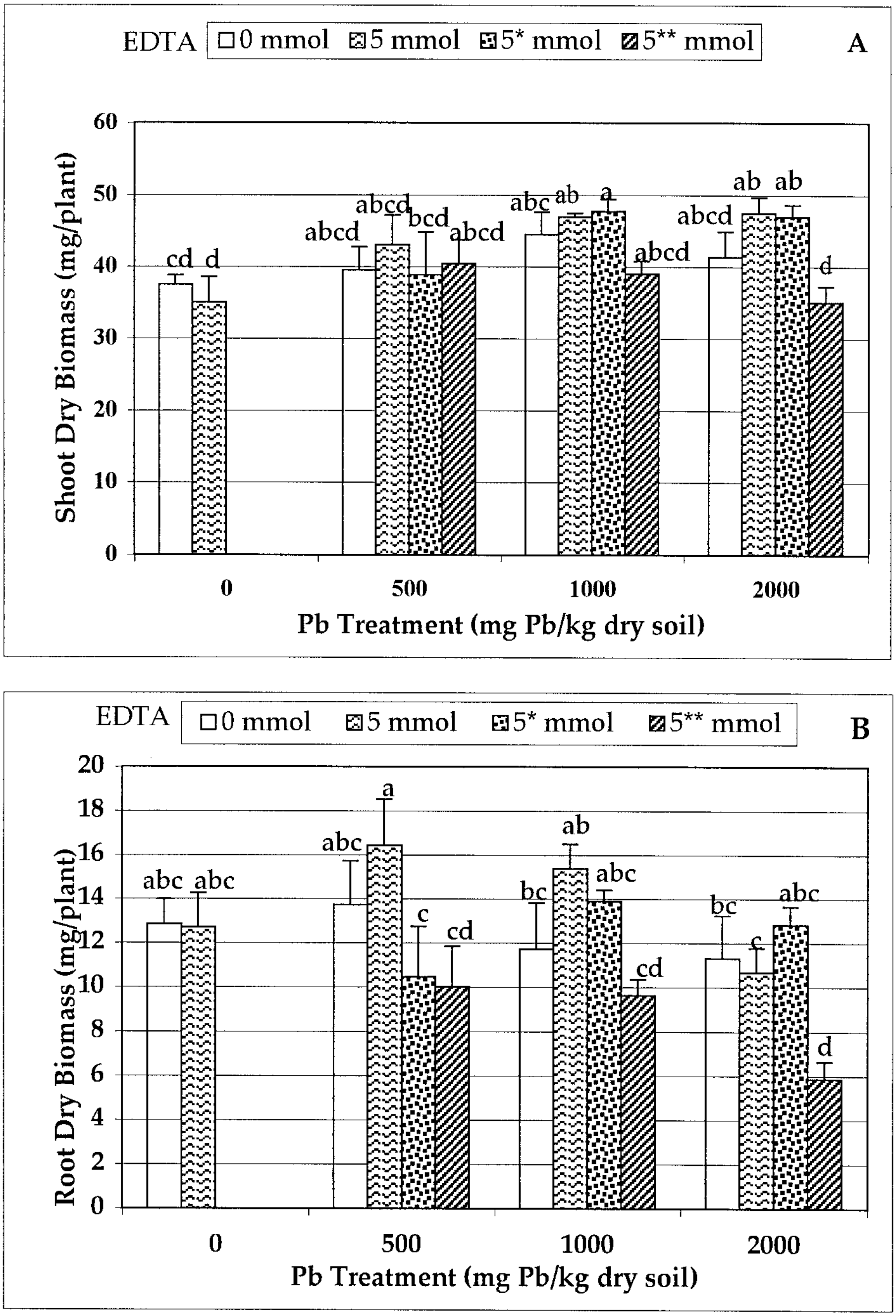
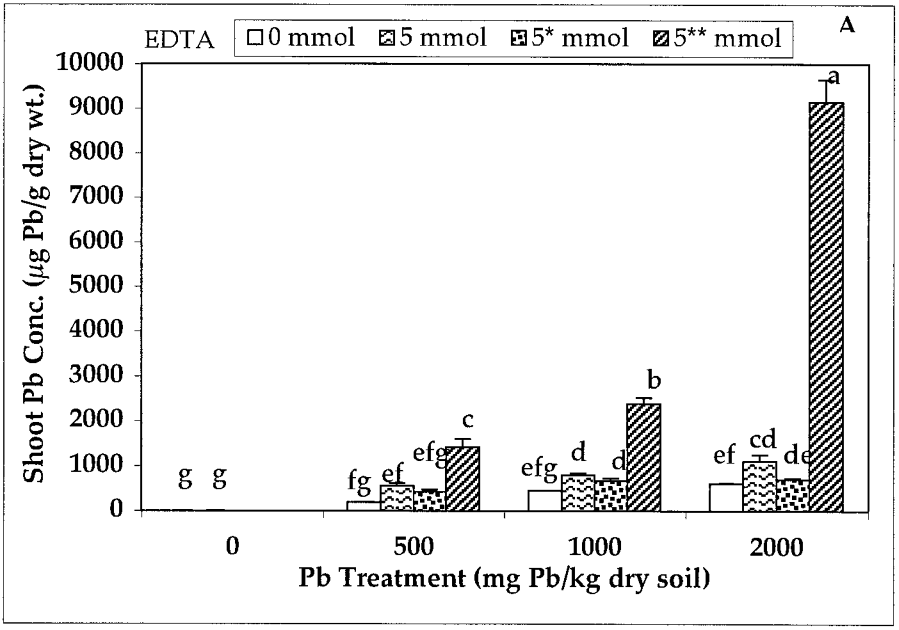


Figure 1. Effects of various concentrations of Pb and EDTA on shoot (A) and root (B) biomass. Vertical bars indicate SE (n=4). EDTA (5*) and EDTA plus acetic acid (5**) were applied one wk before harvest. Means with a similar letter do not differ significantly (LSD test, $p < 0.05$).



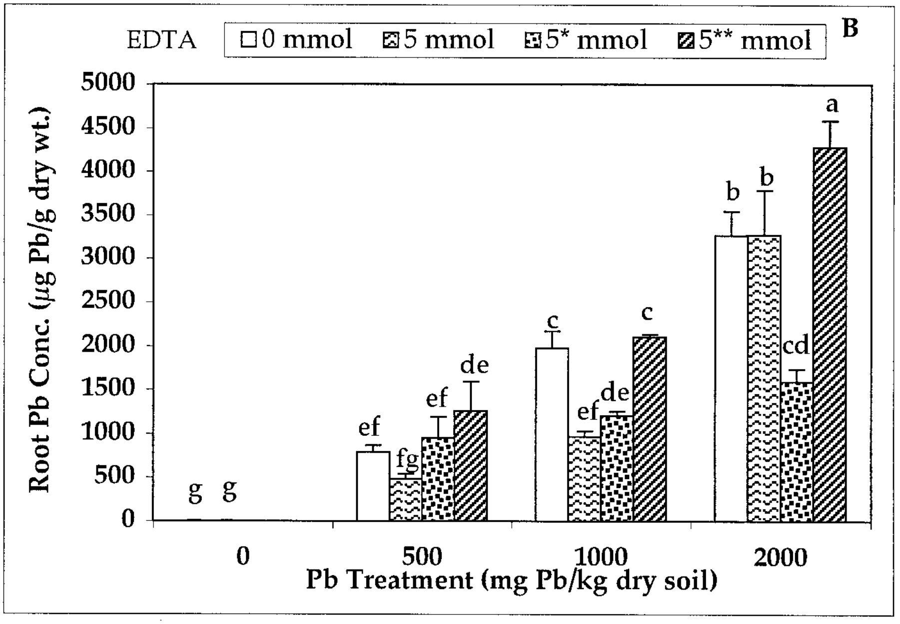


Figure 2. Effects of various concentrations of Pb and EDTA on shoot (A) and root (B) Pb concentrations. Vertical bars indicate SE (n=4). EDTA (5*) and EDTA plus acetic acid (5**) were applied one wk before harvest. Means with a similar letter do not differ significantly (LSD test, $p < 0.05$).

treated corn transplants 24 h following EDTA application. However, when both EDTA and acetic acid were applied a wk before harvest, there was a significant increase in root Pb uptake especially at the highest soil Pb treatment. 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 mainly in the form of Pb carbonates deposited on the cell walls as previously demonstrated (Dushenkov et al. 1995).

Another requisite 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, Pb uptake in the shoot increased with increasing levels of soil-applied Pb. EDTA amendment significantly increased shoot Pb uptake especially in plants treated with the two highest soil Pb treatments (Fig. 2A). Strikingly remarkable was the tremendous enhancement of shoot Pb uptake when both EDTA and acetic acid were applied one wk before harvest especially in wheat grown at 2000 mg Pb/kg soil. This additive effect of acetic acid on EDTA-mediated shoot Pb uptake by wheat is currently being undertaken in our laboratory, looking at the correlations between the amount of available soil Pb and Pb shoot uptake at different periods after acetic acid addition. We believe that EDTA especially in combination with acetic acid enhanced Pb desorption from soil to soil solution, facilitated transport into the xylem, decreased binding of Pb by the root tissue, and increased Pb translocation from the roots to shoots as previously demonstrated in EDTA-mediated phytoextraction studies using corn (*Zea mays* L. cv. Fiesta), pea (*Pisum sativum* L. cv. Sparkle) and Indian mustard [*Brassica juncea* (L.) Czern.] (Blaylock et al. 1997; Huang et al 1997).

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