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Chemically-Enhanced Phytoextraction of Cadmium-Contaminated Soils Using Wheat (*Triticum aestivum* L.)

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Toxic heavy metal contamination of soils poses a major environmental and health risk, and as such, still needs an economical and environmentally safe technological solution (Salt et al., 1995; Raskin et al, 1994). Despite 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 or horticultural attributes. Moreover, there is scarcity of known hyperaccumulating plants for Cd, one of the most environmentally important metallic pollutants in soils (Baker and Walker, 1990; Salt et al., 1998).

Previous studies revealed that uptake and translocation of heavy metals in plants are enhanced by increasing heavy metal concentration in the nutrient or soil solution (Huang et al., 1997; Kumar et al., 1995). Therefore, successful phytoextraction must include mobilization of heavy metals into the soil solution that is in direct contact with the roots. In most soils capable of supporting plant growth, the readily available levels of heavy metals 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),

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facilitate metal transport into the xylem, and increase metal 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).

In our previous study (Begonia et al., 2000), we identified wheat as a potential phytoextraction species because of its high biomass yield under elevated Cd levels and its ability to translocate high amounts (e.g., 76.8 % of total plant uptake) of Cd into its shoots. The main objective of this study was to further evaluate the effectiveness of wheat as a phytoextraction species. Specifically, this experiment was conducted to determine the effective concentration of [ethylenebis (oxyethylenenitrilo)] tetraacetic acid (EGTA) that maximizes the shoot accumulation of Cd by wheat grown on a Cd-contaminated soil.

MATERIALS AND METHODS

Plants were maintained on a laboratory bench with 25°C/20°C day/night temperatures. Supplemental light for 12 hrs were provided by high intensity super halide lamps (1000W 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 900 µmol photons m^{-2} sec^{-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 subsample tube or supercell (Stuewe and Sons, Inc., Corvallis, OR) containing a growth medium composed of 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 2 plants per tube at 5 d after planting. Using a hand trowel, three concentrations (0, 500, 1000 mg Cd/kg dry growth medium) of Cd (supplied as cadmium nitrate) and four concentrations (0, 1.0, 2.5, 5.0 mmol/kg dry growth medium) of EGTA were thoroughly mixed with the growth medium before planting. 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. One d before harvest, the youngest most fully expanded leaf blade from each plant (8 leaf blades per treatment) was sampled for chlorophyll analyses. Chlorophyll contents of leaf blades were extracted by soaking them in 95% ethanol solutions for 48 h in the dark at room temperature. Chlorophyll concentrations were quantified spectrophotometrically according to the procedures of Eihellig and Rasmussen (1979). 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. Cadmium contents of each 200 mg dry, ground plant tissue were extracted using modified nitric acid-hydrogen peroxide procedures (Begonia et al., 1998). Cadmium concentrations of digestates were quantified using atomic absorption spectrometry (Thermo Jarrell Ash Model AA Scan 4) and expressed as μg Cd/g dry wt of plant tissue. This analytical system had a 98% recovery efficiency and detection limit of 5 ppb Cd.

In this experiment, each treatment replicate consisted of a row of 4 subsample tubes (2 plants per tube) arranged on a RL98 tray (Stuewe and Sons, Inc., Corvallis, OR), giving a total of 8 plants per treatment. Treatments (i.e., rows of 4 subsample tubes) were arranged in a 3 Cd x 4 EGTA factorial in a Randomized Complete Block (RCB) design with four replications. Data were analyzed using Statistical Analysis System (SAS, software version 8). Treatment comparisons were done using Fisher's Protected Least Significant Difference (LSD) test. In this study, a probability $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

One of the requisites for efficient phytoextraction is the tolerance of the test plant species to the metal contaminant (e.g., Cd) under consideration. Generally, wheat was relatively tolerant to both Cd and EGTA as shown by non-significant differences ($p>0.07$) in root biomass among treatments (Table 1). Also, leaf chlorophyll concentrations did not vary ($p>0.48$) among Cd/EGTA treatments (Table 2) indicating that the plants did not suffer from a metal-mediated toxicity. However, a slight reduction in shoot biomass was noted in plants grown at the highest Cd/EGTA treatment combination (Table 1). Monocotyledonous species are usually more tolerant to metals than dicotyledonous species (Marschner, 1995) and this observation is consistent with our previous studies of wheat and coffeeweed (*Sesbania exaltata* Raf.) exposed to various levels of soil-applied Pb and EDTA (Begonia et al., 2002a,b). In the present study, wheat plants were able to grow normally, showing no visible signs of metal and/or chelate phytotoxicity. This observation is in direct contrast with other studies, whereby dicots such as sunflower (Chen and Cutwright, 2001) and radish (Zaman and Zereen, 1998) showed significantly reduced growth and lower chlorophyll concentration with high levels of soil Cd contamination.

The success of phytoextraction as an environmental cleanup technology, also depends on metal bioavailability for uptake into roots, and plant ability to intercept, absorb, and accumulate metals in shoots (Ernst, 1996). The potential for phytoextraction of several major metal contaminants including Cd is adversely

Table 1. Effects of various concentrations of Cd and EGTA on wheat root and shoot biomass.

Treatment		Dry Biomass (mg/plant)	
Cd (mg/kg)	EGTA (mmol/kg)	Root±SEM	Shoot±SEM
0	0	29.5 ±0.7	67.2 ±10.1
0	1	27.7 ±3.3	77.9 ±5.2
0	2.5	28.1 ±2.4	55.8 ±1.6
0	5.0	38.4 ±2.4	61.9 ±1.5
500	0	36.4 ±4.7	62.5 ±2.0
500	1.0	36.8 ±3.3	76.3 ±4.1
500	2.5	37.7 ±6.0	69.9 ±5.6
500	5.0	35.8 ±4.7	70.0 ±5.6
1000	0.0	48.2 ±5.7	80.7 ±7.9
1000	1.0	45.7 ±4.2	79.7 ±7.0
1000	2.5	39.6 ±5.5	69.2 ±5.1
1000	5.0	33.6 ±7.6	52.7 ±5.6
LSD (0.05)*		NS	16.3

* Fisher's Protected Least Significant Difference at $P \leq 0.05$; NS = not significant; SEM = standard error of the mean of 4 replications.

affected by metal adsorption to soil solids and/or precipitation as insoluble compounds. Addition of synthetic chelates has been shown to stimulate the release of metals into soil solution and enhance the propensity for uptake into roots (Blaylock et al., 1997; Huang et al., 1997; Lasat, 2002). In the present study, Cd concentrations in both roots and shoots increased with increasing concentration of applied EGTA (Table 2). Chelator enhancement of metal accumulation in plants is plant- and metal-specific. For example, Chen and Cutright (2001) found that EDTA at 500 mg/kg increased the shoot concentration from 34 to 115 mg/kg in sunflower. Chaney et al. (2000) reported that one of the high Cd:Zn genotypes accumulated nearly 1800 mg Cd/kg dry shoot. EGTA treatments in this study increased Cd concentrations in the roots 2- to 6-fold more than in the shoots, suggesting that EGTA was far more efficient in overcoming the diffusion limitation of metal to root surface than the barrier of root to shoot translocation. These results are consistent with those of Kayzer et al. (2000) who showed that nitrilotriacetate (NTA) and elemental S increased the solubility of Cd in the soil by a factor of 58 but accumulation of this metal in maize, Indian mustard, and other plants was only increased by a factor of 2 to 3. Also, our results are compatible with previous studies on chelate-enhanced phytoextraction of Cd by *Thlaspi caerulescens* (Lombi et al., 2001) which showed that EDTA alone increased metal availability in soil and accumulation in roots, but did not

Table 2. Leaf chlorophyll concentrations of wheat grown in various levels of Cd and EGTA.

Treatment		Chlorophyll Concentration
Cd (mg/kg)	EGTA (mmol/kg)	(μg/mg leaf dry wt ± SEM)
0	0	6.3 ± 0.60
0	1.0	5.4 ± 0.78
0	2.5	6.2 ± 1.53
0	5.0	4.3 ± 0.25
500	0	4.5 ± 0.09
500	1.0	5.6 ± 0.78
500	2.5	5.3 ± 0.47
500	5.0	5.4 ± 0.91
1000	0.0	4.7 ± 0.58
1000	1.0	5.2 ± 0.50
1000	2.5	4.0 ± 0.53
1000	5.0	5.2 ± 0.69
LSD (0.05)*		NS

Table 3. Root and shoot Cd concentrations of wheat grown in various levels of Cd and EGTA.

Treatment		Cd Concentration (mg/kg dry wt.)	
Cd (mg/kg)	EGTA (mmol/kg)	Root ± SEM	Shoot ± SEM
0	0	2.8 ± 0.2	3.2 ± 0.6
0	1.0	1.8 ± 0.4	2.6 ± 0.5
0	2.5	2.1 ± 0.1	2.9 ± 0.6
0	5.0	2.7 ± 0.4	2.1 ± 0.3
500	0	64.3 ± 13.4	11.1 ± 0.5
500	1.0	120.0 ± 3.5	55.4 ±10.3
500	2.5	298.3 ± 13.4	71.8 ± 9.7
500	5.0	308.0 ± 13.0	81.8 ± 8.0
1000	0.0	62.1± 10.7	36.7 ±12.2
1000	1.0	276.8 ± 37.0	61.6 ± 6.1
1000	2.5	317.3 ± 13.8	78.2 ± 6.0
1000	5.0	346.3 ± 40.1	111.2 ± 4.4
LSD (0.05)*		51.2	22.3

*Fisher's Protected Least Significant Difference at $P \leq 0.05$; SEM = standard error of the mean of 4 replications.

substantially increase the transfer of metals to shoots. Metal transport to shoot primarily takes place through the xylem. Movement of metal ions, particularly Cd, in xylem vessels appears to be mainly dependent on transpiration-driven mass flow (Salt et al., 1995). Because xylem cell walls have a high cation exchange capacity (CEC), they are expected to retard severely the upward movement of metal cations. Although Cd accumulation in the plant tissues increased with increasing level of EGTA, the amount of Cd in the shoot did not reflect an efficient transport of a non-cationic metal-chelate complex in the transpiration stream. Experiments are currently conducted in our laboratory, exploring ways of improving shoot accumulation of Cd by wheat.

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