Phytoremediation of Lead-Contaminated Soil using *Typha latifolia* (Broadleaf Cattail)

by

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We certify that we have read this study and that it conforms to acceptable standards of scholarly presentation and is fully acceptable, in scope and quality, as a thesis for the degree of Master of Arts.

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ABSTRACT

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The development of industry, mining activity, irrigation of waste water, and the application of sewage sludge to agricultural lands have increased the release of metals into our ecosystems causing serious environmental problems and posing threats to human health. Phytoremediation has become one possible remediation strategy to address lead toxicity. The heavy-metal tolerance of *Typha latifolia* (broadleaf cattail) has made it a potential candidate for chemically enhanced lead phytoremediation. Soil was collected from a wetland area located in Arcata, California and contaminated with $PbCO_3$ to create 0, 1000, 2000, and 4000 ppm lead-contaminated soils. Cattails were grown from seed in the lead-contaminated soil for twelve weeks. There were three treatments for each of the four contaminant levels. The first treatment group (shoot removal and regrowth) had the cattail shoot tissue removed after the first six weeks of growth and a new shoot was allowed to grow during the second six-week period. The second treatment group (chemically enhanced phytoremediation) had 15 mmol ethylene diaminetetraacetic acid (EDTA) injected into the soil with a hypodermic needle during the tenth week of growth

and was allowed to continue growth and lead uptake during the remainder of the twelve-week growth period. The third treatment group (control) was allowed to grow for the full twelve weeks. There was a significant difference ($p=0.0344$) in cattail height after the twelve weeks of growth for the four different lead-contamination levels. There was a significant difference ($p=0.0001$) for the shoot biomass produced; the control group produced the greatest mass and the 2000 ppm lead produced the least (5.64 and 4.61 grams, respectively). There was a significant difference in lead uptake between the EDTA and the non-EDTA groups. The 4000 ppm EDTA treatment cattails translocated a significantly greater ($p<0.0001$) quantity of lead (4.38 mg) into their shoot tissues than any other treatment group or contamination level. The increased lead uptake was significantly greater with the chemically enhanced treatment (EDTA) but was not sufficient for an effective phytoremediation approach (to achieve acceptable soil-lead levels, EPA limits, within 20 years) for lead-contaminated soil in mason jars under greenhouse conditions. It would require 376 years to reduce the lead concentration in the soil within the mason jars from 4000 to 2000 ppm. However, the cattails did reach hyperaccumulator status with the addition of EDTA by accumulating 2418 ppm lead in shoot tissues when grown in 4000 ppm lead contaminated soil.

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INTRODUCTION

Metals are naturally found in the earth's crust at various concentrations. The development of industry, mining activity, irrigation with waste water, and the application of sewage sludge to agricultural lands have increased the release of metals into our ecosystems, causing serious environmental problems and posing threats to human health (Chen et al, 2000; Lantzy and MacKenzie, 1979; Nriagu, 1979; Ross, 1994). Although many metals are essential for cell functions (i.e. Cu, Mn, Ni, Zn), all heavy metals are toxic at higher concentrations (Marschner, 1995). As heavy-metal soil toxicity becomes more prevalent, our society is learning to adapt to this pollution through control, minimization, and various remediation techniques (Bontidean et al., 2003).

Most heavy metals, including lead (Pb), remain persistent in soils and unavailable for plant uptake because of their immobile nature (Garcia et al, 2003). Lead is considered one of the most frequently encountered heavy metals of environmental concern and is the subject of much remediation research (Huang et al., 1997; Xei et al., 1999; Panich-Pat et al., 2003, Epstein et al., 1999). It has no known biological function, but investigations have shown that plants can accumulate lead, and plant tissue concentrations are significantly related to the lead levels in the environment (Xiong, 1997a). Numerous studies have shown that higher lead concentrations in the soil translates into more lead entering a plant (Xei et al., 1999).

Lead is a naturally occurring, bluish-gray, lustrous metal found in small amounts within the earth's crust. It can be found in all parts of our environment as much of it

comes from human activity such as the burning of fossil fuels, mining, and lead-based paints. Other uses of lead include plumbing, ammunition, batteries, x-ray shields, solder, containers for corrosive liquids, weapons manufacturing, and stained glass windows. Once lead is free in the environment, it does not break down or become metabolized by living organisms. As a dust-sized particle (< 20μm) it can travel long distances before settling to the ground. If lead gets into the upper troposphere, it may remain there for 10-14 days. During this period of time, winds and storms can disperse the metal thousands of kilometers (Xei et al., 1999). Once in contact with soil it becomes highly adsorbed to the cation exchange sites. Lead will often stay in the upper 2-5 cm of soil if left undisturbed and will remain immobilized creating a potentially toxic top layer of soil. When lead is in the soil it behaves like a Lewis acid (Sharma and Dubey, 2005) and tends to form ionic bonds with organic compounds, hydroxides, phosphates, and carbonates. If and when these precipitates form, plants cannot absorb the metal from the soil (Xei et al., 1999).

According to the Agency for Toxic Substances and Disease Registry (ATSDR), lead is second on the list of the “Top Twenty Hazardous Substances from the 2003 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances.” This list is based on the frequency of occurrence, toxicity, and the potential for human exposure. It should be noted that this priority list is not a list of "most toxic" substances, but rather a prioritization of substances based on a combination of their frequency, toxicity, and potential for human exposure at National Priority List (NPL) sites (ATSDR, 2003). Lead has also been found in at least 1,280 of

the 1,662 NPL sites identified by the Environmental Protection Agency (EPA) (ATSDR, 2003).

The primary health concerns for lead exposure are inhalation or ingestion of lead-containing substances or absorption of organic lead (i.e. tetraethyl lead) through the skin . Lead can affect many organs in the human body, the most susceptible being the central nervous system. Continued exposure can also inhibit mental and physical growth, damage the kidneys and reproductive systems, decrease reaction time, cause weakness in fingers and wrists, affect memory, cause anemia, disrupt the biosynthesis of hemoglobin, increase blood pressure, increase chances of miscarriages, diminish learning ability in children, and can enter a fetus through the placenta of the mother (ASTDR, 2003; EPA, 2004). Children are more susceptible to lead poisoning than adults because their central nervous systems are growing faster and slight metabolic changes will have greater and longer-lasting negative effects. The main concern for lead exposure in children comes from the ingestion of lead-based paint chips from older houses, playing in and inhaling lead-particulates from contaminated soil near walls that had been painted with a lead-based paint, or through “pica” behavior.

Another concern of lead-contaminated sites is the possibility of lead entering the food chain. Although plants, animals, and microbes have no biological need for lead, it can still be taken up and sequestered into the cells of living organisms. Lead can be moved from plants to herbivores and once it has entered the food chain, it can move to secondary and tertiary predators. The lead concentration in higher organisms can

consistently increase to reach toxic levels with continued feeding on lead-containing organisms, a process known as bioaccumulation.

Excessive lead accumulation in plant tissue is toxic to most plants, leading to a decrease in seed germination, root elongation, decreased biomass, and inhibition of chlorophyll biosynthesis (Xiong, 1997b). In lead and other metal-contaminated sites, vegetation structure and biodiversity are usually reduced, barren patches of soil occur, and trees are absent (Wickland, 1990). While inside a cell, lead affects photosynthesis, respiration, mineral nutrition, and enzymatic reactions as well as a number of other physiological factors (Sharma and Dubey, 2005). If these effects become too severe, growth may discontinue and the plant may die (Seregin et al., 2004).

Lead absorption by plants is retarded by several factors, including: raising the soil pH, increasing organic content, and adjusting both temperature and the amount of light a plant receives (Gray, 2002). In addition, the ability of roots to take up lead may be affected by soil composition/particle size, surface area of roots, substances released by roots including organic acids, mycorrhization, the rate of transpiration, and the quantity of lead in a growth medium (Sharma and Dubey, 2005).

One of the main pathways for lead to enter the plant is through the roots by crossing the cell's plasma membranes via voltage gated calcium ion channels (Rudakova, et al., 1988). This may inhibit calcium uptake through competition for the ion channels (Marshall, et al., 1994; Huang et al., 1997). Once in the roots, lead is not translocated to the shoots of a plant but tends to be sequestered in root cells. The endodermis is a contributing factor for lead sequestering in the plant roots. The Casparian strips within

the endodermis restrict heavy metals, including lead, from passing into the central cylinder tissues and translocating to shoot tissue (Seregin et al., 2004). Carboxyl groups within the cell wall can also reduce the movement of lead by binding to the lead itself. Carbohydrates such as galacturonate and glucuronate bind to the lead and reduce the toxic effects on other cell components. The accumulation of lead-carbohydrate complexes will inhibit lead mobility but will also reduce overall root growth by decreasing the plasticity of the cell wall, which reduces cell size and in turn inhibits root growth (Seregin et al., 2004). Approximately 95% or more of absorbed lead, in most plants, is located in the plant roots unless the plant is a hyperaccumulator or chelate-assisted translocation processes (discussed later) are taking place (Godbold and Kettner, 1991).

High lead levels affect plants in many ways. At higher lead concentrations, the selectively permeable barrier function of the cell membrane is compromised and a large amount of lead can enter the plant cells. Increased lead concentrations will also affect a variety of essential physiological processes by both chemical and physical means such as enzymatic activity, photosynthesis, mineral nutrition, water potential, hormonal status, membrane permeability, and energy production (Sharma and Dubey, 2005).

One of the most drastically affected plant processes from lead-toxicity is photosynthesis. Reduced photosynthesis results from: distorted choloplast ultrastructure, diminished synthesis of chlorophyll, plastoquinone, and carotenoids, obstruction of the electron transport system, inadequate concentration of carbon dioxide via stomatal closure, inhibition of Calvin cycle enzymatic catalysis, and decreased NADP+ reductase

activity (Stefanov et al., 1995). Lead-contaminated plants produce chloroplasts with distorted thylakoid membranes and decreased amounts of both grana stacks and stroma from reduction in magnesium and iron uptake (Burzynski, 1987). Chlorophyll is also degraded faster in lead-contaminated plants due to an increase in chlorophyllase activity (Drazkiewicz, 1994). The inhibition of electron transport seems to arise from effects that occur at donor and acceptor sites of Photosystems I and II, and the cytochrome b/f complex (Mohanty et al., 1989; Sersen et al., 1998).

Induced stomatal closure from lead toxicity is one of the most important factors contributing to decreased photosynthetic rates (Bazzaz et al., 1975). Lead toxicity tends to negatively impact stomatal size by reducing guard cell size (Sharma and Dubey, 2005). Floating an epidermal peel in a lead solution can induce stomatal closure. The lead will inhibit K^+ transport through membranes and/or interfere with an energy system (Bazzaz et al., 1974). Stomatal closure will result in decreased carbon dioxide level, which leads to a decline in photosynthetic activity (Stefanov et al., 1995).

Mineral nutrient deficiency also impairs photosynthesis and overall plant growth through lower nutrient absorption and nutrient translocation. *Picea abies* (Norway spruce) root tips showed decreased iron, zinc, and calcium in root tips when exposed to a 0.1 µM lead solution and showed reduced magnesium levels in 2.0 µM lead solutions (Godbold and Kettner, 1991). Sieghardt (1988) found reduced magnesium in needles in lead treatments. Walker et al. (1977) found decreased uptake of potassium, calcium, magnesium, iron, and nitrate in seedlings as well as declined translocation of potassium, calcium, magnesium, and phosphorus in *Zea mays* (Maize corn) subjected to lead

solutions. Seregin et al. (2004) found reduced zinc levels in leaves of *Phaseolus vulgaris* (Kidney bean) grown in lead solutions.

Reduced uptake of nutrients can be caused by both chemical and physical mechanisms. Physical mechanisms of reduced uptake result from competition for ion-channels from ions with similar radii, as suggested by the lead/calcium competition for the calcium ion channels. Chemical mechanisms of reduced uptake rely on the metal-induced disturbance of cellular metabolism, which causes changes in membrane structure and membrane-enzyme activities. The release of K^+ from roots (via lead-induced interactions with potassium-ATPase and –SH groups of membrane proteins) is an example of this mechanism (Sharma and Dubey, 2005).

Finally, the rate of transpiration and water potential tend to decrease in lead-contaminated plant tissues. Reduced transpiration rates occur from decreased leaf area and stomatal number (Iqbal and Mushtaq, 1987). Water potential in a cell is lowered due to the metal disabling a cell's ability to maintain both proper turgor pressure and cell wall plasticity (Sharma and Dubey, 2005).

The most common method previously used for contending with metal-contaminated soils was to physically remove the soil and transport it to a hazardous waste site where it was buried. Other conventional remediation techniques include solidification and stabilization, soil flushing (water injected *in situ* to extract and treat contaminants), electrokinetics (direct electrical current used to concentrate contaminant cation in one region for mechanical removal), chemical reduction/oxidation, soil washing (*ex situ* method of washing soil to remove contaminants), low temperature thermal

desorbtion, incineration, vitrification, pneumatic fracturing, and excavation/retrieval (Prasad and Freitas, 1999). Estimated clean-up costs of U.S sites contaminated with heavy metals alone were $7.1 billion in 1995 while mixtures of heavy metals and organics added an additional $35.4 billion to the cost (Salt et al., 1995). Glass (1997) estimated that the United States was spending between $7 to $8 billion per year for remediation cost and according to the USGS (2006), cleaning up existing contamination in the US could cost more than one trillion dollars. More than 50,000 metal-contaminated sites await remediation in the U.S. alone and 80% of U.S. superfund sites (designated by the US EPA as priority sites for cleanup) contain heavy metals often mixed with other organic pollutants (Ensley, 2000). The remediation of heavy-metal contaminated soils represents a significant expense and therefore new and alternative methods of remediation are being pursued.

Phytoremediation is one potentially promising lead-remediation method. Phytoremediation is the process of using plants for heavy-metal decontamination in ecosystems and it has emerged as a cost-effective technology alternative to physical remediation or removal (Flathman and Lanza, 1998; Meagher, 2000). Phytoremediation is a low-impact technique requiring more time to remediate a contaminated environment and is dependent upon the specific contaminant, contaminant concentration, and mobility. There are three main approaches currently being pursued within phytoremediation: (1) phytostabilization, (2) phytovolatization, and (3) phytoextraction.

The first type of phytoremediaion is phytostabilization. Phytostabilization of metals does not reduce the concentration of the contaminants existing in a contaminated

environment but prohibits the contaminants from moving off-site. Plants can restrict movement of metals through various methods: prevention of leaching through the upward water flow created by plant transpiration, reduced runoff due to above-ground vegetation, and reduced soil erosion via stabilization of soil by plant roots (Berti and Cunningham, 2000). In another form of phytostabilization, metals are converted to a less bioavailable/toxic form such as the formation of insoluble precipitates on root surfaces (Horne, 2000).

The second type of phytoremediation is phytovolatization. During phytovolatization, heavy metals are taken up from the soil and converted into less toxic vapors, which are released into the atmosphere through the plants' transpiration process. The use of phytovolatization with *Nicotiana spp*. (tobacco) or *Liriodendron tulipifera* (tulip poplar) has been tested for mercury contaminated sites. Sources of elemental mercury and mercury ions (Hg^{2+}) released into the environment include gold mining, burning of fossil fuels, and dumping of medical wastes (Memon et al., 2000). Plants containing the merA and merB bacterial genes are highly resistant to mercury and can volatilize 100-1000 times more Hg than wild-type plants or controls expressing either gene alone (Bizly et al., 1999; Rugh et al., 1996). Transgenic plants possessing the bacterial genes merA and merB may be used increasingly to remediate mercury-contaminated sites. Plants capable of phytovolitization are still being researched and this field within phytoremediation will continue to grow as more suitable plants are found for specific metal contaminants. The use of the transgenic genes within *Spartina schreber* (cordgrass), *Typha latifolia* (cattail), *Scirpus spp*. (rushes) and *Salix spp*. (willows) can

create many suitable mercury-resistant phytovolatilizing plants within wetlands (Memon et al., 2000). There are no currently known, feasible lead-phytovolatization plants available.

The third type of phytoremediation is phytoextraction. Phytoextraction makes use of metal-tolerant hyperaccumulators with the ability to take up metals into their roots and shoots. A lead hyperaccumulator is defined as any plant that accumulates over 0.1% lead on a dry weight basis (1000 mg lead/kg dry weight) (Brooks et al., 1977). The goal of lead phytoextraction is to reduce lead levels in soils to acceptable levels within a reasonable time frame of less than 20 years (Huang et al., 1997). The US EPA standards for acceptable lead concentrations in soil as of 2004 are: 400 ppm for child play areas, 1200 ppm for residential non-play areas, and 2000 ppm for commercial areas. Scientists are looking to solve several drawbacks to phytoextraction with hyperaccumulators. One challenge to phytoextraction is that many hyperaccumulators tend to be slow growing and produce low biomass (Lombi et al., 2001). Also, if a hyperaccumulator is continuously taking lead into shoot tissue, there is a risk of introducing lead into the food chain through herbivory. Another drawback is the affinity of lead to remain fixed on the soil cation exchange sites and extraction is limited by solubility and diffusion to root surfaces.

The key to phytoextraction is to increase and maintain lead concentration in the soil solution (Huang et al., 1997) to enhance uptake by a high-biomass crop. Chemically enhanced phytoextraction has been investigated as a way to overcome some of the drawbacks to non-enhanced phytoextraction. This approach makes use of high-biomass crops induced to take up large amounts of metals in a short period of time. The mobility

of a metal in the soil is enhanced by chelating agents such as: ethylenediaminetetraacetic acid (EDTA), cyclo-hexyl-ethylenediaminetetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), ethyleneglycoltetraacetic acid (EGTA), ethylenediaminedi (0-hydroxyphenylacetic) acid (EDDHA), and nitrilotetraacetic acid (NTA) (Lombi et al., 2001). Previous studies have reported dramatic increases in plant accumulation of Cd, Cu, Ni, Pb, and Zn from soil in the presence of added synthetic chelates (Blaylock et al., 1997). A chelate can free a heavy metal from the soil cation exchange sites by forming a complex with the heavy metal and allowing it to move freely in the soil solution as a chelate-metal complex (Figure 1). Once the metal has been removed from the cation exchange sites it can be taken up by plant roots in the immediate vicinity. One of the most powerful and commonly used chelating agents is EDTA, which forms complexes with many of the metal contaminants within the natural environment. Application of EDTA to lead-contaminated soils increases lead uptake from the soil to more than 1% (w/w) of shoot dry biomass (Huang and Cunningham, 1996; Blaylock et al., 1997; Huang et al., 1997). Vassil et al. (1998) found the majority of lead transported to plant shoots within *Brassica juncea* (Indian mustard) was greatly enhanced and was transported in correlation with EDTA. EDTA allows plants to accumulate high concentrations of lead in shoots. Epstein et al. (1999) found that in soil amended with 4.8 mmol/kg lead and 5 mmol/kg EDTA, transpiration of *B. juncea* was unaffected and the concentration of EDTA and lead in shoot tissue increased. Epstein et al. (1999) also found that maximized lead accumulation by plants occurs by maximizing the concentration of lead-EDTA complex based on the EDTA extractable soil lead. Lestan

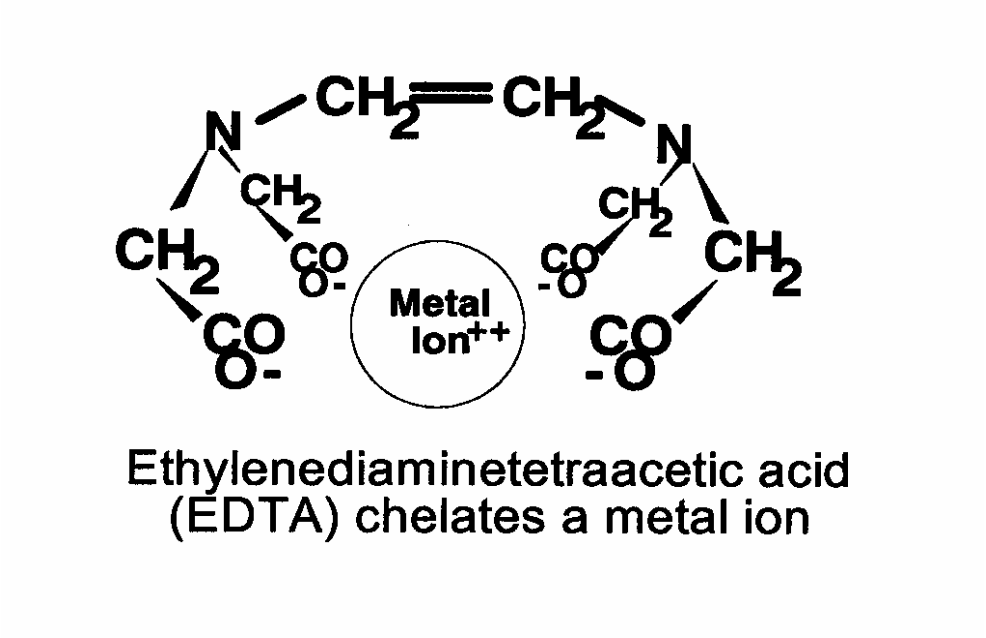

Figure 1. EDTA forms a complex with metal ions to form a chelate-metal complex which moves freely with the soil solution.

and Grcman (2002) found that single doses of 10 mmol EDTA and EDDS (ethylene diamine dissuccinate)/kg soil caused the concentration of lead in plants grown on disturbed soils to increase 94.2- and 102.3-fold, respectively, relative to the control. Lestan and Grcman (2002) also found that EDDS caused less leaching of lead than EDTA at 0.8% and 22.7% of total lead concentration, respectively.

The use of chelating agents reduces the drawbacks of natural hyperaccumulation in several ways. Many different high-biomass crops can be used, especially those that are tolerant to a specific metal contaminant. The use of a chelating agent also reduces the amount of time that lead is present in shoot tissue. The lead will stay in the soil until the chelating agent is applied, upon which time the lead-chelate complex will rapidly move from the roots to the shoots. The crop can then be harvested, thus reducing the overall time lead has a chance of moving into the food chain through herbivory.

There are several ways that plants can take up and sequester heavy metals within plant tissues and the goal of phytoremediation is to utilize and/or enhance these natural metabolic plant functions. Certain plants are better suited and genetically designed to deal with specific contaminants and much of the research conducted today focuses on finding the most appropriate hyperaccumulator for each heavy-metal contaminant.

Existing plant processes that limit phytoremediation of a soil contaminant (i.e. root uptake, root-shoot translocation, sequestration in specific tissues or cell compartments, biomass transformation, plant-microbe interactions) can be accelerated as pathways and genes involved in the phytoremediation are discovered (Pilon-Smits and Pilon, 2000). Plants naturally make metals more bioavailable for uptake by releasing

organic acids (e.g. malate or citrate) that act as metal chelators and decrease the rhizosphere pH, making metal cations more bioavailable (Ross, 1994). There is a positive correlation between the amount of organic chelate a plant produces and the amount of metal a plant is able to uptake. Plants can also change the pH in their immediate root zone through proton pumps in the root cell membrane (Pilon-Smits and Pilon, 2000). Rhizosphere microbes can also affect plant uptake of metals: bacteria have been reported to enhance uptake of Se and Hg (de Souza et al., 1999). The direct transport of metals across the root cell membrane is still being researched as the energetics of transport are not fully understood and often more than one transport system exists for a given metal (Maser et al., 2001). The genome of the model species *Arabidopsis thaliana* (Thale Cress or Mouse-ear Cress) encodes for over 150 different cation transporters in at least nine different families (Pilon-Smits and Pilon, 2002) so research for individual phytoremediation hyperaccumulators may still be years in the future. The ability to enhance uptake of the metal into the root symplast is the first step to phytoextraction and increasing the overall uptake of metal contaminants.

Transportation of metals from the roots to the shoots requires movement through the xylem and is likely transpirationally driven (Salt et al., 1995). Uptake is assisted by chelate-complexation through either natural chelates (e.g. malate or citrate) or synthetic chelates such as EDTA. Root-shoot translocation requires metal transporters from the root symplast to the xylem apoplast (Marschner, 1995) and transportation from the xylem apoplast to the shoot symplast is mediated by metal transporters in the shoot cell membrane (Pilon-Smits and Pilon, 2002). Phytoremediation can be increased by

increasing metal translocation from the roots to the shoots by enhancing the already present metal transporters or by utilizing synthetic chelates to enhance metal movement.

With increased research for enhancing phytoremediation in natural hyperaccumulators or by enticing fast-growing, high-biomass, metal-tolerant plants to take up metals there are still definite drawbacks and concerns. First, lead has limited solubility in soil solutions due to: complexation with organics and inorganic soil colloids, sorption on oxides and clays, and precipitation as carbonates, hydroxides, and phosphates. Also, there are not many metal hyperaccumulators because in most soils and under "normal" conditions there is less than 1% available lead of the total lead in soil and uptake by plants is very limited (Lestan and Grcman, 2002). Another concern is the addition of synthetic chelates to increase the bioavailability of lead also increases the movement of lead within the soil allowing it to move off-site by overland flow or through leaching into the groundwater. The amount of chelate added must not free up excessive lead beyond what the accumulating plants are able to take up. Finally, the appropriate time of chelate application in regards to plant growth as well as the quantity of chelating agent added are two factors which must be taken into consideration before synthetic chelates are added to a contaminated environment.

Typha latifolia (broad-leaf cattail) is an easily grown aquatic plant that has the potential to be an effective lead accumulator. The biomass produced from a stand of cattails is one characteristic favorable for phytoremediation (Wang et al., 1997). *T. latifolia* grows rapidly as monotypic stands, tolerates high levels of lead contamination in the soil, and has the ability to regrow new shoot tissue (after its existing shoot has been

removed) by two means. First, the apical meristem is located very close to the soil level, so most of the shoot can be harvested and the cattail will be able to produce more shoot tissue. The second way cattails can recover from shoot mass removal is by its rhizome, which enables new shoots to emerge via utilization of stored carbohydrate energy. This ability provides a unique situation to germinate seeds in contaminated soils and to harvest shoots continuously from the same root mass. Continuous harvesting would save money and time by removing many labor intensive steps of continuous replanting and harvesting. Exberger (2006) found cattails to be hyperaccumulators in hydroponic studies of lead and zinc-contaminated water. Lead and zinc accumulated in plant tissues up to 5,465 ppm and 330 ppm in the rootstalk tissue and the mature shoot tissue, respectively. Through the use of chelates, lead phytoextraction with *T. latifolia* may become a feasible option for field applications.

As previously stated, traits for an ideal phytoremediation crop are rapid growth, high biomass, and a high tolerance to contaminated soils. Finding a plant with these traits and the natural ability to take up a contaminant is the most difficult obstacle in phytoremediation. The addition of a chelating agent such as EDTA will help overcome this obstacle and make *T. latifolia* a possible candidate for phytoremediation of lead-contaminated soils.

The objectives of this study are (1) to determine if *T. latifolia* will germinate and fully develop in lead contamination of up to 4000 ppm, (2) to determine the increase of lead uptake and movement from root to shoot through the use of EDTA in a lead-contaminated soil system, and (3) to determine the feasibility of continuous shoot

harvesting for lead uptake while allowing the existing root mass to regrow new and/or auxiliary shoots.

METHODS AND MATERIALS

This experiment was conducted over a 12-week growth period with three treatments to *T. latifolia* in four levels of lead contamination. The first treatment group of *T. latifolia* was allowed to grow for the full 12 weeks. Tetrasodium ethylenediaminetetraacetic acid (EDTA) was applied to this first treatment group during the 10th week of growth. In the second treatment group, the primary vegetative shoot was harvested after the 6th week of growth and the *T. latifolia* plants were allowed to regrow the primary shoot and/or auxiliary shoots for the second 6 weeks (regrowth). The third and final treatment group was grown for the full 12 weeks with no additions and no preliminary shoot harvesting (control). The four lead-contamination levels were 0, 1000, 2000, and 4000 ppm lead. Ten replicate plants were used in each treatment group at each lead-contamination level.

Plant and Soil Collection/Preparation

Typha latifolia seeds as well as soil were collected from the Healthsport/Arcata Community Center wetland area (Figure 2) as detailed by the Department of Environmental Services of Arcata, California. The seeds were collected by clipping off the mature female cylindrical inflorescence (seed spike) from 10 different mature plants to ensure genetic variation. The soil was collected from the top 12 inches of the marsh area and stored in five gallon buckets. Both the seed spikes and the soil were then taken back to Humboldt State University for further preparation. The soil was oven dried at

105º C for 48 hours, passed through an electric, belt-driven, rock crusher and then passed through a #10 (2 mm) sieve to remove gravels and large pieces of organic matter. A sample of the soil was sent for further analysis to Dellavalle Laboratories (1910 W. McKinley, Suite 110, Fresno, CA 93728) with the following results: the soil was a clay loam, pH 6.0, electrical conductivity (EC) 0.99 ds/m, and 1.29 mg/kg (ppm) lead. The seed spikes were pulled apart and the pappus was separated from the seeds by stirring the seeds and pappus in a beaker with water. The mature seeds sunk to the bottom of the beaker where they were removed with an eyedropper and allowed to dry on filter paper. The seeds were kept in a cool dark place until they were needed.

Nitric Acid Rinse

All glassware was washed and then rinsed thoroughly with deionized water. Subsequently, the glassware was then rinsed in a 20% nitric acid bath for 30 seconds and re-rinsed with deionized water to remove any traces of lead on the glassware.

Personal Protection

Nitrile gloves were used to prevent lead from entering through small cuts on the hand and half-face mask personal respirators with 99.97% efficiency (HEPA filters) were worn to prevent lead inhalation via dust particles when the soil was dry throughout the experiment.

Figure 2. Soil was collected from the marsh at the Healthsport Pond in Arcata, CA.

Soil Contamination

Seven hundred grams of the oven-dry soil were placed into one-quart mason jars (previously acid washed with a 20% nitric acid bath). Lead in the form of lead carbonate ($PbCO_3$) was then added to the soil to achieve the following lead-contamination levels; 0 ppm, 1000 ppm, 2000 ppm, and 4000 ppm. The amount of $PbCO_3$ added was 1.16 g $PbCO_3$ for 1000 ppm, 2.32 g $PbCO_3$ for 2000 ppm, and 4.64 g $PbCO_3$ for 4000 ppm contamination. The soil and $PbCO_3$ were stirred and mixed with deionized water to bring the soils to saturation. After the soil and $PbCO_3$ were mixed, another 200 g of soil was added to the mason jars to bring the total soil to 900 g. The soil was brought to saturation again. The contaminated jars were then randomly assigned to one of the three treatments (EDTA, shoot regrowth, and control). The jars were then wrapped in aluminum foil to the neck of the mason jar to reduce algal growth. The mason jars were allowed to sit for one week in darkness to allow for the soil solution and $PbCO_3$ to come to equilibrium, and then moved to the Humboldt State University experimental greenhouse.

Plant Propagation and Growth Period

The jars were put into 41 cm x 31cm plastic boxes for ease of movement on the greenhouse table. There were 12 randomly chosen jars placed into each plastic box and the 10 plastic boxes were placed under the growth lights in the greenhouse (Figure 3). The soils were then seeded with 4-10 *T. latifolia* seeds and the grow lights were placed 1.5 meters from the table where the *T. latifolia* were growing. As the *T. latifolia* grew in size, the lights remained 1.5 meters from the table throughout the duration of the

experiment. The lights were programmed to simulate a 16 hour day. The grow lights were tested for light output (microeinsteins/m^2/sec) on three random days at a height of 20 cm and 80 cm above the table with the *T. latifolia* to collect light intensity. The average output of the lights plus natural light at 9:00 am was 128 microeinsteins/m^2/sec at 20 cm above the table and 348 microeinsteins/m^2/sec at 80 cm above the table. The average output of the lights plus natural light at 2:00 pm was 376 microeinsteins/m^2/sec at 20 cm and 506 microeinsteins/m^2/sec at 80 cm. The average output of the lights plus natural light at 6:00 pm was 124 microeinsteins/m^2/sec at 20 cm and 325 microeinsteins/m^2/sec at 80 cm. The plastic boxes were rotated every other day to ensure equal light exposure to each jar. The jars were filled with water as needed to keep the soil inundated with approximately one to two cm of water. The watering and jar rotation continued for the entire 12 weeks of growth. The height of each plant was recorded during each week of the experiment.

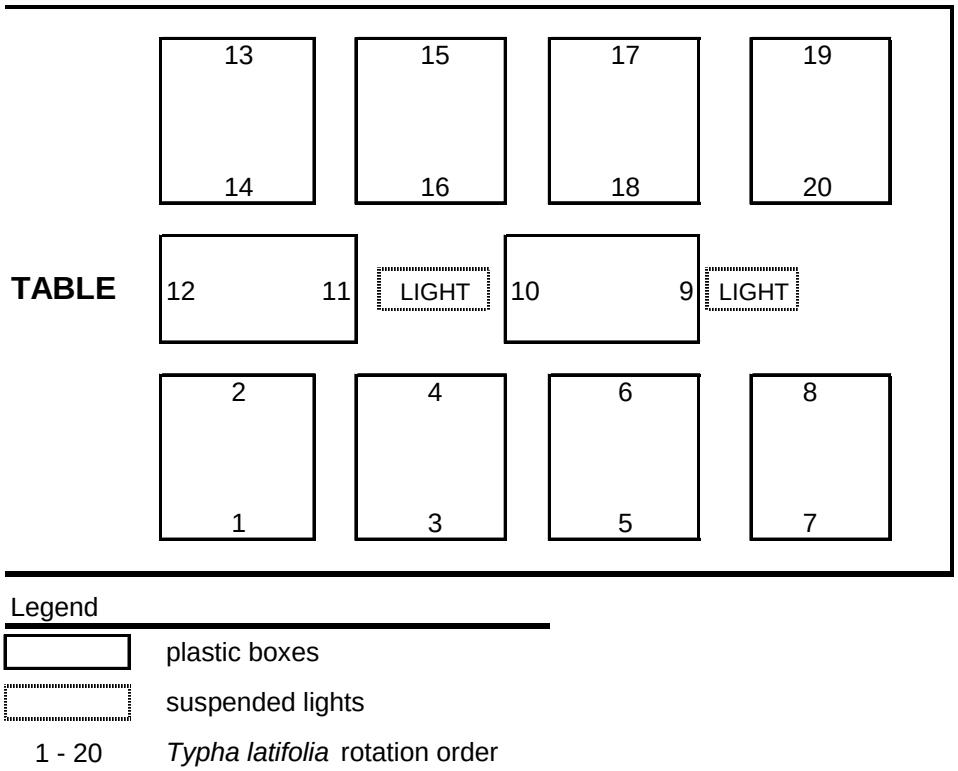

Figure 3. The light placement and table rotation of *Typha latifolia* in the Humboldt State University experimental greenhouse.

The *T. latifolia* were thinned to three plants per jar after ten days and after 21 days the plants were culled to the healthiest plant. At week six, the primary vegetative shoots of the regrowth treatment were collected by cutting the shoots 3.5 cm above the surface of the soil. The harvested shoots were taken to the laboratory for analysis of lead content as described later in "Plant Tissue Analysis." The regrowth treatment plants were then allowed to re-grow the primary shoot or an auxiliary shoot from the rhizome. At week 10, 5.03 g of EDTA were dissolved in 20 ml of deionized water and added to each EDTA treatment jar through an 18 gauge hypodermic needle to make a 15 mmol/kg soil solution. The final concentration in the 900 g of soil was 13.5 mmol EDTA. The needle was injected into the soil as the plunger of the needle was depressed in order to reduce blockage of the needle. Three injections were utilized to achieve an even distribution of the EDTA throughout the soil in the jar. After 12 weeks of growth, all of the shoots were harvested and separated into top shoot (newest growth) and bottom shoot (older growth). The top shoot was harvested by collecting the top 30 cm of the shoot. The bottom shoot was harvested by collecting the remaining shoot not taken by the top-shoot harvest. The bottom shoot was clipped 3 cm from the soil surface. If the shoot was not greater than 30 cm tall, the entire shoot was considered part of the top shoot. Auxiliary shoots from rhizomes were harvested as well and kept separate from the primary shoot for independent analysis.

Plant and Soil Collection

The vegetative shoots were collected, placed into brown paper bags and allowed to dry for 48 hours at 65˚ C. The glass jars were taken to the lab and broken to allow for the separation of the roots, rhizomes, and soil (Figure 4). Once the jars were broken, samples of the soil were placed into sealable plastic sample bags for soil-lead analysis. The roots and rhizomes were separated from the soil and washed of any superfluous soil still attached. The roots were removed from the rhizomes and the rhizomes were then placed into paper bags and dried at 65˚ C for 48 hours. As many roots as possible were collected and placed into paper bags to be dried at 65˚ C for 48 hours. Once all the top shoots, bottom shoots, and rhizomes were oven dried, the weights were recorded to analyze biomass using ANOVA in the NCSS/PASS (Hintze, 2005) software system (roots were not included because not all of the roots could be isolated from the soil).

Plant Tissue Analysis

Each pre-weighed dried plant tissue sample was placed into a pre-weighed, nitric-acid washed crucible and ashed in a muffle furnace at 500˚ C for at least 8 hours. After the samples were allowed to cool in a fume hood, the ash was dissolved in 10 mL of 20% HCl and warmed on a hot plate between 80-90˚ C (still under a fume hood) to ensure the ash was completely dissolved (approximately 10 to 15 minutes). The acid solution was then filtered through a Whatman 44 filter paper and the filtrate was brought to exactly 25 mL with glass distilled water. A new filter paper was used for each sample and the funnels were washed in a 20% nitric acid bath between each filter session. The filtrate

was collected in sterile 50 mL Falcon tubes (plastic disposable graduated cylinders). The filtrate was analyzed for lead using a Perkin-Elmer Model 103 atomic absorption spectrophotometer (AAS) with a Fisher hollow cathode (Ne-Pb) lamp and an air-acetylene flame. If the metal concentration was too high to be accurately analyzed by the AAS, dilutions were completed to bring the lead concentration within the readable range of 0-20 ppm.

Figure 4. The roots and rhizomes were separated from the soils in the laboratory and then prepared for dry tissue biomass analysis of rhizomes and lead concentrations in roots and rhizomes.

Soil Analysis

The soil was allowed to air dry for at least one week in the greenhouse. Approximately 5.0 g. of soil was weighed and then pulverized in a clean, nitric acid washed mortar and pestle. The soil was wrapped in a paper towel before being pulverized with the mortar and pestle to minimize the nitric acid washing necessary for the mortar and pestle. The soil was placed into a 100 mL erlenmyer flask and 10 mL of 1 M ammonium acetate (NH_4OAC) was added to the soil. The flask with the soil/ammonium acetate mixture was then placed on a mechanical shaker at 125 rpm at room temperature for 30 minutes. The soil slurry was then passed through a Whatman 44 slow filter into a sterile 50 mL Falcon tube. The filtrate was brought to exactly 50 mL with the extraction solution, and the filtrate was analyzed using the AAS.

Statistical Analysis

The height of the plants, the mass of the total shoot biomass, and the biomass of the rhizomes were analyzed using ANOVA in the NCSS/PASS (Hintze, 2005) software system. The concentration of lead in each plant portion was analyzed using two-sample T-tests for specific comparisons between lead concentration in the roots and shoots. The overall total lead accumulation (mg lead) was analyzed using ANCOVA (shoot biomass was used as the covariate) in the NCSS/PASS (Hintze, 2005) software system.

RESULTS

Visual Observations

During the first six weeks of cattail growth, the only visual sign of plant stress was reduced growth in the plants subjected to the 2000 and 4000 ppm lead (discussed later in the cattail height and biomass section). Browning and death of shoot tips and yellowing of the margins were detected in all cattails in the EDTA treatment group after the EDTA was applied to the soil (Figure 5). The tip death was due to a salt effect and heavy-metal uptake from the EDTA-lead complex.

Cattail Height and Biomass

All of the *T. latifolia* lead treatments were used for the height analysis at week six as the only factor influencing their growth was the lead content. At week ten, the regrowth treatments were not included in the height analysis because the primary vegetative shoots had been harvested after the height measurements were completed at week six. The EDTA was applied in week ten and the tips of the plants were burned and the growth of the plants in the EDTA treatments stopped. The growth of the cattails began to slow as their height approached 90 cm (week 10 through week 12). The reduced height increase was attributed to space constraints of the mason jars so the height of the cattails at week 12 was not analyzed as other variables beside the lead concentration were contributing to cattail height differences.

Figure 5 Yellowing and burning of *T. latifolia* leaves after twelve weeks of growth and the addition of EDTA at the tenth week.

Reduced cattail height was visibly more noticeable during the first six weeks of growth as compared to height differences at week ten. There was a significant difference in height between all the lead contamination levels at week six except between the 2000 and 4000 ppm lead-contamination levels whereas there was only a significant difference between the 0 and 2000 ppm lead at week ten.

There was a significant difference ($p < 0.00001$) in the cattail height after six weeks of growth (Figure 6). According to the Tukey-Kramer multiple comparison test there was a significant difference between the 0 and 1000 ppm treatments (56.9 cm and 50.9 cm, respectively). The 2000 and 4000 ppm treatments with were not significantly different from each other (44.1 cm and 40.6 cm, respectively) but were significantly different from both the 0 and 1000 ppm treatments. The reduction in height was likely due to the presence of the lead in the soil limiting the roots' ability to take up adequate nutrients from the soil in the initial stages of plant growth.

There was a significance difference ($p = 0.0464$) in plant height after ten weeks of growth. The only significant difference was between the 0 and 2000 ppm (87.8 cm and 83.9 cm, respectively). There was no significant difference between 1000 and 4000 ppm (85.3 cm and 85.25 cm, respectively) and these two treatments were not significantly different from either the 0 or 2000 ppm treatments. At week 10 the growth of the plants slowed down as they approached 90 cm. The cattails were likely reaching their maximum growth while contained in mason jars. The slower growth due to space constraint allowed the higher concentration lead treatments to catch up to the faster growing 0 and 1000 ppm treatments.

At week twelve the constraints of the mason jars slowed the growth of the cattails as they approached 90 cm and the slow growing 4000 ppm became the tallest of the treatments. The height difference between the tallest and shortest cattail in the control treatment group was only 4.3 cm. Due to the space constraints from the mason jars and the slowed growth, the height of the cattails after twelve weeks of growth was not statistically analyzed.

Overall, lead had a tendency to slow the growth of the cattails in the early growth stages but did not seem to negatively affect the potential maximum growth of the cattails (Figure 7). *T. latifolia* has been known to have strong resistance to phytotoxicity from lead contamination and under contamination levels at 4000 ppm (double the commercial contamination limits) the cattails were the tallest and not significantly different from the control lead levels at 0 ppm.

There was a significant difference ($p = 0.0001$) in the shoot biomass for the lead level in the control plants (Figure 8). The 0 ppm lead control plants had the greatest biomass after 12 weeks of growth at 5.64 g and were significantly greater than the 1000, 2000, and 4000 ppm lead levels at 4.99 g, 4.61 g, and 4.85 g, respectively.

There was a significant difference ($p < 0.0001$) in the shoot biomass among all three treatment methods. The mean of all lead levels for the control treatment produced 5.01 g, the EDTA produced 4.05 g, and the regrowth treatment (combining both the six week and twelve week harvests) produced 1.83 g of shoot biomass. The small biomass produced by the regrowth treatment was caused by the removal of the vegetative shoot

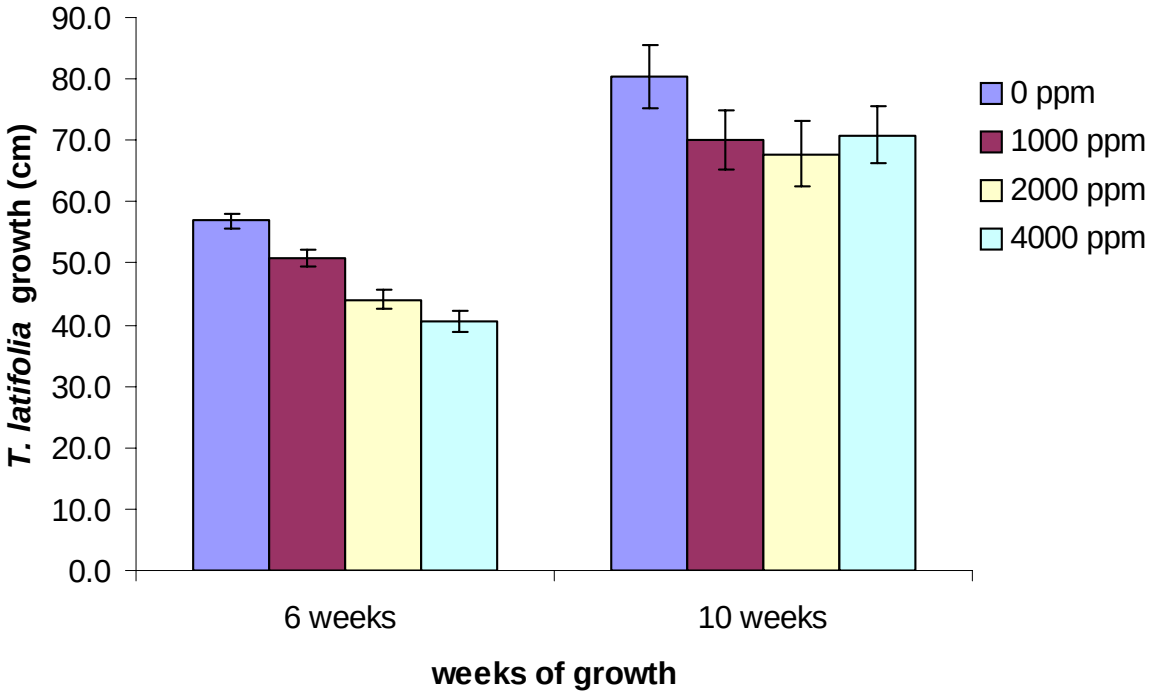

Figure 6 Height (cm) of *T. latifolia* after 6 and 10 weeks of growth in 0, 1000, 2000, and 4000 ppm lead contamination (week 6, n = 120; week 10, n = 80).

Figure 7 Representative *T. latifolia* growth after week 6 (left), week 10 (center), and week 12 (right). In each picture the lead contamination levels are 0, 1000, 2000, and 4000 ppm lead soil contamination from left to right respectively. (Note: the week 12 picture of 0 and 2000 ppm lead soil contaminants showing shoot tip death and yellowing along margins due to the application of EDTA.)

mass at week six. The removal of shoot mass retarded the growth of the cattails by removing the photosynthetic portion of the cattail and making it rely on the minimal stored carbohydrate produced in the first six weeks of growth. Fourteen of the regrowth treatment plants were not able to recover from the initial harvest and only two of the 0 ppm lead contamination level remained alive. The 0 ppm had such a high mortality rate because they were not slowed in growth during the initial six weeks of growth and when the shoot was harvested, the apical meristem may have been damaged. The smaller cattails subjected to the 2000 and 4000 ppm lead levels grew slowly in the first six weeks and because their apical meristem was much lower, the plant was able to recover from the shoot removal.

The EDTA treatment growth was stunted in the tenth week of growth and did not appear to produce additional biomass after this time. Overall these plants were able to grow uninhibited for ten weeks as compared to the six weeks of uninhibited growth in the regrowth treatment.

Lead Accumulation

The lead accumulation was analyzed in two different ways. The first was by lead concentration (mg/kg dry tissue or ppm) in each of the plant tissues (roots, rhizome, shoot base, and shoot top). Two sample T-tests were used to analyze differences in lead concentration between different plant tissues. The second method was actual mg lead present in the rhizome and shoots. The actual mg lead uptake was analyzed as well to determine feasibility of *in situ* remediation. Lead concentration (ppm) can be high while

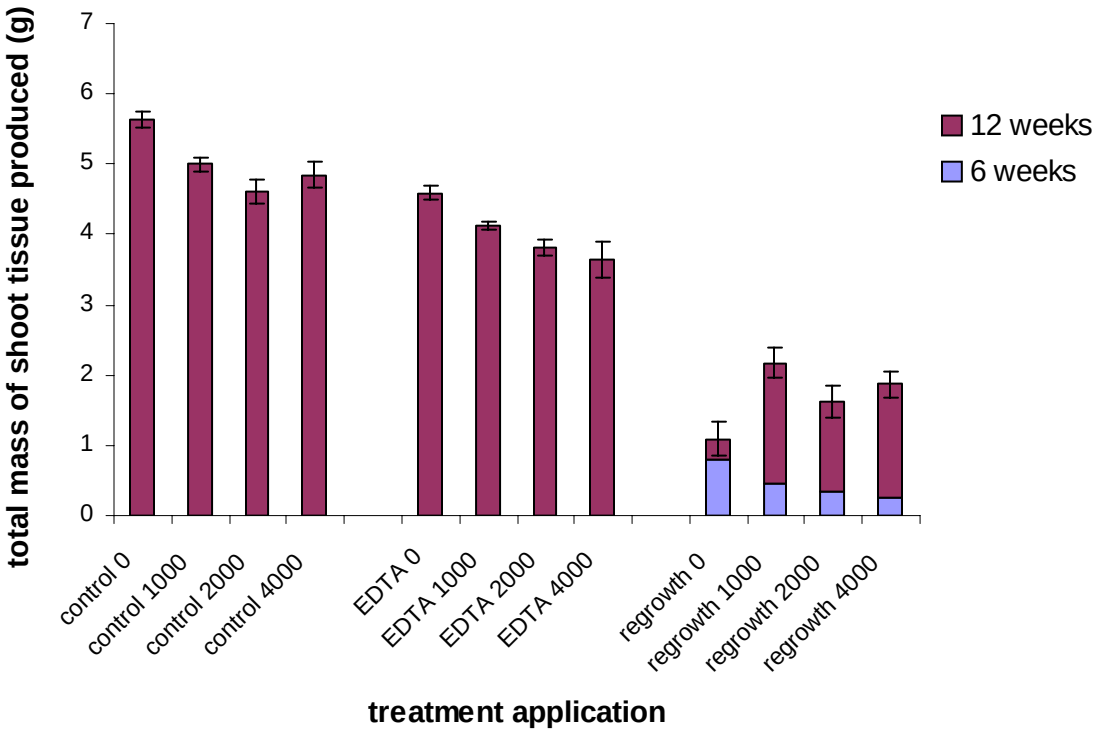

Figure 8 Biomass (g) of shoot tissue produced by *T. latifolia* with 3 treatments in 0, 1000, 2000, and 4000 ppm lead-contaminated soil (Note: only 2 plants survived the regrowth treatments at 0 ppm lead.) (n = 11 for control 2000 ppm and EDTA 1000 ppm; n = 10 for control 0, 4000 ppm, EDTA 0, 4000 ppm; n = 9 for control 1000 ppm and EDTA 2000 ppm; n = 8 for regrowth 1000, 2000, 4000 ppm; and n = 2 for regrowth 0 ppm.)

biomass is low and therefore very little lead may have actually been taken into the plant tissues and out of a contaminated site. ANCOVA was used to analyze the lead accumulation (mg lead) because the size of the shoots was a significant co-variate in lead uptake.

The lead concentration (ppm) in the control treatment was significantly greater in the root tissue for all lead-contamination levels and decreased in concentration when moving from the roots to the rhizomes and then into the shoot tissues (Figure 9). There was a significant difference ($p < 0.0001$) between the roots and all other tissues for each of the lead levels. This supports other investigators' findings that lead is sequestered in the root tissue when there is not a chelating agent (natural or chemical) to mobilize the lead from the roots to the shoots. The greatest concentration of lead was found within the root tissue in the 4000 ppm lead-contamination level at 1515.2 ppm.

The lead concentration (ppm) in the EDTA treatment was not significantly different at the 4000 ppm lead level ($p = 0.890$) and the 1000 ppm lead level ($p = 0.194$) between the roots and the shoot tops (Figure 10). The lead concentration for the roots and shoot tops was 2483.5 and 2418.5 ppm, respectively, for the 4000 ppm lead level and 702.8 and 557.2 ppm, respectively, for the 1000 ppm lead level. There was a significant difference at the 2000 ppm lead level ($p = 0.009$) and the 0 ppm lead level ($p < 0.0001$) between the roots and the shoot tops. The lead concentration for the roots and shoot tops was 1374.8 and 811.2 ppm, respectively, for the 2000 ppm lead level and 81.4 and 14 ppm, respectively, for the 0 ppm lead level. The increased amount of lead transported

to the shoots is evidently due to the lead-EDTA complex, which is taken up freely by the cattail and subsequently moved through the plant tissues and into the shoot tissue.

The lead concentration (ppm) in the regrowth treatments was significantly different ($p < 0.0001$ for 1000, 2000, 4000 ppm lead; $p = 0.0006$ for 0 ppm lead) between the roots and the shoot tops in all of the lead levels (Figure 11). The greatest concentration of lead was found at the 4000 ppm lead level in the roots at 2370.6 ppm lead. The regrowth treatment had greater concentrations overall than the control treatment including shoot tops, but this is also due to the smaller biomass generated in the regrowth treatment. The concentration was likely greater in the regrowth than the control because the roots worked at the same rate initially when the vegetative shoot tissue was removed and thus created a greater concentration in the remaining and newly emerging vegetative growth.

Figure 12 and Figure 13 are both shown as actual mg lead taken into the plant tissues. The results were log transformed (not shown in Figure 12 and Figure 13) to meet variance and normality assumption of ANCOVA.

There was a significant difference in the total mg lead uptake ($p < 0.00001$) for the three treatments and the four lead concentrations using ANCOVA on the log of the total lead uptake (Figure 12). The total shoot weight was a significant covariate ($p = 0.007518$). The total lead uptake was significantly greater in the EDTA treatments at the 1000, 2000, and 4000 ppm soil lead contaminations (1.92 mg, 2.83 mg, and 6.52 mg lead, respectively) according to the Tukey-Kramer Multiple Comparison Test. There was not a significant difference between total lead uptake in the control and the regrowth

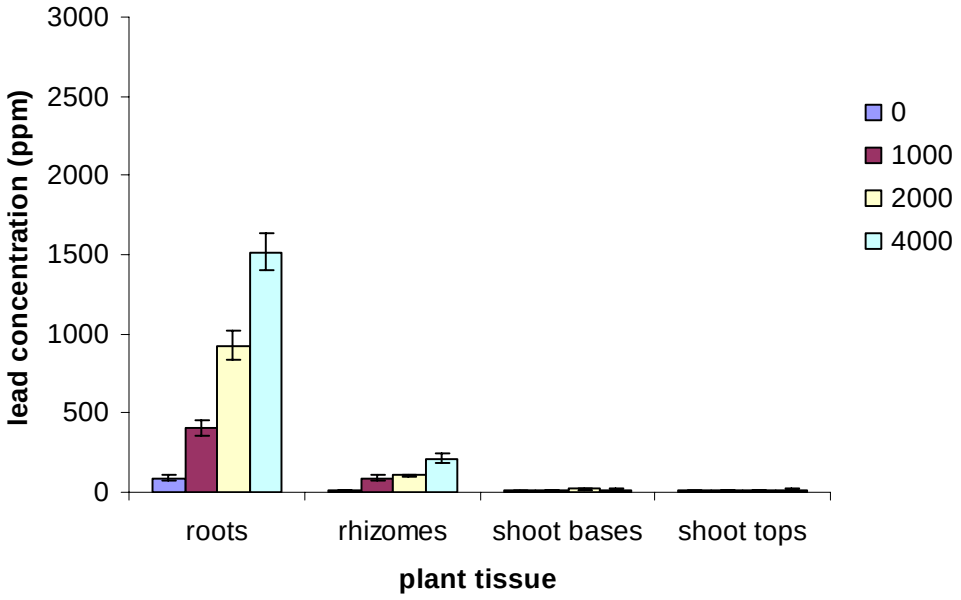

Figure 9 Lead concentration (ppm) in control cattail plant tissue (without EDTA addition or previous shoot harvest). (n = 10 for 0, 4000 ppm; n = 9 for 1000 ppm; n = 11 for 2000 ppm).

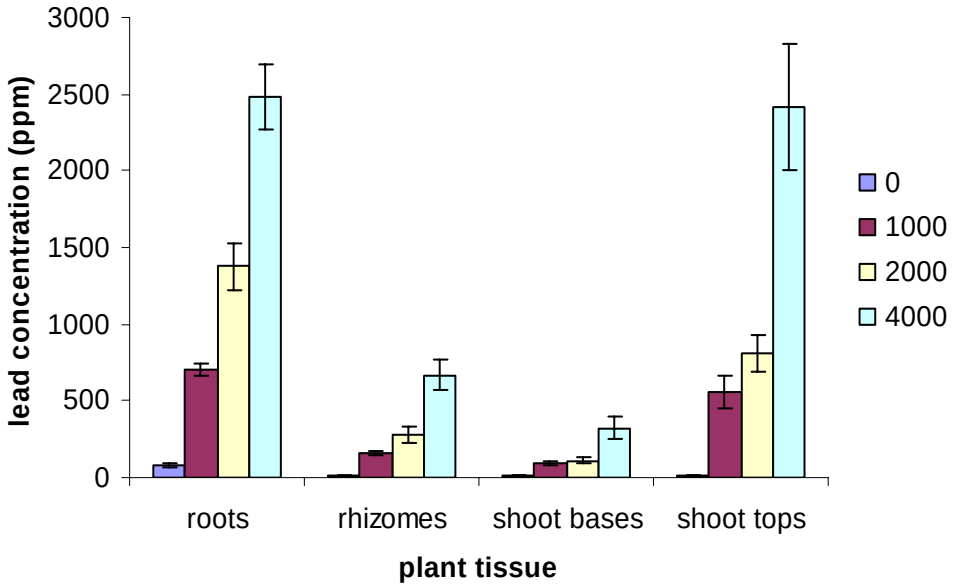

Figure 10 Lead concentration (ppm) in cattail tissue after 12 weeks of growth with an addition of EDTA (15 mmol/kg soil) at 10 weeks of growth. (n = 10 for 0, 4000 ppm; n = 11 for 1000 ppm; n = 9 for 2000 ppm).

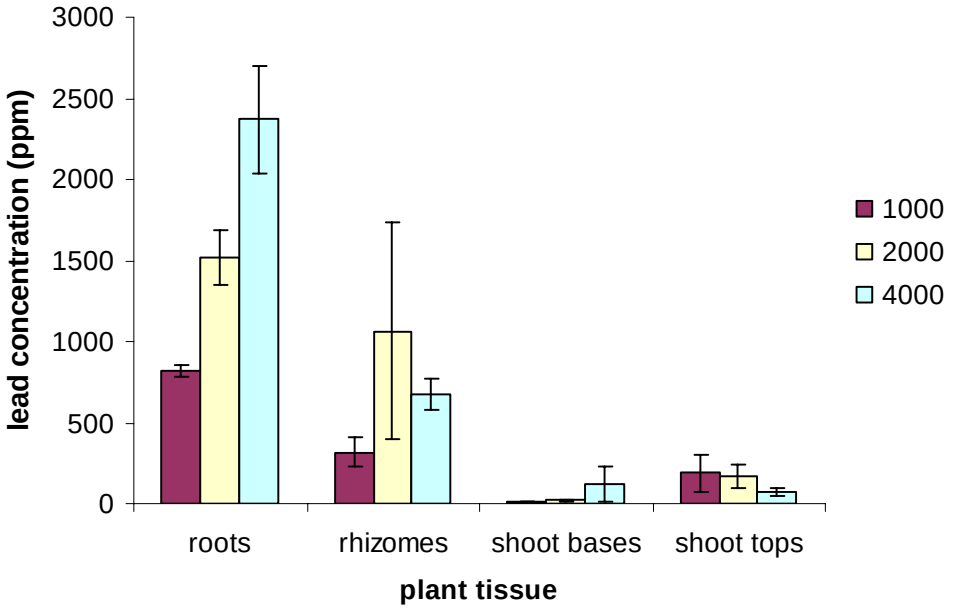

Figure 11 Lead concentration (ppm) in *T. latifolia* tissue after 12 weeks of growth in 1000, 2000, and 4000 ppm lead after a shoot harvest on the sixth week of growth. (Note: 0 ppm lead was not included as only two plants survived the six week harvest, only one produced a rhizome, and the biomass collected was too small to analyze accurately with available technology). (n = 10 for 1000, 2000, and 4000 ppm).

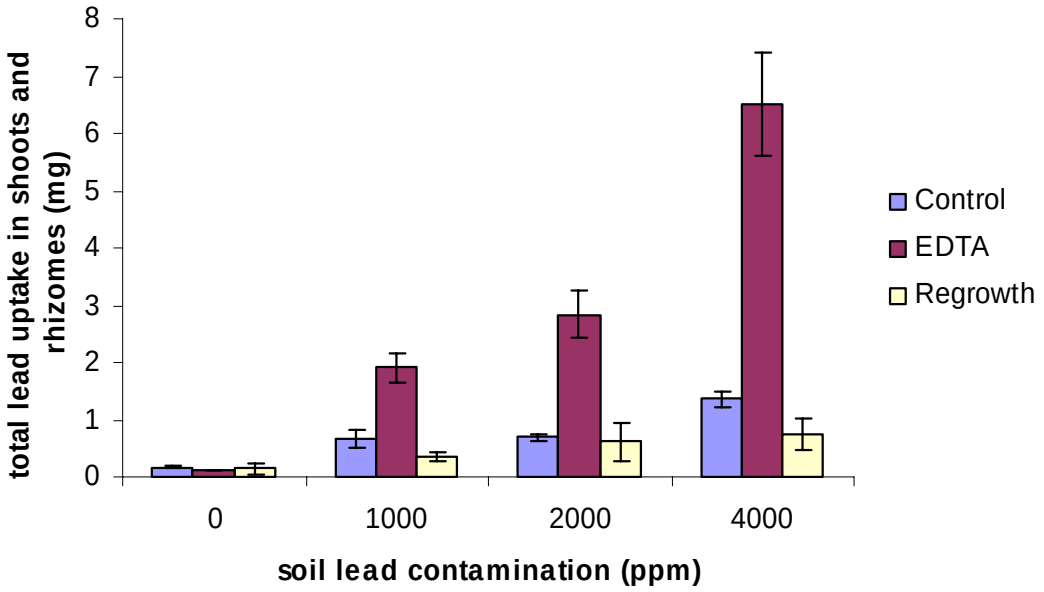

Figure 12 Total lead uptake (mg) in the rhizomes and shoots of *T. latifolia* at 0, 1000, 2000, and 4000 ppm lead contamination levels with 3 treatments. The lead uptake was log transformed before analysis with ANCOVA with biomass as the covariate (n = 10 for control 0, 4000ppm, EDTA 0, 4000 ppm, regrowth 1000, 2000, 4000 ppm; n = 9 for control 1000 ppm, EDTA 2000 ppm; n = 11 for control 2000 ppm, EDTA 1000 ppm; n = 2 for regrowth 0 ppm).

at the 0 ($p = 0.19609$), 1000 ($p = 0.793512$), or 2000 ($p = 0.884813$) ppm lead contamination. There was a significant difference between the total lead uptake in the control and the regrowth at the 4000 ppm lead contamination ($p = 0.026649$) with more lead entering the control plants.

There was a significant difference in the quantity of lead taken up into the shoot tissue of the three treatments at the 1000 ppm lead contamination ($p = 0.000012$); the total shoot weight was not a covariate ($p = 0.528926$) (Figure 13). The EDTA treatment uptake was significantly greater at 1.19 mg lead according to the Tukey Kramer multiple comparison test. There was a significant difference in the quantity of lead taken up into the shoot tissue of the three treatments at the 2000 ppm lead contamination ($p < 0.000001$); the total shoot weight was not a covariate ($p = 0.894569$). The EDTA treatment uptake was significantly greater at 1.58 mg lead according to the Tukey Kramer multiple comparison test. There was a significant difference in the quantity of lead taken up into the shoot tissue of the three treatments at the 4000 ppm lead contamination ($p < 0.000001$); the total shoot weight was not a covariate ($p = 0.334240$). The EDTA treatment uptake was significantly greater at 4.38 mg lead according to the Tukey Kramer multiple comparison test. There was not a significant difference at the 0 ppm lead contamination level ($p = 0.661479$) and the total shoot weight was not a covariate ($p = 0.434503$).

Exchangeable Lead in the Soil

The exchangeable lead within the soil was significantly increased with the addition of 15 mmol EDTA (Figure 14). The EDTA creates a lead-chelate complex which frees the lead from the soil cation exchange capacity (CEC) and allows for movement with the soil solution and into the cattails as they photosynthesize and respire. If the lead is not freed from the CEC, then the uptake of lead will be greatly reduced as shown earlier in the reduced concentration of lead within the shoot tops in the control group when compared to the EDTA treatment (12.2 ppm lead and 2370.6 ppm, respectively). The application of 15 mmol EDTA to the soil only released approximately 10% of the total lead (4000 ppm; 17.3 mmol lead) in the soil from the CEC by forming a lead-EDTA complex. The EDTA did not release all of the lead into the soil solution because it is not a lead-specific chelator so it will create a chelate-metal complex with a wide range of metals within the soil. Also, there was not enough EDTA injected into the soil to create a chelate complex for all of the lead in the soil. The fact that not all of the lead was released from the soil CEC is important because it limits the quantity of lead that may be chelated, not taken up by the plant, and able to move freely with the soil solution (possibly off-site). As noted in the introduction, one of the main concerns with chemically enhanced phytoremediation is the formation of a contaminant-chelate complex and subsequent movement off-site in the groundwater.

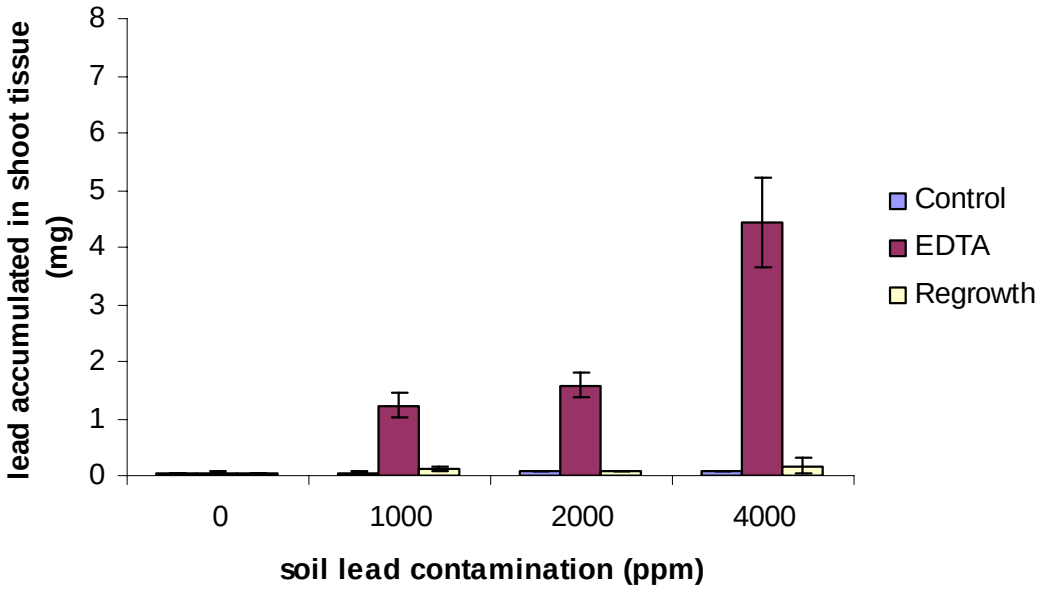

Figure 13 Total lead uptake (mg) in the shoots of *T. latifolia* at 0, 1000, 2000, and 4000 ppm soil lead contamination levels with three treatments. The lead uptake was log transformed before analysis with ANCOVA with biomass as the covariate (n = 10 for control 0, 4000 ppm, EDTA 0, 4000 ppm, regrowth 1000, 2000, 4000 ppm; n = 9 for control 1000 ppm, EDTA 2000 ppm; n = 11 for control 2000 ppm, EDTA 1000 ppm; n = 2 for regrowth 0 ppm).

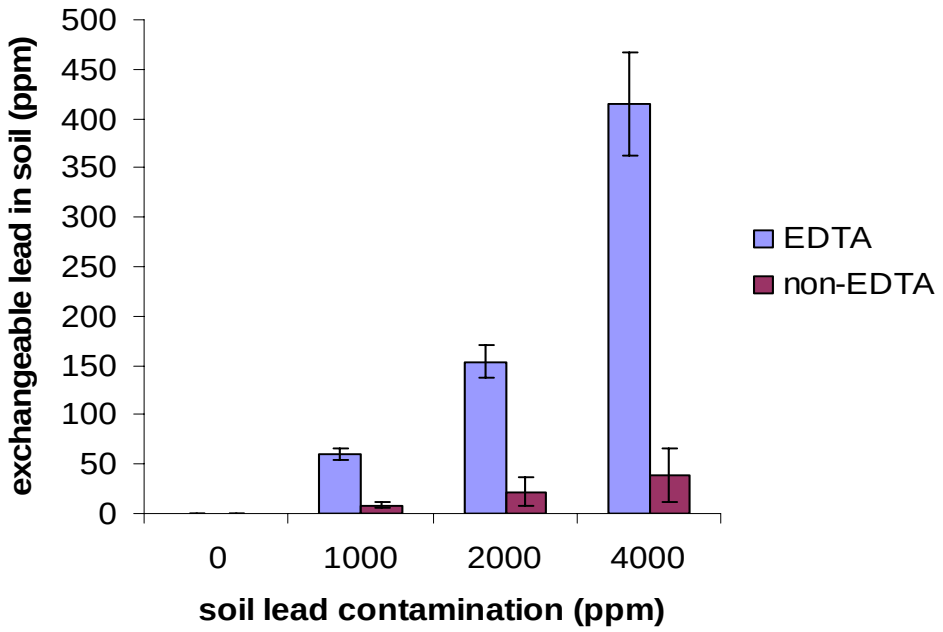

Figure 14 Exchangeable lead concentration (ppm) in the soil at 0, 1000, 2000, and 4000 ppm soil lead contamination levels with and without the application of 15 mmol EDTA. (n = 40 for EDTA; n = 80 for non-EDTA.)

DISCUSSION

This experiment directly compared a chemically enhanced lead-phytoremediation approach (EDTA application to the lead-contaminated soil) with a physically enhanced lead-phytoremediation approach (shoot removal to encourage an increase in lead uptake by the plant) by growing *T. latifolia* from seed in lead-contaminated soil. Similar studies have been conducted hydroponically (Exberger, 2006) or with *Typha angustifolia* (narrow leaf cattail) as a chemically enhanced lead-phytoremediation approach only (Panich-Pat et al., 2003). The cattails within the physically enhanced lead-phytoremediation approach did not reach hyperaccumulator status when grown in lead-contaminated soil, but accumulated a significantly greater concentration of lead within the shoot tissue when compared to the control plants. The chemically enhanced phytoremediation approach reached hyperaccumulator status and accumulated significantly greater quantities as well as higher concentrations of lead in shoot tissue than either the control or the regrowth methods.

The cattails proved to be very tolerant to increased levels of lead contamination. The overall height was only slowed in the first six weeks by the increased contamination of lead levels to 4000 ppm. In the twelfth week of growth the cattails grown in the 4000 ppm contaminated soils were approaching the same height as the cattails grown without lead contamination. Although the growth was slowed due to space constraints of the mason jars, the cattails did not show stunted growth when exposed to high soil-lead levels. Cattails have a strong tolerance for many heavy metal contaminants and this study

corroborates previous reports of the cattail's tolerance to lead. The dry shoot biomass produced in the control group was less for the 4000 ppm contaminated soil as compared to the 0 ppm control treatments. The control group (0 ppm lead contamination) produced the greatest shoot biomass whereas the lowest shoot biomass produced was from the 2000 ppm lead contamination. Panich-Pat et al., (2003) found that in general, lead did not have a significant effect on the biomass; increased lead concentration and longer exposure time did not result in decreased biomass of *Typha angustifolia* (narrow leaf cattail). Given time, a cattail stand would be able to establish at a site with lead-contaminated soils with only limited biomass reduction, which is the first step for cattail phytoremediation of lead-contaminated soils.

The control group was able to sequester 1515 ppm lead in the root tissue and only translocated 17 ppm lead to the shoot tips. Without natural or synthetic chelates, the cattail does not have a significant ability to absorb and translocate lead into shoot tissues. Pinach-Pat et al. (2003) found lead accumulates more in roots than in shoots or rhizomes. They determined that *T. angustifolia* grown in 267 ppm lead-contaminated soil accumulated 8937 ppm lead in its roots, 118 ppm lead in its rhizomes, and 146 ppm lead in its shoots. One of the concerns of phytoremediation using hyperaccumulators is the accumulation and sequestering of heavy metals in the shoot tissue where herbivory would introduce lead into the food chain. The sequestering of lead in the roots and not moving lead continuously to the shoots as traditional hyperaccumulators function would significantly decrease the probability and quantity of lead entering the food chain through herbivory. Specifically, cattail seeds are a food source for several species of ducks; geese

and muskrats feed on cattail stems and roots; and moose and elk will eat fresh spring shoots.

The regrowth treatment had a severe drop in overall shoot tissue biomass produced (total biomass including the harvest at six weeks and the final harvest; cattails that did not survive the harvest at six weeks were not included). There was an overall increase in the lead concentration in this treatment due to the overall lower biomass produced in the regrowth treatment. The regrowth treatments averaged 250 ppm lead in the shoot tissue whereas the control group averaged less than 20 ppm lead in shoot tissue. Exberger (2006) found that metal accumulation in new shoots increased lead and zinc content by greater than 10-fold when compared to the concentration in mature shoots. This can be explained by the smaller overall shoot biomass with an equivalent lead uptake.

A study by McNaughton et al. (1998) found there was no evidence that grazing reduced root productivity over the annual cycle in native grasses of the Serengeti. They concluded that intense herbivory does not inhibit root biomass or below-ground productivity of the Serengeti grasslands over either the short or the long-term. A study by Detling et al. (1979) also found that no statistically significant changes in root respiration occurred following defoliation of *Bouteloua gracilis* (blue grama) in the laboratory over a 10-day period following clipping to a 4-cm height to simulate grazing by large herbivores. The biomass of unclipped plants nearly doubled during the 10-day study period, while that of defoliated plants increased 67%, so biomass would be affected, but not initial root respiration. In a study using two tundra graminoids, *Eriophorum*

vaginatum (tussock cottongrass) and *Carex aquatilis* (water sedge), Chapin and Slack (1979) suggest that the stimulation of root respiration and phosphate absorption immediately following defoliation resulted from lowered root phosphorus status as nutrient reserves were reallocated to support shoot regrowth. They also found that root growth was affected more severely by defoliation than was root activity and two or more defoliations were required for reduced root elongation, initiation and weight per unit length, but root mortality increased only after four defoliations. Another study by Engel et al. (1998) determined that defoliation of plants during the growing season did not affect the number of tillers, weight per tiller, above-ground weight, number of buds, or weight of rhizomes, in *Andropogon hallii* (sand bluestem).

Several of the above-mentioned studies noted that multiple defoliations during a single growing season would affect the root activity but a single defoliation did not significantly affect root activity. The allocation of resources in a defoliated plant may be altered due to shoot removal, physiological changes may occur, and after time the roots would adjust and slow processes due to decreased photosynthesis, but the quantity of lead moved into the shoot tissues would still be a greater concentration due to the reduced shoot biomass and continued nutrient and metal uptake due to root activity. Therefore, lead was still being transported into the existing shoot tissue and significantly raising the lead concentration but the amount of lead taken up was not significantly greater than the control group. If the regrowth treatment were allowed to grow to its maximum potential, it might take up an equivalent amount of lead as the control group, but harvesting the shoot tissue would not necessarily increase the cattail's ability to take up substantial lead

to a create a practical remediation option unless physiological changes were taking place within the cattail due to defoliation. Exberger (2006) also reported an increase in lead concentration in shoot tissue after defoliation and if physiological changes are taking place over a longer period of time after defoliation, then longer growing times and multiple harvests should be studied for cattail phytoremediation options. Another possible strategy could include the use of both EDTA and shoot removal approaches combined. Repeated harvest with applied EDTA could prove to be a practical phytoremediation technique and should be investigated.

The EDTA treatment was the best treatment for lead uptake and translocation from the soil, to the roots, and into the harvestable shoot tissue. There was not a significant difference between the concentration of lead in the shoots and the roots for the EDTA treatment at the 4000 ppm soil contamination, which means the plant was translocating a high quantity of lead to the shoot tissues. Sequestering contaminants at the shoot tips enables the plant to lose the contaminants through leaf senescence. Essentially, the plant is attempting to rid itself of as much contaminant as possible and then recover with fresh shoot tissue. Studies have shown that transpiration is the driving force for lead-EDTA uptake (Vassil et al., 1998 and Epstein et al., 1999) and it would follow that those leaves transpiring more would accumulate a higher concentration of the metal. Bouchier (2003) showed that younger leaves have higher transpiration rates than do older leaves after EDTA application. Bouchier's work corroborates the findings reported here of higher lead concentrations in younger shoot tissue when compared to older tissue.

The application of EDTA allows cattails to be classified as a hyperaccumulator of lead (lead concentration greater than or equal to 1000 ppm) as the cattails in 4000 ppm lead contamination level sequestered 2742 ppm in the shoot (top and base) tissue. Exberger (2006) also found that cattails can be considered a lead hyperaccumulator because cattails grown hydroponically in lead contamination sequestered 5465 ppm in rootstalk tissue and that new shoot tissue after initial harvesting accumulated up to 1079 ppm lead in lead-contaminated solutions and up to 2092 ppm lead when growing in lead/zinc-contaminated solutions.

One of the goals of phytoremediation is to have a plant that will tolerate high levels of a contamination and *T. latifolia* has proven to be capable of high tolerance to lead-contaminated soils. Researchers have studied cattail growth in the environment and determined that they grow well in a variety of contaminated conditions (Lee and Bukaveckas, 2002; Hotchkiss and Dozier, 1949; Yeo, 1964) and have a natural affinity to accumulate heavy metals in their root and shoot tissues (Kufel, 1991; McNaughton, 1974). Another goal of phytoremediation is to be a fast-growing, high-biomass crop. *Typha latifolia* has also proven to grow quickly and to produce a large biomass (up to six to ten megatons per hectare per year) when compared to natural hyperaccumulators (Dickerman and Wetzel, 1985). *T. latifolia* also sequesters lead in its root tissue until a chelating agent such as EDTA is applied. The concentration of lead at the 4000 ppm contamination level without the application of EDTA was 16 ppm in the shoot tissue and 1515 ppm lead in the root tissue. The concentration of lead at the 4000 ppm contamination level with the application of EDTA was 2742 ppm in the shoot tissue

(shoot top and base) and 2483 ppm lead in the root tissue. The sequestering of the lead in its roots keeps the lead out of the food chain until a chelating agent is applied. There would be a short time of lead availability in the shoots due to an EDTA application but this time span would be limited when compared to a hyperaccumulator which has a contaminant continuously sequestered in shoot tissues. Although there was a high mortality rate in the regrowth treatments at the 0 ppm lead-contaminated soil, *T. latifolia* has the ability to recover from shoot removal as demonstrated when the shoots were removed in the 1000, 2000, and 4000 ppm lead soil contaminations. If the shoots were removed at a height of greater than 12 inches, it would allow for removal of the shoot tips where the EDTA-lead complex would be maximally sequestered and allow for shoot regeneration by leaving the apical meristem in place and undamaged.

One final goal of phytoremediation is to restore a contaminated site back to acceptable concentrations (EPA allowable lead concentration in soil), within a 20-year time frame (Huang et al., 1997). At the 4000 ppm lead (4000 mg lead /kg) soil-contamination level, 4.38 mg lead was taken into the shoot tissue. If cattails were continuously grown in the contaminated soil within the mason jars and if there were only one harvest a year, the total lead taken out of the soil after 20 years for each cattail would be 87.6 mg lead. If there were two harvests per year; 175.2 mg would be taken up and if there were three harvests per year; 262.8 mg lead would be taken up per cattail. At a rate of one harvest per year, it would take approximately 376 years to reduce the lead concentration in a mason jar from 4000 to 2000 ppm lead (EPA commercial limit); two harvests per year would require 188 years. Therefore cattails grown in mason jars with

lead-contaminated soil may not lead to the possibility of becoming a practical phytoremediation approach.

Although this may sound discouraging there are several other factors that could be considered for in an *in situ* experiment as opposed to a mason jar/greenhouse experiment. The height of the cattails was limited to approximately 90 cm while growing in the mason jars. Cattails in natural settings have the capacity of reaching heights in excess of two meters, which would increase the overall capacity of lead uptake. This additional cattail height and biomass could easily cut the remediation time in half thus reducing the time frame to 94 years. Another possibility for increased lead uptake is to increase the concentration of EDTA to allow for more EDTA-lead complex formation. If EDTA were applied at a concentration of 30 mmol/kg soil, then the additional EDTA-lead complex would increase the available lead for uptake and would also facilitate increased translocation of lead from root tissue to shoot tissue. An increased concentration of EDTA could cut the remediation time in half again, reducing the remediation time to only 47 years. Another factor not yet considered is the density of cattails in soil. If more than two cattails can grow in one kilogram of soil, then the remediation time could theoretically be reduced to approximately twenty-four years. Twenty-four years is almost within the time frame for an acceptable phytoremediation approach. Furthermore, cattails can be easily adapted to harvesting in various climatic conditions using existing agricultural practices and can be regularly harvested during the growing season to capture large amounts of lead and potentially other metals at a contaminated site. (Dickerman and Wetzel, 1985; Boyd and Hess, 1970; Lombardi et al., 1997).

CONCLUSION

The direct comparison of chemically enhanced lead phytoremediation and physically enhanced lead phytoremediation revealed several new concepts to be explored with lead and cattails. The *T. latifolia* regrowth treatments sequestered a significantly greater lead concentration than the control treatments even though they did not reach hyperaccumulator status when grown in lead-contaminated soil. *T. latifolia* did achieve hyperaccumulator status with the addition of 15 mmol EDTA/kg soil. A combination of shoot harvesting and EDTA application combined could prove very successful in future experiments.

Chemically enhanced lead phytoremediation using cattails may soon become a practical approach for cleaning lead-contaminated sites. *Typha latifolia* demonstrated its tolerance to lead-contaminated soil as there was only a slight difference (although statistically significantly different) for the final height achieved after 12 weeks of growth in 0, 1000, 2000, and 4000 ppm lead. The regrowth group generated the least biomass, below the EDTA and control groups. The regrowth treatment did achieve a significantly greater lead concentration than the control (but due to the lack of biomass, the actual quantity of lead removed by the shoot tissues was not significantly different than the control group). The cattails reached hyperaccumulator status with the addition of EDTA (15mmol/kg soil; 13.5 mmol EDTA/mason jar) but unfortunately did not enable the cattails to uptake and translocate sufficient quantities of lead to be used as an effective phytoremediation system for lead-contaminated soil in mason jars under greenhouse

conditions. However, as presented in the discussion, there are several aspects that are very promising for lead phytoremediation using *Typha latifolia*: cattails in a natural setting can grow to over twice the height of greenhouse-grown cattails in mason jars; multiple harvests per year as opposed to one harvest per year may double or triple lead removal from the soil depending on the length of the growing season; an increased EDTA application may enhance lead uptake; an increase in the density from one cattail/kg soil to two or more cattails per kg soil may reduce the remediation time for a contaminated site. With the above-mentioned considerations, the remediation time required for an *in situ* lead phytoremediation approach with cattails could be theoretically reduced to 24 years.

In hydroponic experiments *T. latifolia* achieved hyperaccumulator status after shoot removal and regrowth (Exberger 2006) and with the addition of EDTA they achieved hyperaccumulator when grown in lead-contaminated soil. *Typha latifolia* may be considered a weed in many places, but it is a promising lead-phytoremediation candidate that is likely to find a home in the world of chemically enhanced phytoremediation.

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