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Cadmium and Lead Bioaccumulation In Cabbage Plants Grown In Metal Contaminated Soils

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Abstract

Phytoremediation, a relatively new green technology, uses plants to remove contaminants from the environment. In this study, we examined the potential of cabbage plants (*Brassica oleracea*) to remove cadmium (Cd) and lead (Pb) from contaminated soils. Plants were grown in soil containing 0 (control), 100, 250, 500, and 1000 mg/kg Pb; and 100, 250, and 500 mg/kg Cd. The plants were grown in the laboratory under 240 W color-corrected lights on a 16:8 hr. light: dark cycle. Plants were harvested after a 30-day growth period. Plants were then dehydrated for one week at 70^0 C in a laboratory incubator. Dry plant tissues were acid digested and analyzed for tissue metal content using Inductive Coupled Plasma Mass Spectrometry. Results showed that cabbage plants tolerated and extracted Cd and Pb from the soil and the metal uptakes were dose related. Therefore, these two plant species deserve attention to further explore their potentials in the remediation of Cd and Pb contaminated soils.

Keywords: Phytoremediation, Cabbage, *Brassica Oleracea*, Cadmium, Lead.

1.0 Introduction

Heavy metal contamination of soils is a serious problem in areas of intense industry and agriculture. Soils polluted with heavy metals pose health hazards to humans and often require remediation practices (Greman, 2005). The conventional approaches that are currently used for remediating metal contaminated soils are: (1) Landfilling: the excavation, transport and deposition of contaminated soil in a permitted hazardous waste landfill; (2) Fixation: the chemical processing of soils to immobilize the metals, usually followed by treatment of soil surface to eliminate penetration by water; (3) Leaching: using acid solutions or proprietary leachants to desorb and leach metals from soil followed by return of clean soil residue to the site (Salt et al., 1995). All these methods are prohibitively expensive (Salt et al., 1995). Because of the high cost, there is a need for less-expensive cleanup technologies. Phytoremediation is a cost-effective and environment-friendly cleanup alternative.

Phytoremediation uses plants to remediate environments contaminated by various pollutants. The identification of metal hyperaccumulator plants that are capable of accumulating extraordinarily high levels of metal contaminants, demonstrates that some plants have the genetic potential to cleanup contaminated soil (Lasat, 2002). This technology has been receiving attention lately as an innovative, cost-effective alternative to other established but expensive treatment methods to cleanup hazardous waste sites. Phytoremediation is receiving attention because it is cost effective (Adams *et al.*, 2000) and also provides opportunities for economic gain through landscape reclamation, employment opportunities and improved air and water quality (Licht & Isebrands, 2005).

Plants pump water, solutes, and organic matter from the surrounding medium as part of their natural physiological processes. The success of phytoremediation of metal contaminated soil depends on bioavailability (for uptake by roots) of the contaminants and plant's ability to intercept, absorb, and accumulate metals (Ernst, 1996; Khan *et al.*, 2000; Robinson *et al.*, 2003).

Previous studies in our laboratory indicated that Indian mustard (*Brassica juncea*), a member of *Brassica* family, is a Cd and Pb accumulator (Zaman et al., 2003; Shumaker *et al.*, 2009; Zaman & Lockett, 2010). Kumar *et al.* (1995) reported that

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Indian mustard plants were able to concentrate chromium, cadmium, nickel, zinc, and copper in the shoots. Since cabbage belongs to the same family, this research is focused to evaluate the potential of this plant to accumulate Cd and Pb from contaminated soils.

2.0 Methods

2.1 Experimental Plant and Growing Medium

The cabbage plants were grown from seeds purchased at a local Farmers Co-op store. The growing medium was Memphis silt loam soil. This soil was collected from an undisturbed forest area of Alcorn State University campus, in Southwest Mississippi. This is a well characterized soil containing about 70% silt, 20% clay, 9% sand, and 1% organic matter with a pH of 6.9 (Panicker, 1992).

2.2 Treatment Procedure

Plants were divided into several Cd and Pb treatment groups. Sixteen plants were used for each group. Pb treated plants were grown in soils containing 100 mg/kg, 250 mg/kg, 500 mg/kg, and 1000 mg/kg Pb. Cadmium treated plants were grown in soils containing 100 mg/kg, 250 mg/kg, and 500 mg/kg Cd. A 1000 mg/kg Cd treated group was not used in this experiment as previous studies in our laboratory indicated that Cd was more phytotoxic as compared to Pb and plants might not survive such toxicity. The control group was not treated with Cd or Pb.

2.3 Planting and Harvesting

Cabbage seeds were pre-germinated on moist paper towels. Germinated seeds were sown in porous-bottom planters containing 166 g of soil (dry weight) per planter. Five depressions were made in the soil of each planter and one pre-germinated seed was placed in each depression. Planters of each treatment group were then placed on separate reservoir trays under 240 W color corrected grow-lights (Hydrofarm Inc. Petaluma, CA, USA) on a 16:8 hr light: dark cycle. Distilled water was added to the reservoir tray every other day and once a week the plants were given Hoagland nutrient solution (Hoagland & Arnon, 1950).

Plants were harvested after a 30-day growth period. Plants were thoroughly cleaned and the root and shoot parts were separated by cutting at the shoot/root interface. Plants were then dehydrated for one week at 70^0 C using a laboratory incubator and then used for tissue metal content analysis.

2.4 Tissue Metal Content Analysis

Dried plant materials were then acid digested (US EPA Method 3050A), filtered, and then used to determine tissue metal content analysis [0.25 to 0.50 g of dried plant materials were placed in a conical flasks, to which 10 ml of distilled water and 15 ml of nitric acid (Fisher Scientific, IL, USA) were added and heated at 95°C for about 45 minutes or until the samples were dissolved. Samples were allowed to cool down for 3 minutes and then 1 ml of distilled water and 2 ml of Hydrogen Peroxide (H_2O_2) were added to the samples. The digested samples were heated again until the effervescence was stopped. Samples were allowed to cool down for another 3 minutes and 3 ml of H_2O_2 were added. Samples were heated again and sample volumes were adjusted to approximately 5 ml by evaporation. Twenty ml of distilled water was added to the samples, samples were filtered and the filtrate volumes were adjusted to 50 ml by adding distilled water]. Lead and Cd concentrations in plants were determined using Inductively Coupled Plasma Mass Spectrometry following modifications of US EPA Method 6020 using a Perkin Elmer Elan DRC-II ICP-MS (Waltham, MA). Briefly, the digestate was diluted 1:10 with 1% ultrapure nitric acid prior to analysis. Cadmium was quantified using the ^{111}Cd isotope, with ^{114}Cd monitored for confirmation (after correction for ^{114}Sn); ^{208}Pb was monitored for quantitation with ^{206}Pb used for confirmation. Rhodium, Terbium, and Holmium were added on-line using a mixing-T as internal standards to correct for instrumental drift. The instrument was calibrated using NIST-traceable mixed element standards purchased from SPEX CertiPrep (Metuchen, NJ) and CPI International (Santa Rosa, CA), each isotope was calibrated at 0, 1, 10, and 100 µg/L, with correlation coefficients greater than 0.999. A second-source standard was also analyzed to verify

instrument calibration, with analyte recoveries within 10% of the nominal concentration.

2.5 Statistical Analysis

Data was analyzed by one-way Analysis of Variance (ANOVA) and Tukey test (SigmaStat Statistical Software, SPSS, Chicago, IL, USA). Differences were considered to be significant at P d" 0.05.

3.0 Observations

3.1 Plant Tissue Cd Accumulation

Plants grown in Cd contaminated soils accumulated high concentrations of Cd in plant tissues (root plus shoot). Cadmium accumulations were dose related and the accumulation levels in all Cd treated plants were significantly higher when compared to control plants (Figure 1). Moreover, Cd concentrations in the plants were between 300% and 400% higher than the soil Cd concentration. Chlorosis and leaf necrosis was observed in some 250 mg/kg and 500 mg/kg Cd treated plants. This was probably caused by Cd phytotoxicity as plants absorbed more Cd than the Pb.

3.2 Plant Tissue Pb Accumulation

Lead accumulation in Pb treated plants was dose related. Lead accumulations by all Pb treated groups were significantly higher when compared to control plants (Figure 2). Lead concentrations in the plants were 25% to 40% higher than the soil Pb concentrations. Necrotic or chlorotic leaves were not observed in Pb treated plants as plants accumulated considerably lesser quantities of Pb than the Cd. Further details on plant morphology and metal accumulations of cabbage plants will be presented in a follow-up paper.

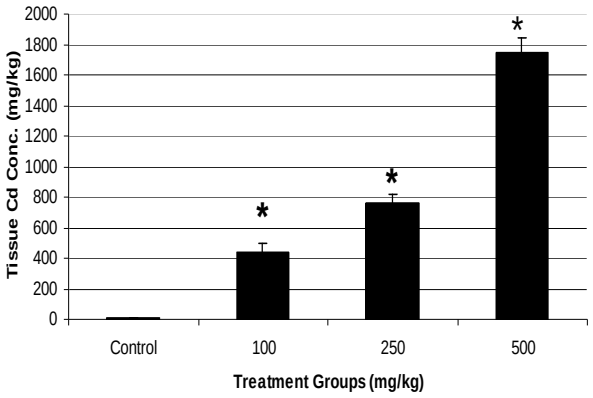

Figure 1: Cadmium accumulation (mean ± SEM, n = 16) in cabbage plants grown in 0, 100, 250, and 500 mg/kg Cd contaminated soils (* Means are significantly different from the control at $p \leq 0.05$ level: Tukey Test).

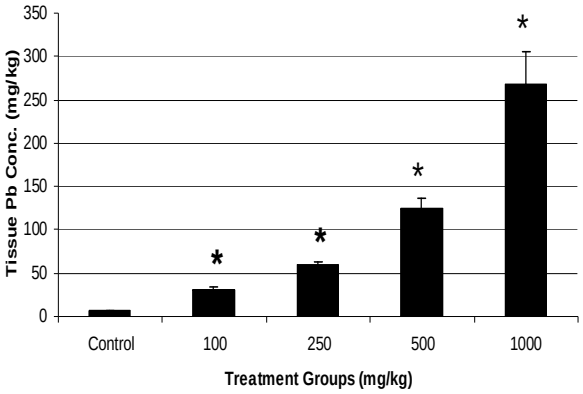

Figure 2: Lead accumulation (mean ± SEM, n = 16) in cabbage plants grown in 0, 100, 250, and 500 mg/kg Cd contaminated soils (* Means are significantly different from the control at $p \leq 0.05$ level: Tukey Test).

4.0 Discussions

The results from this study indicated that cabbage plants were able to absorb considerable quantities of Cd and Pb from contaminated soils. Plants absorbed and accumulated more Cd than Pb. However, higher Cd contents in plants instigated morphological changes such as necrosis and chlorosis. Less Pb accumulation was probably due to relatively low mobility of this metal caused by tight binding of Pb to soils clay particles. In soils, Cd is more mobile as it is present mainly as a free ion and soluble complexes or adsorbed at ion exchange sites of inorganic soil constituents (Tessier *et al.*, 1979).

Cabbage plants tolerated high levels of Cd and Pb in contaminated soils and accumulate considerable amounts of these metals in plant tissue. This observation suggested that both of these metals were bioavailable to the plants and the plants had the affinity for these metals. Similar results were reported by Xian (1989) using Cd, Pb, and Zinc.

The current study indicates that cabbage plants can tolerate high concentrations of Cd and Pb in soil and bioabsorb these metals from soil. Therefore, the phytoremediation potential of this plant species

needs to be further investigated. Studies also need to be conducted to evaluate bioabsorption of other toxic metals by cabbage plant species. Even though phytoremediation is a relatively new field, it shows the promise to be a reliable environmental cleanup technology and should be established as an alternative technology to cleanup environmental pollutants in the near future.

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