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Effects of Chelate Application Time on the Phytoextraction of Lead-Contaminated Soils

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Anthropogenic emissions of metals into the environment occur at increasing rates, causing serious problems for environmental and human health (Nriagu 1979; Ross 1994). Currently, there are more than 50,000 metal-contaminated sites in the U.S.A. that await remediation, many of them Superfund sites (Ensley 2000). For metal-contaminated soils, the commonly used engineering-based remediation technologies include soil washing, excavation and reburial (Glass 2000). Present U.S. remediation costs range from $7 billion to $8 billion per year, approximately 35% of which involves remediation of metals (Glass 2000).

Phytoremediation is a relatively new, inexpensive, solar-driven technology that utilizes plants for clean up of metals. One of the two principal phytoremediation strategies for metals is phytoextraction which involves harvesting of shoot biomass containing the accumulated metals (Salt et al. 1998; Blaylock and Huang 2000). The harvested plant material may be used for non-food purposes, or it can be ashed followed by recycling of the metals if economically feasible, or disposal in a landfill (Chaney et al. 2000). Commercial implementation of phytoextraction technologies are already being used effectively (Salt et al. 1998; Blaylock 2000).

The goal of phytoextraction is to reduce heavy metal levels below regulatory limits within a reasonable time frame. To achieve this goal, 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 Pb, one of the most environmentally important metallic pollutants in soils (Baker and Walker 1990; Salt et al. 1998).

Previous studies demonstrated that uptake and translocation of heavy metals in plants are enhanced by increasing heavy metal concentration in the soil solution (Huang et al. 1997; Kumar et al. 1995). Therefore, successful phytoextraction

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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, 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; Begonia et al. 2000a; Begonia et al. 2000b).

Chelate-enhanced phytoextraction, however, increases the risk of possible transference of Pb and other heavy metals from soil to ground water and promotion of off-site migration (Cooper et al. 1999; Huang and Cunningham 1996; Kulli et al. 1999). Thus, effective implementation of phytoextraction must consider measures to minimize leaching. One strategy is to limit metal mobility in the soil by applying the chelate only when plants had attained maximum biomass, then harvest the plants a few days after chelate amendment. Therefore, the main objective of this study was to compare the effects of chelate application time on the root uptake and subsequent translocation of Pb to the shoots of wheat plants grown on a Pb-contaminated soil.

MATERIALS AND METHODS

Plants were maintained inside a greenhouse with day/night temperatures set at 30°C/25°C and 50% relative humidity. 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 2:1 (v/v) mixture of sieved silty clay loam soil (pH 8.2; 1.5% organic matter) and peat. Emerged seedlings were thinned out to 2 plants per tube at 5 d after planting.

Two wk before planting, two concentrations (1000 and 2000 mg Pb/kg dry soil) of Pb (supplied as lead nitrate) were thoroughly mixed with the soil using a hand trowel. For treatments 2 and 3 (see Tables 1 to 4), ethylenediamine tetraacetic acid (EDTA) was also mixed as a powder (5 mmol/kg dry soil) before planting, or as a 100 mL aqueous solution 42 days after planting (DAP), respectively. A combination of EDTA (5 mmol/kg) and acetic acid (5 mmol/kg) was also applied as an aqueous solution to the Pb-spiked soil at 42 DAP. Tubes in treatment 1

received no chelate amendments other than the appropriate amounts of Pb mixed with the soil 2 wk before planting. Based from the evaporative demand, 5 mL of nutrient solution were periodically 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, necrosis, stunting) exhibited by plants were visually noted during the experimental period. All plants were harvested at seven 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. 2002a,b). Lead concentrations of digestates 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 both experiments, 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 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

Phytoextraction requires that test plant species must be able to tolerate toxic metal levels, and produce substantial amounts of biomass especially in the shoots. Root biomass of plants grown at both Pb levels were slightly reduced (Tables 1 and 2). Also, greater reductions in root biomass were observed at higher Pb level, and where a combination of chelates (EDTA and acetic acid) was used. Roots were more susceptible to mid-season (42 DAP) compared to early-season (i.e., before planting) chelate application. This could be explained by a surge of bioavailable Pb that were in close proximity to the roots right after mid-season chelate application.

Generally, shoot biomass of wheat plants grown at both Pb levels were neither affected by the chelate amendments nor time of chelate application (Tables 1 and 2). This observation is also substantiated by the absence of any discernible metal

Table 1. Root and shoot biomass of wheat grown in a lead (1000 mg Pb/kg)-spiked soil that had been amended with chelates at different times (Expt. 1).

Treatments Chelates (mmol/kg)	Application Time	Root biomass (mg/plant±SEM) at harvest 42 DAP	45 DAP	49 DAP
1.EDTA (0)	--	68.5a±2.0	70.9a±2.8	70.2a±5.5
2.EDTA(5)	BP	53.3a±8.6	66.4a±2.5	71.3a±3.5
3.EDTA(5)	42DAP	36.1b±2.1	40.9b±3.2	43.6b±2.1
4.EDTA (5) + Hac(5)	42 DAP	32.4b±4.0	29.8c±0.1	22.8c±2.4
		Shoot biomass (mg/plant±SEM) at harvest		
1.EDTA (0)	--	52.1ab±4.5	60.4b±1.1	64.5a±3.1
2.EDTA(5)	BP	40.0b±2.5	64.2ab±3.1	66.2a±1.2
3.EDTA(5)	42DAP	60.8a±4.1	73.8a±3.9	63.9a±3.7
4.EDTA (5) + HAc (5)	42 DAP	56.0a±5.1	72.2ab±6.6	45.5b±2.6

For each tissue, means in a column followed by a common letter are not significantly different from each other according to Fisher's Protected Least Significant Difference (LSD) test ($P \leq 0.05$); SEM = standard error of the mean of 4 replications; BP = before planting; DAP = days after planting; HAc = acetic acid.

Table 2. Root and shoot biomass of wheat grown in a lead (2000 mg Pb/kg)-spiked soil that had been amended with chelates at different times (Expt. 2).

Treatments Chelates (mmol/kg)	Application Time	Root biomass (mg/plant±SEM) at harvest 42 DAP	45 DAP	49 DAP
1.EDTA (0)	--	25.1b±0.1	36.6a±3.5	34.2a±3.3
2.EDTA(5)	BP	29.1a±1.3	27.3bc±1.3	34.6a±2.3
3.EDTA (5)	42DAP	22.1b±1.1	28.6b±0.2	25.7b±1.1
4.EDTA(5) + HAc (5)	42DAP	22.0b±1.0	22.2c±1.2	21.0b±1.1
		Shoot biomass (mg/plant±SEM) at harvest		
1.EDTA (0)	--	66.5ab±2.7	63.7b±5.4	82.8a±4.3
2.EDTA(5)	BP	59.5b±1.4	58.9b±5.4	66.1a±6.8
3.EDTA (5)	42DAP	68.7a±3.6	80.7a±2.9	78.5a±5.8
4.EDTA (5) +Hac (5)	42DAP	58.1b±3.1	57.6b±4.4	71.8a±5.6

For each tissue, means in a column followed by a common letter are not significantly different from each other according to LSD test ($P \leq 0.05$). See Table 1 caption for nomenclatures.

toxicity symptoms exhibited by the metal- and/or chelate-treated plants. This is not surprising since in our previous findings (Begonia et al. 2000a; Begonia et al. 2000b) this wheat cultivar was relatively tolerant to moderately high levels (e.g., 1,000 - 2,000 mg Pb/kg) of soil Pb.

Plants grown at 1000 mg Pb/kg exhibited increasing root Pb concentrations (Table 3) with longer periods of Pb exposure (i.e., longer interval between chelate application time and harvest date). Plants that received chelates before planting had the highest root Pb concentrations compared to the other treatments. A similar increasing trend of root Pb concentrations were also observed in plants exposed to 2000 mg Pb/kg (Table 4). However, root Pb concentration was highest in plants that received a combination of chelates at 42 DAP.

Table 3. Root and shoot Pb concentrations of wheat grown in a lead (1000 mg Pb/kg)-spiked soil that had been amended with chelates at different times (Expt.1).

Treatments Chelates (mmol/kg)	Application Time	Root Pb conc (µg Pb/g ±SEM) at harvest 42 DAP	45 DAP	49 DAP
1.EDTA (0)	--	95c± 8	155c ±16	187d±26
2.EDTA (5)	BP	463a±45	579a ±98	773a±54
3.EDTA (5)	42 DAP	148c±13	300bc±31	360c±13
4.EDTA (5) + HAc (5)	42 DAP	281b±39	336b ±19	629b±35
		Shoot Pb conc (µg Pb/kg±SEM) at harvest		
1.EDTA (0)	--	68c± 4	216a±10	244c±20
2.EDTA (5)	BP	251a±28	281a±33	451b±44
3.EDTA (5)	42 DAP	86c± 6	225a±20	247c± 6
4.EDTA (5) + HAc (5)	42 DAP	184b±11	214a±42	754a±42

For each tissue, means in a column followed by a common letter are not significantly different from each other according to Fisher's Protected Least Significant Difference (LSD) test ($P \leq 0.05$); SEM = standard error of the mean of 4 replications; BP = before planting; DAP = days after planting; HAc = acetic acid.

In both experiments, shoot Pb concentrations increased with increasing time interval between chelate application time (e.g., either before planting or 42 DAP) and harvest date (Tables 3 and 4). This observation is compatible with the work of Shen et al. (2002) which indicated that soil Pb could be solubilized by EDTA in a short time and maintained at a high level over the experimental period up to 120 h (5 d). Except at 42 DAP, shoot Pb concentrations were highest in plants

Table 4. Root and shoot Pb concentrations of wheat grown in a lead (2000 mg Pb/kg)-spiked soil that had been amended with chelates at different times (Expt. 2).

Treatments Chelates (mmol/kg)	Application Time	Root Pb conc (µg Pb/kg±SEM) at harvest 42 DAP	45 DAP	49 DAP
EDTA (0)	--	305c±62	643b ±29	604c±65
EDTA(5)	BP	597b±46	788ab±76	1225b±82
EDTA (5)	42 DAP	375c±26	814ab±44	1415a±31
EDTA (5) + HAc (5)	42 DAP	734a±28	902a±128	1552a±48
		Shoot Pb conc (µg Pb/kg±SEM) at harvest		
EDTA (0)	--	79c± 6	129d±9	148c±28
EDTA(5)	BP	351a±45	569b±4	685a±27
EDTA (5)	42 DAP	162b± 7	287c±33	355b±19
EDTA (5) + HAc (5)	42 DAP	138bc±14	783a± 2	785a± 69

Means in a column followed by a common letter are not significantly different from each other according to Fisher's Protected Least Significant Difference (LSD) test ($P \leq 0.05$); SEM = standard error of the mean of 4 replications; BP = before planting; DAP = days after planting; HAc = acetic acid.

that received a combination of two chelates at 42 DAP. This implies that chelates amended to a Pb-contaminated soil in order to enhance phytoextraction may not necessarily be applied early in the growing season. Since chelate-triggered Pb accumulation in plants is rapid (Huang et al. 1997; Lombi et al. 2001), chelates can be applied to the root zone when the plants have attained maximum biomass. Such short resident time for the chelates lessens the mobility of bioavailable metals that can potentially migrate and serve as sources of secondary pollution to the ground water.

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