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Phytoremediation of Lead-Contaminated Soil in West Atlanta

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B.S., University of California, Davis, 2018

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An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
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Abstract

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The HERCULES pilot soil project at Emory University has discovered lead (Pb) contamination in residential and garden soils within West Atlanta neighborhoods. Phytoremediation has been shown to be a cost-effective remediation method to remove Pb contamination in soil. However, the plant species with strong potential for phytoremediation of Pb contamination have not been comprehensively investigated in the literature. The goal of this study was to determine the intervention potential of cultivating commonly-used plants to reduce concentration and mobility of Pb in soil. A greenhouse study was conducted to assess the efficacy of four plant species (*Helianthus annuus* (sunflower)*, Gomphrena globose* (globe amaranth)*, Brassica pekinensis* (Chinese cabbage)*, and Vigna unguiculata* (cowpea)) for the removal and immobilization of Pb in contaminated soil as well as to evaluate the efficiency of EDTA and compost applications as phytoremediation enhancement methods. We used soils with a concentration of 515 ppm (mg/kg) Pb sampled from a residential site in West Atlanta and cultivated plants in the contaminated soil for 60 days. After the harvest, the mean Pb concentrations in four plant species were 23.5, 25.7, 50.0, and 58.1 ppm, respectively, and the root was the major site of Pb accumulation. The ratio of Pb concentrations in the shoot to the root, referred to as translocation factor (TF), was less than 1 for all species, suggesting their potentials for phytostabilization. The highest Pb concentration, TF, and biomass were found in cowpea (*V. unguiculate*). The soils growing sunflower (*H. annuus*) were additionally treated with ethylenediaminetetraacetic acid (EDTA) solution (0.1 g/kg) and compost (20% soil blend), respectively. EDTA treatment resulted in a significant increase in the total Pb uptake by sunflowers. Compost treatment increased biomass production and reduced the bioavailability of Pb in soil. We found that: 1) cowpeas (*V. unguiculate*) were the most favorable for Pb uptake and immobilization compared to the other three species; and 2) the addition of EDTA was shown to improve phytoextraction and the application of compost was shown to enhance phytostabilization.

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Introduction

Lead (Pb) contamination is an adverse consequence of human activities. Pb was widely used as an additive to gasoline in the United States until it was phased out in the late 1970s and totally banned in 1996 (Newell and Rogers, 2003). As it was in the 1900s, Pb is still used in many industrial products nowadays, such as batteries and pigments. Pb-based painting on household surfaces from old houses and point source emitters, including metal smelters and mine tailings, are major sources of Pb (Zhang et al., 2015). The biogeochemical cycling of Pb has been accelerated by diverse applications of Pb. As a result, Pb has accumulated in soil ecosystems, especially in urban areas (Jaworski et al., 1987). Since Pb is an element, it is a non-biodegradable contaminant and does not degrade or disappear over time. Pb can generally remain in the soil for thousands of years (Sipos et al., 2005), and thereby the contaminated soil, as a long-term source of Pb exposure, leads to a significant public health problem.

Pb has two main oxidation states: Pb (II) and Pb (IV), and Pb (II) is very toxic to humans and other living organisms at high concentrations. Pb poisoning can occur through acute or chronic Pb exposure. The definition of acute exposure is that high doses of Pb are absorbed by human bodies over a short period of time, while chronic exposure is defined as the long-term intake of Pb at low levels. The exposure pathways of Pb in soil include the direct ingestion or inhalation of Pb-contaminated soil, the food chain, or Pb-contaminated drinking water (Lanphear and Roghmann, 1997). Children are especially vulnerable to the toxicity of Pb, since the proportion of Pb absorbed in the empty stomach has been estimated to be 5-10 times higher in young children than in adults (Ziegler et al., 1978). Pb poisoning can lead to serious neuropsychological effects on children, such as lowered IQ and learning efficiency (Mason et al., 2014). Children are more likely to be exposed to Pb in soil because they can accidentally ingest Pb-contaminated soil through hand-to-mouth behaviors when playing on the ground. Although adult Pb poisoning is less severe than childhood Pb poisoning, Pb is still a concern for pregnant women because it can affect the development of the fetal nervous system (Assi et al., 2016). Pb contamination in soil also addresses an important food safety issue. Some vegetables, especially leafy greens, can accumulate a large amount of heavy metal and metalloid (HMM) in tissues which can enter the food chain afterwards (Itawongse and Dean, 2006). Thus, the consumption of vegetables growing in the contaminated soil becomes a pathway of human Pb exposure.

Several methods have been developed to clean up HMM contaminants in soil. The conventional methods involve physical processes and the most common technique is soil replacement, which requires excavation and landfill disposal (Salt et al., 1995). This method is not only expensive and laborious but also associated with possible secondary contaminations and the disturbance of soil ecosystems. Chemical and thermal technologies, such as soil washing and vitrification, have been used as alternatives to soil replacement (Dermont et al., 2008; USEPA, 1992). However, these costly techniques still cannot avoid destroying the nature of the soil and disrupting biological activities in soil.

Phytoremediation has been developed as a green approach to remediate contamination without disturbing the ecosystem. It refers to the technology that use living plants to clean up contaminated soil, air, and water. Compared to other remediation methods, phytoremediation has many economic and ecological advantages. First, it is very cost-effective. For example, according to Salt et al. (1995), to clean up one acre of soil with a depth of 50 cm, the cost of phytoremediation is approximately $80,000, while the cost of soil excavation and landfill disposal is at least $400,000. In addition, phytoremediation results in minimal land disturbance and produces less waste compared to conventional methods. Instead of direct disposal, there are other disposal techniques for contaminated plant biomass produced by phytoremediation, including liquid extraction, composting, and combustion which are associated with much less risk of causing secondary contamination (Kovacs and Szemmelveisz, 2017). More importantly, phytoremediation turns brownfields into greenfields. It has the potential of recovering soil ecosystems, because plants are capable of improving soil quality by reducing soil erosion and adding organic matter (Salt et al., 1998). Nevertheless, the effectiveness of phytoremediation is influenced by several soil- and plant-associated factors, including the bioavailability of contaminants in soil and the capability of plants to absorb contaminants and survive in the contaminated soil. To overcome these limitations, phytoremediation can be enhanced through the addition of soil amendments, such as ethylenediaminetetraacetic acid (EDTA) and compost. Previous research has used EDTA to increase the bioavailability of contaminants in soil and used compost to facilitate the plant growth in the contaminated soil (Turgut et al., 2004; Kumpiene et al., 2007).

As a part of a pilot grant project that was funded by Emory's HERCULES Exposome Research Center (5P30ES019775, Marsit PI) and started in April 2018, Emory University researchers, working with a community partner Historic Westside Gardens Atlanta Inc., have discovered Pb contamination in residential and garden soils within West Atlanta neighborhoods (English Avenue and Vine City). Pb contamination in soil here is defined as soil Pb concentrations above the residential screening level of 400 ppm (mg/kg) established by the United States Environmental Protection Agency (EPA), which is considered to pose health risks to residents living in these neighborhoods, especially to young children who can unintentionally ingest soil containing Pb. Moreover, there are many urban farmers in West Atlanta neighborhoods cultivating vegetables in the native soil for daily consumption, and they can be exposed to Pb through consuming vegetables growing in the Pb-contaminated soil.

To provide urban farmers and communities in West Atlanta with potentially cost-effective and green approaches to reduce their exposure to Pb in soil, the phytoremediation research of Pb-contaminated soil was conducted as a part of the HERCULES pilot soil project. The goal of this study was to determine the intervention potential of cultivating commonly-used plants to reduce the total concentration and bioavailability of Pb in soil. There were two main aims: 1) to assess the phytoremediation potentials of four plant species for Pb-contaminated soils; and 2) to evaluate methods that enhance phytoremediation of Pb including the addition of EDTA and compost. The plant species with strong bioaccumulation capacities of Pb and high tolerance to Pb levels are preferred for phytoremediation. The following plants which have been shown to possess these characteristics were selected for this study: *Helianthus annuus* (sunflower), *Gomphrena globose* (globe amaranth), *Brassica pekinensis* (Chinese cabbage), and *Vigna unguiculata* (cowpea) (Alaboudi et al., 2018; Fatnassi et al., 2014; Xiong et al., 1998; Adejumo et al., 2019). Important findings in the previous phytoremediation studies of these species are discussed in the next section. In addition, as ornamental plants, flowering species are suitable remediation candidates for urban farmers to grow in their gardens because of their combined beautification potential. Vegetables are favored in phytoremediation because they generally produce larger biomass which is associated with higher amount of HMM accumulated in plants (Sheoran et al., 2016). Furthermore, the associated risk of consuming vegetables growing in Pb-contaminated soil was assessed through measuring Pb bioavailability in edible parts of plants, which refers to the fraction of Pb that can be absorbed in the gastrointestinal tract.

Literature Review

Phytoextraction vs. Phytostabilization

There are two common subsets of phytoremediation for soil contamination: phytoextraction and phytostabilization. Each phytoremediation process has its own advantages and disadvantages. Phytoextraction refers to the process that plants remove HMM from soil through the uptake and accumulation of HMM from soil into the plant tissues (Garbisu and Alkorta, 2001). Hyperaccumulator species are capable of accumulating excessively high amounts of HMM in aerial parts. The accumulation rate, defined as the ratio of HMM concentration in plants to that in soil, ranges from 1,000 to 10,000 times for certain HMM, such as copper (Cu), manganese (Mn), mercury (Hg), aluminum (Al), arsenic (As), cadmium (Cd), and zinc (Zn). Because of the hyperaccumulation capability, these species are ideal candidates for phytoextraction. For example, as a hyperaccumulator species for Cd, Cu, Pb and Zn, *Brassica juncea* (Indian mustard) has been widely used in the phytoextraction research. However, hyperaccumulators tend to have less biomass production due to the toxic effect of HMM described as the term phytotoxicity (Raskin and Ensley, 2000). Phytotoxicity inhibits plant growth and thereby plant biomass decreases when plants grow in soil with higher HMM concentrations. Therefore, the duration of time taken to remediate contaminations can be very long due to the low biomass yield of hyperaccumulators, which decreases the efficiency of phytoextraction.

The effectiveness of phytoextraction for contaminated soils is significantly influenced by the bioavailability of HMM for plant uptake (Lasat, 2002). In soil science, bioavailability is defined as the fraction of total HMM that is available for absorption into biota (Davis et al., 1994). Generally, total HMM concentrations are not equal to bioavailable HMM concentrations. Chemical speciation is one method to classify all chemical forms of HMM in soil into defined fractions in terms of biological extractability (Cottenie et al., 1980). Chemical speciation provides information on the bioavailability of HMM which is related to the mobility and solubility of HMM in soil. Bioavailability is determined by the ability of HMM to re-enter the soil solution from more stable phases which include solid phase and forms associated with other species (Ashraf et al., 2012). According to Tessier et al. (1979), HMM in soil can be classified into five fractions: exchangeable metals, carbonate-associated, Fe-Mn oxide-associated, organic-associated, and residual fraction. The exchangeable fraction of HMM is commonly considered to be bioavailable for biota. Exchangeable or soluble HMM are ions weakly adsorbed on organic or mineral particle surfaces and they can be easily dissolved or exchanged by cations in the soil solution. Since plants can only absorb free HMM ions and soluble HMM complexes from the soil solution, a higher percentage of the exchangeable fraction of HMM in soils means higher accumulation of HMM through phytoextraction. However, the percentage of Pb in the exchangeable form is generally small in soil (less than 20%) (Giacalone et al., 2005; Sun et al., 2009). To improve the effectiveness of phytoextraction, chelating agents are usually used to increase the bioavailability of HMM in soil.

Phytostabilization involves a different mechanism, in which plants immobilize HMM by sequestering them in the rhizosphere to reduce resuspension and leaching (Vangronsveld, 1995). In phytostabilization, HMM are absorbed into the roots or adsorbed on root surfaces. Plant roots excrete organic acids that precipitate HMM in the rhizosphere, and the solubility of metals is removed in this process (Hinsinger et al. 2005). Immobilization of HMM can also be achieved through other pathways, which include decreasing wind-blown dust and reducing soil erosion. Contrary to phytoextraction, which requires high bioavailability of HMM in soil, the ideal result of phytostabilization is that the amount of bioavailable HMM is decreased. Through phytostabilization alone, the mobility of HMM can be reduced through the accumulation in plant roots, adsorption onto roots, and precipitation within the rhizosphere. Manipulation of the soil environment to reduce the bioavailability of HMM is important for effective phytostabilization. Previous studies have found that the addition of organic soil amendments, such as compost, biosolid, and manure can decrease the mobility and bioavailability of certain HMM in soil, including Zn, Pb, Cu, Cd, and Cr, therefore enhancing phytostabilization (Kumpiene et al., 2007; Rizzi et al., 2004; Castilhoet al., 1993; Alexander, 1999). Furthermore, plant species that are resistant to the phytotoxicity associated with high HMM concentrations are efficient to perform phytostabilization. Unlike hyperaccumulator species, these plants can maintain their biomass production, even when growing in soil with higher HMM concentrations, but there is a main problem that most HMM stay in soil rather than being removed. HMM that are formerly immobilized by phytostabilization can become bioavailable again over time if there is no re-vegetation (Bolan et al., 2003). Thus, long-term cultivation and monitoring are required to maintain the immobilization of HMM in soil.

Soil Amendments

The efficiency of phytoremediation is significantly influenced by the bioavailability of HMM in soil and the plant biomass. With the goal to overcome the limitations associated with these two factors, the usage of chemical and biomass amendments has been widely studied in the phytoremediation literature. The effectiveness of phytoextraction mainly depends on the bioavailability of HMM in soil, and several chelating agents have been shown to be effective for increasing HMM bioavailability in soil, root uptake, and translocation of HMM to aerial biomass (Wu et al., 2004; Parra et al., 2008). Many have studied the usage of synthetic chelating agents such as EDTA and diethylenetriamine pentaacetate (DTPA) as well as natural chelating agents, including citric acid, nitriloacetic acid, and other organic acids. Among these chelators, numerous studies have reported EDTA to be the most effective chemical for enhancing phytoremediation. Turgut et al. (2004) has shown that EDTA at a concentration of 0.1 g/kg of soil resulted in the highest enhancement in the total plant uptake of HMM including Pb, Hg, Cu, As, and Al. Although EDTA has been extensively used to improve phytoremediation through increasing the bioavailability and mobility of HMM in soil, its application is associated with the risk of affecting the food chain via animal exposures and leaching of HMM that can potentially contaminate ground water due to its long persistence and slow degradation in soil (Zhang et al., 2010; Chen et al., 2004).

The mechanism of EDTA to increase HMM bioavailability and mobility in soil has been investigated in the literature. The effect of EDTA is based on its role as a hexadentate ligand (Zhang et al., 2010). The electron-donor atoms on the $EDTA^{4-}$ molecule, including oxygen and nitrogen, are ligand-binding atoms, which enable $EDTA^{4-}$ to bind to a metal cation to form a coordination complex (Figure 1). For example, $EDTA^{4-}$ binds to Pb (II), forming the Pb (II) anion $[Pb(EDTA)]^{2-}$. The formation of metal-EDTA complex makes metal ions detach from minerals. The strong binding between $EDTA^{4-}$ and metal ion weakens the interaction between metal and mineral structure in soil. Therefore, EDTA promotes the dissolution of metal deposits associated with oxides and carbonates (Zhang et al., 2010), increasing the metal bioavailability and mobility (Zhang et al., 2010). Metal ions are enveloped by $EDTA^{4-}$ within the complexes, which reduces their catalytic properties. Since metal-EDTA complexes are anionic, they have high solubility in water. Although the metal-EDTA complex is hydrophilic, it cannot cross the plasma membrane because of its large molecular size (Bell et al., 1991). Generally, the endodermal Casparian bands in the roots block the movement of large hydrophilic solutes to the xylem. However, the Casparian bands have gaps, providing a bypass flow pathway that allows the metal-EDTA complex in the apoplasm to be transported into the xylem (Bell et al. 1991).

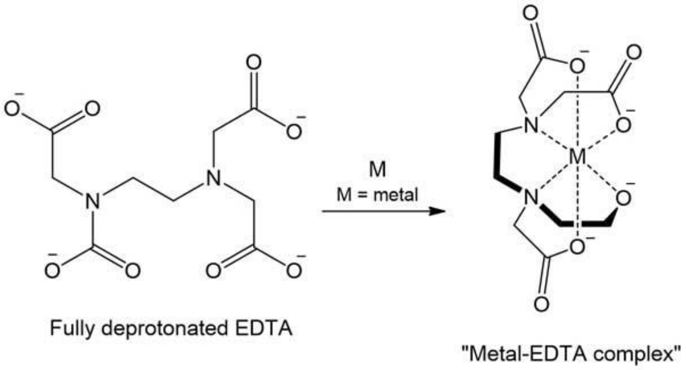

Figure 1. The chemical structure of metal-EDTA complex. (pharmafactz.com, 2019)

Biomass amendments, such as compost, can enhance plant growth and influence the effectiveness of both phytoextraction and phytostabilization. The increase in plant biomass production is associated with higher accumulation of HMM including Zn, Cu, Cr, Cd, and Pb (Sheoran et al., 2016). Masciandaro et al. (2013) showed that soil quality and plant biomass were improved in phytoremediation by increasing the amount of organic matter in soil. As a soil amendment being rich in organic matter, compost is capable of increasing water holding capacity, nutrient availability, organic carbon, and microbial activities in soil, which contribute to better plant growth and higher biomass yield. Yang et al. (2005) revealed that the addition of compost (5%) improved the phytoextraction of Cu in soil with *Elsholtzia splendens* (shiny elsholtzia) through increasing the shoot biomass production by 47.5%.

Compost is, however, more preferable for phytostabilization compared to phytoextraction because of its demonstrated ability to immobilize selected HMM in soil (Castaldi et al., 2005). Attanayake et al. (2015), for example, demonstrated that the compost treatment (44 kg/m2) decreased the bioavailability of Pb in soil by 17% compared to the soil without it. Castaldi et al. (2005) showed that the addition of compost decreased the accumulation of Pb in the shoot of

Lupinus albus (white lupin) by 87% and increased the residual fraction of Pb in soil. Plants treated with compost also produced higher biomass compared to control plants. The main mechanism for compost to immobilize metals is that compost increases soil cation exchange capacity (CEC) by providing more binding sites from organic matter (Benito et al., 2003). CEC is a measure of the capacity of soil to retain metal ions. As soil CEC increases, the bioavailability and mobility of HMM decreases. In addition, Rizzi et al. (2004) found that the addition of compost to soil in an Italian mining area improved soil structural characteristics and resulted in reduced cracking and porosity. These improvements in soil structure helped the formation of water-insoluble metal aggregates, which eliminated the dispersal of HMM (Rizzi et al., 2004). Overall, compost can be applied to phytoextraction to increase HMM accumulation in the plant biomass, and it can be used in phytostabilization to immobilize HMM in soil.

Translocation Factor and Bioconcentration Factor

The translocation factor (TF) and bioconcentration factor (BCF), which assess the translocation and accumulation of HMM, respectively, into the plant tissues, have been widely used to determine the phytoremediation potential of plant species for HMM-contaminated soil. TF measures the capacity of plants to translocate HMM from roots to shoots. It is calculated as the ratio of HMM concentration in the shoot to that in the root (Baker, 1981). TF values have been used to characterize the phytoextraction and phytostabilization potentials of plants (Yoon et al., 2006; Yadav et al., 2009). High TF ($TF > 1$) indicates the capacity of plants to accumulate most HMM in shoots, suggesting the use of these plants in phytoextraction. Low TF ($TF < 1$) indicates the capacity of plants to accumulate most HMM in roots, proposing the use of these plants in phytostabilization.

BCF indicates the efficacy of a plant to absorb HMM from soil and accumulate them in tissues. It is the ratio of the HMM concentration in the plant tissues to that in soil (Baker, 1981). BCF values have been used to determine the capacity of plants to accumulate HMM in the plant tissues. According to Baker (1981), plants with BCFs greater than 1 are classified as accumulators and plants with BCFs smaller than 1 are classified as excluders. In addition, plants with BCFs greater than 10 were classified as hyperaccumulators (Baker, 1981) which are the most ideal candidates for phytoextraction. A plant species may selectively act as an accumulator or an excluder across a range of soil HMM concentration (Baker, 1981). Acting as excluders is a strategy of plants in response to high HMM concentrations. To tolerate the phytotoxicity associated with high HMM concentrations, plants have evolved detoxification mechanisms to suppress the HMM uptake and translocation of HMM from roots to shoots (Baker, 1981). Hence, excluder species generally have higher tolerance to HMM contaminations compared to accumulator species, making them suitable candidates for phytostabilization (Alkorta et al., 2010).

Previous Research of Selected Species

Plant species differ in their capabilities of HMM tolerance and accumulation. For phytoremediation, especially phytoextraction, ideal plant candidates should have high tolerance to HMM, high potential for HMM accumulation, and large biomass production (Nouri et al., 2009). Many plant species, particularly hyperaccumulators, have been studied for their capacities to be used in phytoremediation of Pb-contaminated soil. As a native American annual plant species, sunflower has been widely studied in the phytoremediation literature. Sunflower (*H. annuus)* has high tolerance to HMM (Pilon-Smits, 2005), and it has been well characterized as the hyperaccumulator of Zn, Cr, Cu, and Mn in the literature (McCutcheon and Schnoor, 2003). Recently, the potential of sunflower for phytoremediation of Pb-contaminated soil and water was investigated in some studies. Fulekar et al. (2016) showed that sunflowers were capable of remediating 77-89% of Pb at 5-50 ppm levels in the aquatic environment, and the highest accumulation of Pb was found in roots. Alaboudi et al. (2018) demonstrated that the Pb accumulation in shoots and roots of sunflowers increased as the Pb concentrations in soils increased from 0 to 200 ppm. The authors also found that the root system was the main site of Pb accumulation in sunflower, which accounted for 73% of the total amount of Pb accumulated in the plant tissues (Alaboudi et al., 2018).

Being first introduced to the United States in 1714, globe amaranth (*G. globose*) has been a common ornamental plant grown in the American gardens, and it is a potential species for phytoremediation of Pb contamination. Two species in the genus of Gomphrena - *Gomphrena claussenii* and *Gomphrena celosioides* - were shown to be capable of accumulating Pb, Zn, and Cd (Carvalho et al., 2013; Adejumo et al., 2019). Adejumo et al. (2019) conducted a hydroponic culture experiment where *G. celosioides* was grown in solutions with Pb at 500 ppm, and the subsequent Pb concentration in the plant was 85 ppm, with approximately 98% of Pb accumulated in roots. Because these two species are categorized in the same genus as amaranth, they are closely related to each other in genetics, and therefore they may share some common characteristics.

Amaranth could have similar capability of HMM accumulation. Since these two species are not common in the United States, as a non-invasive species (Cornell University), globe amaranth could be a potential alternative to be used for phytoremediation.

Legumes are popular plant species for phytoremediation research because of their symbiotic relationship with microorganisms in soil, known as plant growth-promoting rhizobacteria (PGPRs) (Gomez-Sagasi, 2015). PGPRs assist with the nitrogen fixation process and they can facilitate the phytoremediation of HMM-contaminated soil that uses symbiotic legumes. Cowpea (*V. unguiculata*) is a common commercial legume crop in the US. It was first studied for phytoremediation of soil contaminated by crude oil which is an organic pollutant (Tanee and Akonye, 2009; Jidere et al., 2012), and it was capable of reducing hydrocarbon concentrations in agricultural soils. Some recent studies showed that cowpea preferably accumulated Pb in the root system, suggesting it as a strong candidate for phytostabilizing Pb-contaminated soil (Fatnassi et al., 2014; Odoh, 2017).

Brassica juncea is the most cited plant species for phytoremediation because of its ability to hyperaccumulate Cd, Cr, Cu, Ni, Pb, and Zn, and many plant species in the genus of Brassica have been studied for their phytoremediation potentials (Vamerali et al., 2010). Xiong et al. (1998) assessed the Pb accumulation in Chinese cabbage growing in multiple concentrations ranging from 0 to 1000 ppm. They found that the Pb accumulation in shoots and roots of Chinese cabbage increased as the Pb concentration in soil increased. The Pb concentration in roots was proportional to the soil Pb. 86% of Pb accumulated in the tissues of Chinese cabbage was found in roots and

14% of that was found in shoots. The main accumulation of Pb in roots suggested the potential of Chinese cabbage for phytostabilization (Xiong et al., 1998).

Materials and Methods

Soil Sampling and Characterization

Pb-contaminated soil was collected from an empty lot in West Atlanta (zip code 30318) where Emory researchers found high Pb concentrations in soil and slag, which are wastes from smelting. The soil was sampled in a depth of 0-15 cm, and EPA incremental sampling method was used to prevent soil heterogeneity from negatively influencing the environmental data (USEPA, 2007). In the sampling site, three decision units (DUs) were defined and each DU was marked into 30 equal area squares where subsamples were taken. Three randomized sample points were chosen in each square, and each point was in the same location in each square. Soil samples were collected from these 30 squares with matching sample locations. Eventually, three sets of 30 subsamples were collected from each DU. In addition, to ensure that all experimental pots contained soils with the same Pb concentration, pH, and organic content, soil samples collected from the urban site were thoroughly homogenized using EPA method, which involves repeated quartering procedures (USEPA, 2007). In this process, each soil sample was divided into quarters and each quarter was mixed individually, and then two of the quarters were mixed to form halves. The same procedure was performed on two halves which were later mixed to form a homogeneous sample. The quartering procedure was repeated to mix two homogeneous soil samples together. This method eventually generated three homogeneous samples representing DUs. Equal amounts of soil were taken from these three soil samples to be well mixed, which was subsequently transferred to a pot. A portion of homogenized soil was air-dried, screened to pass through a 0.15 mm sieve, and analyzed for physicochemical characteristics including soil pH and Pb concentrations. Soil pH was tested to be 7.43 using Hanna Lab pH meter, and Pb concentration in soil was examined to be 515 ± 10 ppm using field portable X-ray fluorescence (XRF) analyzer (Niton® XL3t Series Multi-element XRF Spectrum Analyzer) (EPA method 6200). Approximately 1.5 kg homogenized soil was transferred to each plastic pot with a dimension of 20.32 x 20.32 x 16.51 cm for future cultivation.

Plant Materials and Pot Experiment

Pot experiments were conducted in the greenhouse of Biology Department at Emory University. The treatments comprised of four plant species: *Helianthus annuus* (sunflower), *Gomphrena globosa* (globe amaranth), *Brassica pekinensis* (Chinese cabbage), and *Vigna unguiculata* (cowpea). Mature seeds were purchased from Johnny's Selected Seeds. Each treatment of flowering plants was replicated three times for assessing phytoremediation potential, and each treatment of vegetables was replicated six times for examining phytoremediation capacities and bioavailability of Pb in edible parts. For each plant species, three were grown in normal potting soil which was examined to have no HMM contaminants using XRF analyzer and received the same treatment as experimental plants as a control group. Control groups helped to eliminate the effect of seed variability as well as alternative interpretation of experiment results. To assess the effect of EDTA and compost additions on phytoremediation, the sunflower treatment with EDTA and with compost were replicated three times. Overall, there were 27 experimental treatments and 12 control treatments (Table 1).

Table 1. Pot experiment overview

		Experiments	
Species	Common name	Contaminated (pots)	Control (pots)
Vigna unguiculata	Cowpea	XRF (soil) and acid digestion-ICP-MS (root and shoot): 6 Bioavailability digestion-ICP-MS (fruit): 4	Acid digestion-ICP-MS (root and shoot): 3
Brassica pekinensis	Chinese cabbage	XRF (soil) and acid digestion-ICP-MS (root and shoot): 6 Bioavailability digestion-ICP-MS (shoot): 6	Acid digestion-ICP-MS (root and shoot): 3
Gomphrena globose	Amaranth	XRF (soil) and acid digestion-ICP-MS (root, shoot, and flower): 6	Acid digestion-ICP-MS (root, shoot, and flower): 3
Helianthus annuus	Sunflower	No treatment: 3 EDTA treatment: 3 Compost treatment: 3	Acid digestion-ICP-MS (root, shoot, and flower): 3
		XRF (soil) and acid digestion-ICP-MS (root, shoot, and flower): 9	

Cultivation Practices

All plant seeds were germinated in the sampled soil within pots. Each pot contained 2-4 plants based on the expected size of mature plants. Only tap water was added to soil during cultivation. Soil moisture was measured each week to maintain the same moisture content in all pots, and watering schedule was adjusted based on the soil moisture measurements. Plants were harvested after the cultivation of 60 days. For compost treatment, the compost made from garden debris was mixed into soil to create a 20% soil blend before the cultivation. For EDTA treatment, the 0.1 g/kg-soil level EDTA disodium salt solution was added to soil two weeks before harvest.

Post-Phytoremediation Analyses

After plants were gently removed from pots, they were washed, separated into three to five plant parts (roots, stems, leaves, flowers, and fruits) based on species, and dried at 70 °C for 24 hours in a drying oven. Dry biomass measurements were recorded. Experimental soil was dried at 250 °C until it was completely dry and sieved into fine particles using 0.15 mm sieves for analysis. Pb concentrations in soil were examined using handheld XRF analyzer. Dried plants were ground into fine powders using SPEX 8000M Mixer Mill for further analysis. To determine Pb concentrations in the plant tissues, 0.5 g sieved plant material of each experimental sample was digested by concentrated nitric acid (HNO_3) and 30 % hydrogen peroxide (H_2O_2) (EPA test methods 3050B). The acid-digested samples were diluted and analyzed for Pb concentrations using inductively coupled plasma mass spectrometry (ICP-MS, LEADER Laboratory, Emory University). Plant compartments were digested separately.

The bioavailability of Pb in shoots of cabbages and fruits of cowpeas was assessed using *in vitro* gastrointestinal digestion followed by ICP-MS. Six Chinese cabbage shoot samples and four cowpea fruit samples were digested separately. The digestion results were compared to the Interim Reference Level (IRL) for Pb which is an allowable maximum daily intake established by U.S. Food and Drug Administration (FDA). The FDA has used IRL to evaluate the amount of Pb in a food product that is high enough to increase blood lead level (BLL) to a point that requires clinical monitoring. IRL was determined by considering the amount of a specific food that people consume daily and other factor that can lead to BLL of 5 µg/dL which is the blood lead reference value established by the Centers for Disease Control and Prevention (CDC). There is no safe level of Pb and medical treatment is suggested for children with BLL higher than 5 µg/dL (CDC, 2012).

Determination of Translocation and Bioconcentration Factor

Metal concentrations in plant tissues including roots and shoots and in soil were used to calculate TF and BCF using the formula of Yadav et al. (2009).

Translocation factor (TF) = Metal concentration in the shoot of plant (ppm)/Metal concentration in the root of plant (ppm)

Bioconcentration factor (BCF) = Metal concentration in the plant (ppm)/Metal concentration in soil (ppm)

Determination of Total Lead Uptake

Plant dry biomass and Pb concentration in the plant tissues were used to calculate the amount of Pb accumulated in one individual of selected plant species, using the following equation:

The total uptake of Pb (mg) = Plant dry biomass (kg)*Pb concentration in the plant (mg/kg)

Statistical Analysis

Statistical analyses were performed using Microsoft Excel. The mean Pb concentrations, TFs, BCFs, and biomass productions of study species were compared using t-tests to detect statistically significant differences. A significance level of $p < 0.05$ was used in the study.

Results

Lead in Soil

The post-phytoremediation mean Pb concentrations in soil were 461 ppm in cowpea, 456 ppm in Chinese cabbage, 444 ppm in amaranth, 474 ppm in sunflower, 454 in sunflower with EDTA, and 421 in sunflower with compost.

Biomass Production

The mean dry biomass production of individuals of selected plant species is depicted in Table 2. The highest dry biomass yield was recorded in cowpea. Total and shoot dry biomass of Chinese cabbage were significantly smaller than those of other three species at $p < 0.05$ significance level (Figure 2). Root dry biomass of cowpea was significantly larger than that of other three species at $p < 0.05$ significance level (Figure 2).

Table 2. Mean dry biomass of selected plant species

Species	Common name	Total dry biomass (g)	Root dry biomass (g)	Shoot dry biomass (g)
Vigna unguiculata	Cowpea	32.8	5.43	27.4
Brassica pekinensis	Chinese cabbage	15.5	2.50	13.0
Gomphrena globose	Amaranth	27.6	2.54	25.1
Helianthus annuus	Sunflower	29.4	2.38	27.0

Lead Accumulation in Plant Tissues

Pb accumulation varied in plant species and tissues. The mean total Pb concentrations in plant species were 58.1 ppm in cowpea, 50.0 ppm in Chinese cabbage, 25.7 ppm in amaranth, and 23.5 ppm in sunflower (Figure 3). There was no significant difference between the total Pb concentrations of cowpea and Chinese cabbage and between those of amaranth and sunflower at $p < 0.05$ significance level. The total Pb concentrations in cowpea and cabbage were significantly higher than those in amaranth and sunflower at $p < 0.05$ significance level.

The distribution of Pb concentrations in plant tissues show that the main accumulation site for Pb was the root in all plant species that we studied (Figure 3). Except for cowpea having higher concentrations of Pb in leaves than in stems, the concentrations of Pb in different plant compartments were in the following order: roots > stem > leaves > flower. In addition, the mean Pb concentration in roots were 49.2 ppm in Chinese cabbage, 47.0 ppm in cowpea, 24.9 ppm in amaranth, and 19.8 ppm in sunflower. The mean Pb concentration in shoots were 11.1 ppm in cowpea, 0.864 ppm in Chinese cabbage, 0.768 ppm in amaranth, and 3.66 ppm in sunflower. Root Pb concentrations in cowpea and Chinese cabbage were significantly higher than those in amaranth and sunflower, while shoot Pb concentrations in cowpea and sunflower were significantly higher than those in Chinese cabbage and amaranth at $p < 0.05$ significance level.

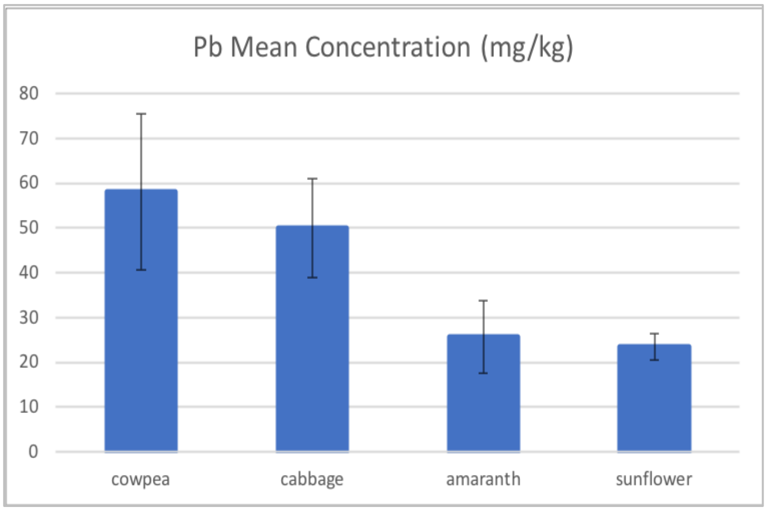

Figure 2. Mean concentration of Pb in selected plant species with one standard deviation

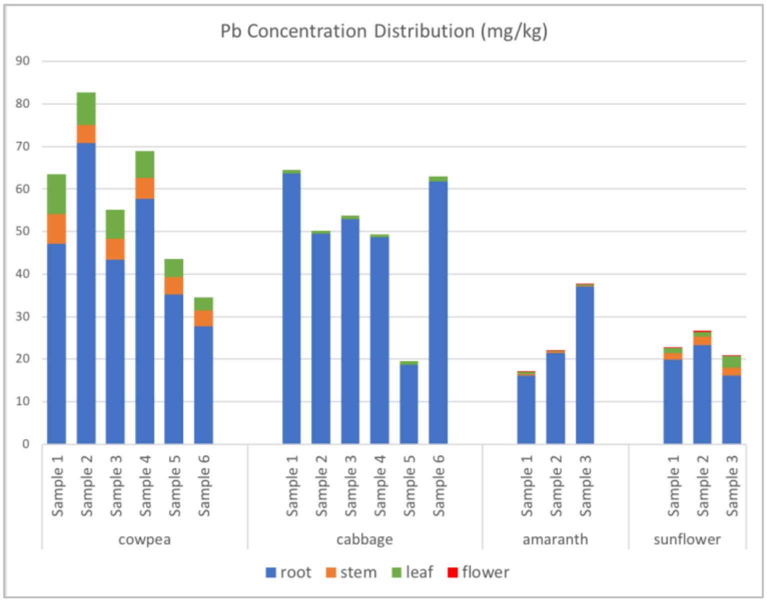

Figure 3. Concentration of Pb in plant compartments of selected plant species

Translocation Factor and Bioconcentration Factor

The TF and BCF of four plant species for Pb concentrations are summarized in Table 3. TFs of cowpea and sunflower were significantly higher than those of Chinese cabbage and amaranth at $p < 0.05$ significance level. The highest TF was recorded in cowpea, which means cowpea was capable to translocate more Pb from roots to shoots compared to other species studied. BCFs of cowpea and Chinese cabbage were significantly higher than those of amaranth and sunflower at $p < 0.05$ significance level. The highest BCF was recorded in cowpea, indicating cowpea had the highest efficiency of Pb accumulation among all plants studied.

Table 3. Translocation factor and bioconcentration factor of plant species for Pb concentrations

Species	Common name	TF	BCF
Vigna unguiculata	Cowpea	0.24	0.13
Brassica pekinensis	Chinese cabbage	0.020	0.11
Gomphrena globose	Amaranth	0.034	0.060
Helianthus annuus	Sunflower	0.19	0.049

Total Lead Uptake

The mean total amount of Pb accumulated in individuals of selected plant species is shown in Figure 4. The amount of Pb in the root and shoot of each cowpea was significantly higher than that of other selected species at $p < 0.05$ significance level. Except for cowpea which accumulated the greatest amount of Pb in both root and shoot, the amount of Pb in the root of each Chinese cabbage was significantly higher than that of amaranth and sunflower, and the amount of Pb in the shoot of each sunflower was significantly higher than that in Chinese cabbages and amaranth at $p < 0.05$ significance level.

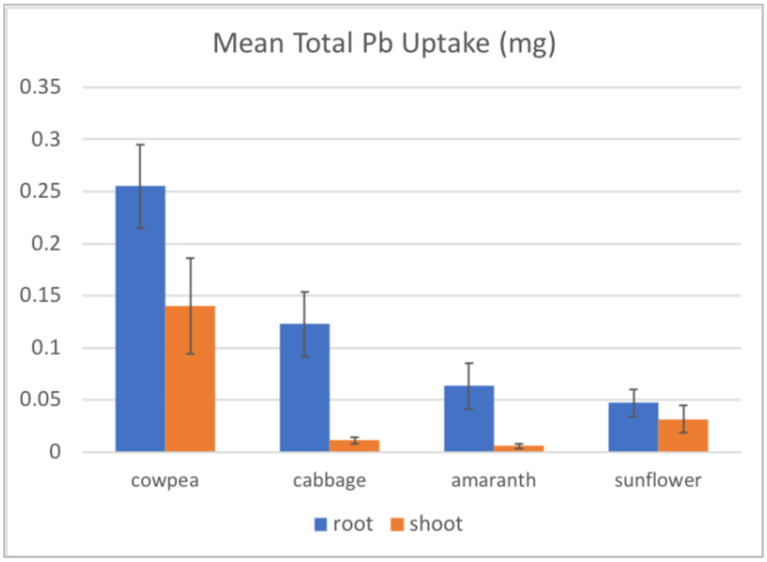

Figure 4. Mean total uptake of Pb by selected plant species with one standard deviation

Bioavailability of Lead in Vegetables

In vitro gastrointestinal extraction of edible parts of selected plant species (fruits of cowpea and shoots of cabbage) provided the concentrations of Pb in vegetables that are bioavailable for human absorption. The results showed that the mean bioavailable Pb concentrations in stomach and intestine were 1.28 µg/kg and 2.2 µg/kg in cowpea and 3.43 µg/kg and 8.66 µg/kg in Chinese cabbage, respectively. Because the sunflower seeds production was not enough for the analysis, the bioavailability of sunflower seeds was not tested in this study.

Enhanced-Phytoremediation with EDTA and Compost

The mean Pb concentrations in sunflower without treatment and with EDTA or compost addition were 23.45 mg/kg, 52.27 mg/kg, and 16.01 mg/kg, respectively (Figure 5). The Pb concentrations in the roots and shoots of sunflower with EDTA increased significantly compared to without treatment at $p < 0.05$ significance level (Figure 6). TF and BCF were also significantly increased by EDTA application and there was no significant reduction in plant biomass at $p < 0.05$ significance level (Tables 4 and 5), which indicates EDTA enhanced the translocation of Pb from the root to shoot and the total uptake of Pb without influencing the biomass production. In contrast, the Pb concentration in the tissues of sunflower with compost decreased significantly compared to without treatment at $p < 0.05$ significance level (Figure 5), meaning compost reduced the bioavailability of Pb in soil via immobilization. Additionally, compost increased the root biomass production of sunflower by 61.6% (Table 5).

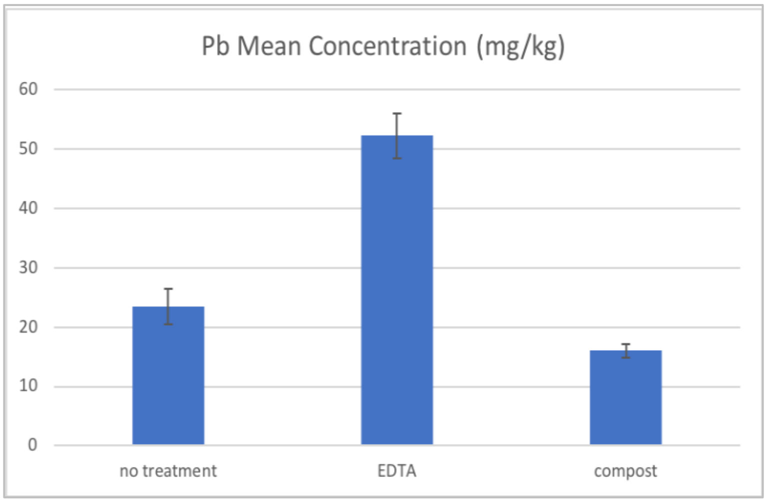

Figure 5. Mean concentration of Pb in sunflower (with and without treatments) with one standard deviation

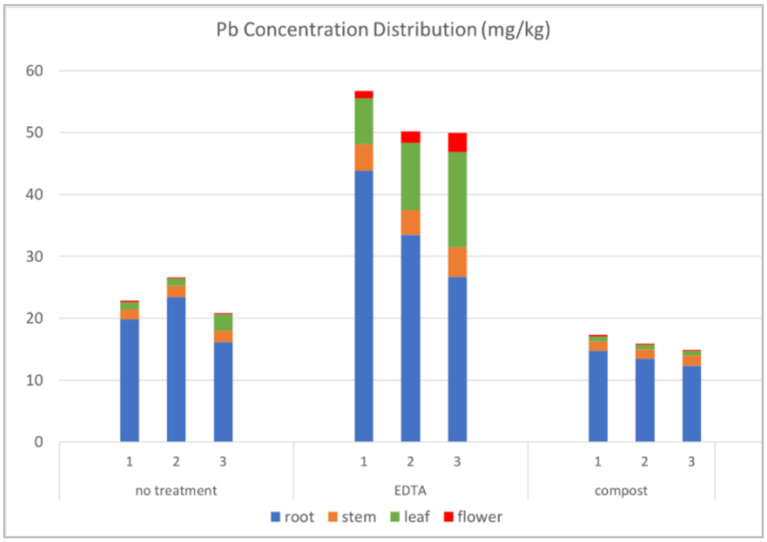

Figure 6. Concentration of Pb in sunflower compartments (with and without treatments)

Table 4. Translocation factor and bioconcentration factor of sunflower (with and without treatments) for Pb concentrations

Species	Treatment	TF	BCF
Helianthus annuus (Sunflower)	No treatment	0.19	0.049
	EDTA	0.56	0.12
	Compost	0.19	0.045

Table 5. Mean dry biomass of sunflower (with and without treatments)

Species	Treatment	Total dry biomass (g)	Root dry biomass (g)	Shoot dry biomass (g)
Helianthus annuus (Sunflower)	No treatment	29.36	2.38	26.98
	EDTA	27.63	2.21	25.42
	Compost	32.52	3.85	28.67

Discussion

The study results revealed that root is the major site of Pb accumulation for all selected plant species. This finding is consistent with the previous studies which showed that sunflower, cowpea, and Chinese cabbage accumulated Pb mainly in roots when growing in Pb-enriched medium (soil or water) with a range of 200 to 500 ppm (Alaboudi et al., 2018; Fatnassi et al., 2014; Xiong et al., 1998). However, Pb concentrations in cowpea, Chinese cabbage, and sunflower growing in soil with around 500 ppm Pb in this study were much lower compared to earlier studies that examined the same species growing in Pb-enriched medium where aqueous solution of Pb was added to create a concentration of 500 ppm (200 ppm Pb in sunflower study) (Alaboudi et al., 2018; Fatnassi et al., 2014; Xiong et al., 1998). The difference can potentially be explained by the difference in the amount of Pb that was bioavailable (Lasat, 2002). In previous studies, Pb ions were added in soluble forms in order to have high bioavailability for plant uptake. In contrast, according to chemical speciation, Pb usually presents in five fractions in the native soil (Tessier et al., 1979). In real-life scenarios, only a small proportion of Pb is in exchangeable forms, which is bioavailable for plant uptake. Pb interacts with minerals, organic matters, and clays in soil, forming complexes that are less bioavailable for plant uptake (Giacalone et al., 2005; Sun et al., 2009). Therefore, the bioavailability of Pb was expected to be lower in the soil sampled from the urban site in this study.

The results of this study showed that all selected plant species had TFs less than 1, suggesting these plants were more suitable for phytostabilization. The TF values of cowpea, Chinese cabbage, and sunflower evaluated in this study agree with previous studies, which showed that these species had TFs smaller than 1 when growing in the medium with similar Pb concentrations (Alaboudi et al., 2018; Xiong et al., 1998). In addition, the results showed that all selected plant species have BCFs < 1, indicating that they were excluders of Pb when growing in soil with Pb around 500 ppm. This finding agrees with previous studies of cowpea and Chinese cabbages which showed BCFs < 1 growing in an environment with 500 ppm Pb (Fatnassi et al., 2014; Xiong et al., 1998). However, Alaboudi et al. (2018) showed that sunflower had BCF > 1 when treated with 250 ppm Pb. The difference in BCFs between this study and previous research suggests that the threshold concentration that distinguishes sunflower as an accumulator or an excluder for Pb is within 250-500 ppm.

The finding that major accumulation of Pb was in the root system and TF values were less than 1 suggests all selected plant species in this study have the potential for phytostabilization. Plants suitable for phytostabilization of soil with high HMM concentrations should be capable of maintaining the translocation of HMM from roots to shoots as low as possible (Rizzi et al., 2004). Therefore, small TF values that are less than 1 are preferred. Removal of contaminants through plant root uptake and accumulation is another capacity favored by phytostabilization. Plant-associated factors are critical to phytostabilization, since plant phenotypic characteristics determine the ability to absorb, bind, and immobilize HMM in soil (Baker et al., 1995). Root depth and density are important for the effectiveness of phytoremediation and thus larger root biomass is desirable for this technology. With dense and deep roots, plants have larger surface areas to penetrate more contaminated soil, facilitating the immobilization of HMM in the rhizosphere. The results of this study showed that cowpea had the best phytostabilization potential among selected plant species because of its highest accumulation of Pb in roots, highest root biomass production, and low translocation of Pb from roots to shoots. Furthermore, the study investigated the phytoremediation potential of globe amaranth, which has not been studied previously to the best of our knowledge. When growing in soil with 500 ppm Pb concentration, amaranth had a similar capacity of Pb accumulation and similar biomass to sunflower. The TF value of amaranth was smaller than that of sunflower, suggesting amaranth is more favorable for phytostabilization compared to sunflower. Root was the major site of Pb accumulation in amaranth, and this finding is consistent with Adejumo et al. (2019) who showed that *Gomphrena celosioides* accumulated most Pb in the root system, suggesting that globe amaranth is a suitable alternative to *G. celosioides* to be used for phytostabilization.

Although plants used for phytostabilization should have low accumulation of HMM in aerial compartments, including the edible parts for leaf greens and fruit vegetables, it is important to investigate the food safety associated with the consumption of these plants. The IRLs for Pb are 3 µg/day for children and 12.5 µg/day for adults (Flannery et al., 2019). With the assumption that adults and children consume at least 1 kg of vegetables per day, the bioavailable concentrations of Pb in cowpea and Chinese cabbage found in this study were higher than the IRL for children. This result suggests that there is a risk associated with the consumption of cowpea and Chinese cabbage growing in soil with around 500 ppm Pb, especially for children. Consumption of edible parts of plants used for phytostabilization is thus not recommended, and it is important to prevent children from accidently consuming these plants.

The results of this study revealed that EDTA application effectively enhanced phytoextraction. The total Pb accumulation, TF, and BCF in sunflower were significantly increased by EDTA treatment. This result is consistent with the study of Seth et al. (2011), which showed that the addition of EDTA (500 µM) enhanced the Pb accumulation in roots and shoots in sunflowers by 12% and 88%, respectively. This result also confirmed the finding that the usage of chelating agents can increase the shoot-to-root ratio of Pb (Luo et al., 2006; Wu et al., 2004). Furthermore, the compost addition effectively improved phytostabilization by increasing root biomass production of sunflower. This result is consistent with Rizzi et al. (2004) who showed that the growth of *Lolium italicum* (Italian ryegrass) and *Festuca arundinacea* (tall fescue) was improved by compost application, which resulted in higher biomass production in roots and shoots when growing in Pb-contaminated soil (Rizzi, 2004). In addition, the reduction of Pb concentration in the plant tissues found in this study suggests that the compost helped to reduce the mobility and bioavailability of Pb in soil, which suppressed Pb uptake by plants. This finding agrees with another study which showed the compost treatment reduced Pb accumulation in *Phaseolus vulgaris* (dwarf bean) from 10.7 ppm to 6 ppm (Ruttens et al., 2006).

The methodology of this study allowed the comparison of phytoremediation potentials of multiple commonly-used species in the US. The study maintained the same treatment for all experimental plants and the soil used for cultivation was thoroughly homogenized to have even distributions of Pb concentration, pH, and organic matter in soil. This study design helped to eliminate biases and alternative interpretation of experiment results. Moreover, the study examined the actual Pb contamination in the urban site and assessed remediation methods accordingly. Therefore, the findings of this study are highly applicable to the soil contamination problem in West Atlanta neighborhoods.

Nevertheless, the study found an inconsistency between the post-phytoremediation Pb concentrations in soil tested by XRF and Pb concentrations in the plant tissues examined by ICP-MS. The total amount of Pb accumulated in the plants was approximately one thirtieth of that reduced in the soil. This result indicates a potential error in the research methodology that caused the excess reduction of Pb concentration in soil. The most possible explanation is that an amount of Pb was washed away along with soil attached to the roots of plants. The main phytoremediation process was identified to be phytostabilization for all species selected in this study, and thus Pb was sequestered in the root zone. A high Pb concentration theoretically presented in the soil surrounding the plant roots, which was lost during the washing process. This error significantly decreased the credibility of the XRF data of Pb concentrations in soil, which is one major limitation in this study. This finding suggests a modification in the method of sample preparation-the soil adhered to the plant roots should be carefully removed and saved for further analysis as an important part of soil sample.

Furthermore, this study has several other limitations. First, the sample size was small which decreases the statistical power of the study. As a result, the results of pot experiment were not completely representative and there might be potential biases. Second, only one soil Pb concentration was examined, and thus the study did not investigate the potential change in phytoremediation potentials of the selected species when these plants grow in different concentrations. Third, there were potential experimental errors associated with acid digestion. Since 100% extraction rate might not be achieved in some samples during acid digestion, the Pb concentration detected by ICP-MS might not be equal to the actual concentration in the plant tissues. Fourth, the study did not investigate the chemical speciation of soil Pb, which requires sequential extraction procedures. Therefore, the bioavailability of soil Pb, which influences the effectiveness of phytoremediation, was not determined. Future studies of Pb speciation in soil are suggested to further investigate the effect of metal bioavailability on phytoremediation as well as the efficacy of EDTA and compost applications to increase and decrease Pb bioavailability, respectively.

Conclusion

This study found that for the soil with approximately 500 ppm Pb concentration, V. unguiculate (cowpea) had the greatest capacity to accumulate Pb in roots and immobilize it in the rhizosphere, compared to other selected plant species. Nevertheless, the bioavailability of Pb in the fruit of cowpea suggests the potential adverse health effects associated with the accidental consumption, which is a disadvantage of using cowpea in phytostabilization. This finding also emphasizes the importance of performing phytoremediation with caution to prevent the human exposure to Pb via the consumption of phytoremediation plant materials. In addition, EDTA application was shown to be an effective method for optimizing phytoextraction by increasing Pb uptake and the translocation of Pb from roots to shoots. However, it is important to know that the usage of EDTA as chelating agent is associated with the risk of entering the food chain and ground water contamination because of its long persistence and low biodegradability. The future research on safe EDTA management in phytoremediation is recommended. Compost addition was also shown to enhance phytostabilization by increasing root biomass production and decreasing mobility and bioavailability of Pb in soil. As a natural soil amendment, compost has no negative impact on the environment, and it is highly recommended to be used for phytostabilization practices.

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