MAGNETIC SEPARATION AS A TOOL FOR METAL REMOVAL FROM SOILS AND SEDIMENTS

By

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Heavy metal contamination of soils and sediments is of increasing concern. The main objective of this research was to investigate the effectiveness of a magnetic treatment method for removing heavy metals from contaminated soils and sediments. The treatment approach was based on adsorbing the metal contaminants onto iron filings seed and removing the metal-laden filings by magnetic separation.

The heavy metal binding capacity of different types of soils was first assessed using the MetPLATE test which responds specifically to heavy metal toxicity. The binding capacities of Cu, Zn, or Hg in different types of soils followed this trend: clay rich soil > organic rich soils > sandy soils.

The magnetic treatment was conducted by using 5% (w/w) iron filings and a 3-h contact time between iron filings and soil. The effectiveness of magnetic separation of Cu, Zn and Cd from spiked soils was evaluated using the MetPLATE assay, the 48-h *Ceriodaphnia dubia* and the 96-h *Selenastrum capricornutum* toxicity tests. Results showed that the magnetic treatment method worked best on Cu-spiked soils, followed by the Zn-spiked soils and the Cd-spiked soils. The toxicity removal for Cu varied between 81.4% and 99.9% after a single treatment, where as the toxicity removal for Zn and Cd were, respectively, 81.4%-98.7% and 80.1%- 96.2% after two

or three treatments. Chemical analysis indicated that the metals were removed from both the soil matrix and the soil extracts, and the energy dispersive X-ray spectroscopy (EDS) further confirmed the adsorption of Cu, Zn, and Cd onto the added iron filings. No significant reduction in magnetic separation efficiency was observed as regards Cu and Zn removal from aged soils. The retrieved iron filings could be regenerated in 1 N HNO_3 for 1 hr and then retreated by 1 M NaOH for 72 hrs prior to reuse.

The magnetic separation method also worked effectively in Pb contaminated shooting range soils and artificially spiked sediments. More than 77.8% of the toxicity produced by Pb was removed from five shooting range soils. For Cu-, Zn-, and Hg- spiked sediments, the toxicity removal after a single treatment varied between 83.0% and 99.98%. The type of sediment and metal did not affect the treatment effectiveness, and the metals were removed from both the sediment matrix and the sediment extracts.

MetPLATE also showed great potential in predicting heavy metal phytoavailability in different types of soil. Results from a plant study showed that if a soil extract showed approximately 90% inhibition by MetPLATE assay, this soil could probably cause phytotoxicity in lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*). The plant study also demonstrated the effectiveness of the proposed magnetic treatment on reducing Cu phytoavailability. After treatment, the growth of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) was significantly enhanced, and the Cu content in plants shoots and roots also significantly decreased after treatment.

CHAPTER 1
INTRODUCTION

1.1 Problem Statement

Heavy metal contamination of soils and sediments is of increasing concern. Sources of heavy metals in soils and sediments mainly include natural occurrence and anthropogenic sources (e.g. mining, smelting, energy and fuel production, vehicle emission, waste disposal, urban wastewater effluents, fertilizer application, military operations, etc.) (Cui et al., 2004a; Hong et al., 2002; Nedelkoska and Doran, 2000). Since heavy metals are not biodegradable, they tend to persist over long-term periods in the soil matrix. They can be transported to groundwater (an important source of drinking water) or taken up by agricultural crops which leads to concerns over human and animal health (Mulligan et al., 2001a; O'Connor, et al., 2003; Sas-Nowosielska et al., 2004). Heavy metal-contaminated sediments have direct adverse effects on aquatic life and ecosystems. Potential problems caused by contaminated sediments include poisoning of food chain and loss of recreational enjoyment (US EPA, 1998a). Table 1-1 lists the health effects of Cu, Cd, Zn, Pb, and Hg on humans, and Table 1-2 provides the U.S. EPA national primary or secondary drinking water standards (US EPA, 2003a; 2003b) and Florida soil cleanup target levels (SCTLs) for these five metals (FDEP, 2005).

A number of remediation techniques have been developed to decontaminate heavy metal polluted soils and/or sediments. These methods have been successful under specific situations, such as in laboratory experiments, but may not be implemented in large scale yet (Virkutyte et al., 2002). Factors like variable soil type and texture, decrease of soil productivity, high cost, and safety concerns can also limit the applicability of some of the existing techniques. Therefore, research is now being directed at developing alternative, low-cost and environmentally safe

methods for heavy metal clean-up for soils and sediments (Nedelkoska and Doran, 2000; Reed et al., 1996; Sas-Nowosielska, 2004).

1.2 Heavy Metal Behaviors in Soils and Sediments

The partitioning of heavy metals in solids can be divided into five fractions, including exchangeable, bound to carbonate, bound to Fe-Mn oxides, bound to organic matter, and residual fraction (Tessier et al., 1979). Factors that affect the speciation of metals in soils are soil texture, pH, organic matter, clay minerals, carbonate, and redox potential (Mulligan et al., 2001a). Metal ions undergo a series of reactions with different soil constituents, including adsorption/desorption, precipitation, biological mobilization and immobilization, redox reaction and penetration into the crystal structure of minerals (Leštan et al., 2003). Moreover, some microorganisms can convert heavy metals into more toxic forms, e.g., methylation of Hg (Zhou et al., 2000). Figure 1-1 displays the interactions between soil constituents and heavy metals.

Table 1-3 shows the background levels of Cu, Cd, and Zn in natural soils and plants. Bioavailability of heavy metals is often associated with their distribution among soil fractions (Tu et al., 2001), and toxicity of heavy metals is associated with their bioavailability. Bioavailable metals are most likely found in water soluble and exchangeable forms, whereas the residual fraction is tightly bound and unlikely to be bioavailable under natural conditions (Ma and Rao, 1997). Therefore, total metal content usually cannot be used directly for determining the bioavailability of a metal. Soil pH, redox potential, clay minerals, organic matter, as well as the presence of hydrous oxides of aluminum, iron, and manganese can affect the solubility, mobility and thereby the bioavailability of heavy metals in soils and sediments. Generally speaking, an increase in soil pH, clay and organic matter content, and a decrease in redox potential can lower the solubility and availability of heavy metals (Morel, 1997; Trivedi and

Axe, 2000). Table 1-4 summarizes the chemical species of trace metals with regard to their bioavailability and potential toxicity to organisms.

Within the sediments, under anoxic conditions, metals usually occur as insoluble sulfides. The bioavailability of sediment-borne metals is related to sediment characteristics, including organic matter and clay content, particle size distribution, pH, cation exchange capacity, etc. Heavy metals in sediments can be transferred to the water column via a variety of processes, such as diffusion, resuspension, and biotransfer through organisms (Adriano, 2001a).

Sequential extraction is a commonly used method to identify metal fractionations in soils and sediments (Li et al., 1995; Ma and Rao, 1997; Nóvoa-Muñoz et al., 2007; Reddy et al., 2001). This technique is based on using a series of reagents with different chemical properties to extract a faction of metals linked to a specific form (Tessier et al., 1979).

1.3 Soil and Sediment Remediation Techniques

Soil and sediment remediation techniques include physical, biological and chemical treatments (Hamby, 1996), and these techniques can also be divided into *ex situ* remediation and *in situ* remediation. A number of soil remediation techniques and their applications are summarized in Table 1-5. Among the numerous remediation strategies discussed, methods such as excavation, containment, bioremediation, vitrification, electrokinetic remediation, soil washing and flushing, chemical immobilization, and phytoremediation are all commonly used remediation techniques for heavy metal-contaminated soils.

The mechanisms of heavy metal removal by microorganisms can be categorized as either metabolic or non-metabolic uptakes. The former is due to the fact that some heavy metals are essential micro-nutrients for microbial growth, while the latter includes organic binding to the cell wall and extracellular biopolymers (Antsuki et al., 2003). Microorganisms are able to convert metals into more soluble or mobile forms by protonation, chelation, or chemical

transformation (e.g., redox reaction), which can be removed from solid matrices. In contrast, immobilization of heavy metals by microorganisms can occur by precipitation, crystallization, sorption, uptake and intracellular sequestration. These immobilization processes may have applications for *in situ* remediation (Gadd, 2004). The microbial reduction of Cr^{6+} to Cr^{3+} and As^{5+} to As^{3+} have been attracting researchers' interests and show promising results for soil remediation (Anderson and Cook, 2004; Chirwa and Wang, 2000; Faisal et al., 2005; Luli et al., 1983; Megharaj et al., 2003). Microbial reduction of U^{6+} and Se^{6+} have provided possibilities to concentrate uranium and selenium from contaminated soils (Abdelouas et al., 1998; Ganesh et al., 1997; Ike et al., 2000; Oremland et al., 1999; Spear et al., 2000). The ability of microorganisms to convert Hg^{2+} to volatile Hg^{0} has shown great potential for mercury bioremediation (Nakamura et al., 1999; Wiatrowski et al., 2006; Zeroual et al., 2001). Although successful experiments have been conducted in laboratories, the application of bioremediation in large scales is still limited so far.

Phytoremediation is an environmentally friendly alternative that uses plants (hyperaccumulators) to remove metals from soils (Chaney et al., 1997; Wong et al., 2004). A hyperaccumulator is defined as a plant whose leaves may contain >100mg/kg Cd >1,000mg/kg Ni, or Cu, or >10,000mg/kg Zn or Mn (dry weight) when grown in metal-rich media. Some of the identified hyperaccumulators include *Zea mays, Brassica juncea, Alyssum bertolonii, Berkheya coddii, Helianthus, and Thalspi caerulescens* (Zavoda et al., 2001). Due to the small size and low biomass of most hyperaccumulators, much research is being conducted to enhance the availability of heavy metals in soils by adding chemical amendments, such as ethylene diaminetetraacetate (EDTA) and nitriloacetate (NTA) (Wong et al., 2004). However, the use of synthetic chelators can enhance the mobility and bioavailability of metals in soils and thus

increase the potential risks of leaching into groundwater and of posing harm to soil microorganisms (Meers et al., 2005; Wong et al., 2004). Organic acids (e.g., citric acid), which can degrade quickly in soils, may be a promising alternative to the persistent aminopolycarboxylic acids such as EDTA for increasing metal bioavailability (Meers et al., 2005). In addition, the disposal of contaminated crop material after harvesting is also a concern (Sas-Nowosielska et al., 2004).

Amendments utilized in chemical immobilization of heavy metal-contaminated soils include alkaline-based products, such as lime and phosphate-based materials (Basta and McGowen, 2004; Eighmy et al., 1997; Hettiarachchi et al., 2000; McGowen et al., 2001; Wang et al., 2001). In addition, organic matter (biosolids) (Brown et al., 2003, 2004; Farfel et al., 2005), as well as various industrial products, such as zeolites (Boularbah et al., 1996; Edwards et al., 1999; Friesl et al., 2003; Oste et al., 2002) and paper mill sludge (Calace et al., 2005), can also be used as soil amendments. Chemical immobilization is a cost-effective alternative and will not present adverse environmental or health efffects (Hamby, 1996).

Remediation techniques for contaminated sediments can also be grouped into *in situ* and *ex situ* treatments. *In situ* treatments avoid handling of sediments and usually cost less than *ex situ* treatments. However, *in situ* treatment is almost always less effective than *ex situ* treatment, and sometimes lacks process control (US EPA, 1993). The categories and techniques used by the U.S. EPA are listed in Table 1-6, among which only a few, such as capping, chemical treatment, ground freezing, washing, and solidification/stabilization, can be used for heavy metal-contaminated sediments. However, each remediation technique has its own applicability and limitations. *In situ* chemical treatment may cause secondary contamination, and is difficult to ensure complete mixing of the treatment reagents with contaminated sediments. Ground freezing

has very limited application due to its high cost. *In situ* solidification/stabilization has not yet been proven or accepted by the U.S. EPA for contaminated sediments since little is known about the cost, effectiveness, and possible by-products for large-scale treatment. Among *ex situ* treatments, only soil washing and solidification/stabilization can be used for heavy metal-contaminated sediments. Sediment washing is not effective for sediments with high contents of fine particles (e.g., silt, clay and humic substances) or sediments with low permeability. The success of solidification/stabilization is very dependent on the selection of proper stabilizing agents and requires a low organic concentration of less than 20 percent (US EPA, 1993).

1.4 Magnetic Separation

Four categories of magnetic separation was reported by Parker (1977), and they are the following: low intensity dry magnetic separation, low intensity wet magnetic separation, high intensity dry magnetic separation, and high intensity wet magnetic separation, which is also known as "high gradient magnetic separation" (HGMS).

Magnetic force acting on particles during their passage through the system is defined by Equation 1-1, provided that the particle is sufficiently small for the magnetic field to be considered uniform throughout its volume. Greater magnetic force generates greater possibility of successful separation (Parker, 1977).

$$F_m = \frac{1}{2} \mu_0 (k - k_m) \upsilon \nabla (H^2) \qquad (1\text{-}1)$$

Where, F_m is the magnetic force, μ_0 is the permeability of free space, υ is the particle volume, k is the susceptibility per unit volume, k_m is the susceptibility of the particle-bearing medium that can be neglected in most circumstances, and H is the magnetic field strength (Parker, 1977).

In a separation process, "tails" refers to the non-magnetic product collected after passing through the system, and the magnetic concentrate is called "mags". The separation efficiency is

measured in terms of "recovery" (R) and "grade" (G). R is the ratio of magnetic material in the *mags* to that in the original feed; G is the fraction of the magnetic component in the *mags* (Parker, 1977).

Table 1-7 lists some conventional and modern applications of magnetic separation in different fields. The advantages of magnetic separation, such as low cost, simplicity and ability to work at high flow rates, have encouraged the application of this technique to environmental problems (Karapinar, 2003). A number of studies have shown that high gradient magnetic separation (HGMS) can be used to remove magnetic and non-magnetic pollutants such as solid particles (DeLatour, 1975), phosphorus (Bitton et al., 1974; Franzreb and Höll, 2000; Karapinar et al., 2004), organic compounds (Sakai et al., 1997), algae (Bitton et al., 1975), bacterial viruses (Bitton and Mitchell, 1974), color and turbidity (Anderson et al., 1983) from water and wastewater effluents. A successful separation of non-magnetic contaminants consists of adding a magnetic seeding agent, such as magnetite, Fe_2O_3, Cr_2O_3, or MnO_2, to which the pollutants will be attached and an additional treatment such as precipitation /flocculation /coagulation (Karapinar, 2003). In addtion, investigations have indicated that the adsorption properties of magnetite can be used in conjunction with a magnetic field to remove heavy metals, metal colloids, and nanoparticles from aqueous effluents (Anand et al., 1985; Navratil and Tsair, 2002; Terashima et al., 1986).

1.5 Research Objectives and Dissertation Outline

The purpose of this doctoral research is to develop an effective approach to remove heavy metals from soils and sediments. The approach is based on using iron filings as an adsorbent and subsequently recovering the iron filings by magnetic separation.

This dissertation is organized into eight chapters. Chapter 1 covered the background and research objectives. Chapter 2 developed a toxicological approach for assessing the heavy metal

binding capacity of soils. Chapter 3 investigated the effectiveness of heavy metal removal from artificially contaminated soils using magnetic separation. Chapter 4 discussed the effect of soil aging on the magnetic separation process. Magnetic separation of heavy metals from Pb-contaminated soils and artificially contaminated sediments were covered in Chapter 5 and Chapter 6, respectively. Chapter 7 investigated the ability of MetPLATE^{TM}, a bacterial toxicity test, in predicting heavy metal phytoavailability, as well as the use of plants to assess the effectiveness of magnetic separation for removing heavy metals from soils. Chapter 8 summaried the findings of all of these research experiments.

Table 1-1. Health effects of selected heavy metals on humans

Toxicant	Exposure routes	Signs and symptoms of exposure	Carcinogenicity by EPA
Copper	Inhalation Oral Dermal	Nose, mouth and eyes irritation, headaches, dizziness, nausea, vomiting, diarrhea, abdominal pain, immune system damage, liver and kidneys damage	Group D (not classable)
Zinc	Inhalation Oral Dermal	Chest pain, cough, dyspnea, reduced lungs volume, nausea, chills, malaise, leukocytosis, vomiting, abdominal cramps, diarrhea, skin irritation, copper deficiency	Group D (not classable)
Cadmium	Inhalation Oral Dermal	Extreme fragility of bones, severe pain in bones and joints, severe nausea, vomiting, salivation, abdominal cramps and diarrhea, renal disturbances, lung insufficiency, osteomalacia, anemia and anosmia	Group B1 (probable human carcinogen)
Lead	Inhalation Oral Dermal	Fatigue, tremor, vomiting, abdominal pain, weight loss, weakness in fingers, wrists, or ankles, small increases in blood pressure, anemia, nervous system damage, brain and kidneys damage, miscarriage	Group B2 (Probable human carcinogen-based on sufficient evidence of carcinogenicity in animals)
Mercury	Inhalation Oral Dermal	Cough, lungs irritation, nausea, vomiting, diarrhea, increase in blood pressure or heart rate, skin rashes, eye irritation, personality changes, tremor, changes in vision, deafness, muscle incoordination, loss of sensation, difficulties with memory, nervous system damage, kidneys damage	Elemental mercury is group D (not classable); Methylmercury is group C (Possible human carcinogen)

Source: US DHHS, 1999a, 1999b, 2004, 2005a, 2005b; Francis, 1994;

Table 1-2. US and FL regulations on drinking water and soil levels of Cu, Cd, Zn, Pb and Hg

Metal	Drinking Water regulations (mg/L)	FL SCTL Direct Exposure (mg/kg)[c]		FL SCTL Leachability based on groundwater criteria[c] (mg/kg)
		Residential	Commercial/Industrial	
Cu	1.3[a]	150	89,000	NA
Cd	0.005[a]	82	1,700	7.5
Zn	5[b]	26,000	630,000	***
Pb	0.015[a]	400	1,400	***
Hg (inorganic)	0.002[a]	3	17	2.1

[a] US EPA national primary drinking water regulations, 2003a; [b] US EPA national secondary drinking water regulations, 2003b; [c] FDEP soil cleanup target levels (SCTLs), 2005; *** Leachability values may be derived using the SPLP test to calculate site-specific SCTLs or may be deterimined using TCLP in the event oily wastes are present; NA= not available at time of rule adoption

Table 1-3. Background level of Cu, Zn, and Cd in natural soils and plants

Metal	Concentration in soils (ppm)	Concentration in plants (ppm)
Cu	2-100	5-30
Zn	30-150	10-150
Cd	<1	0.005-0.02

Source: Mulligan et al., 2001a

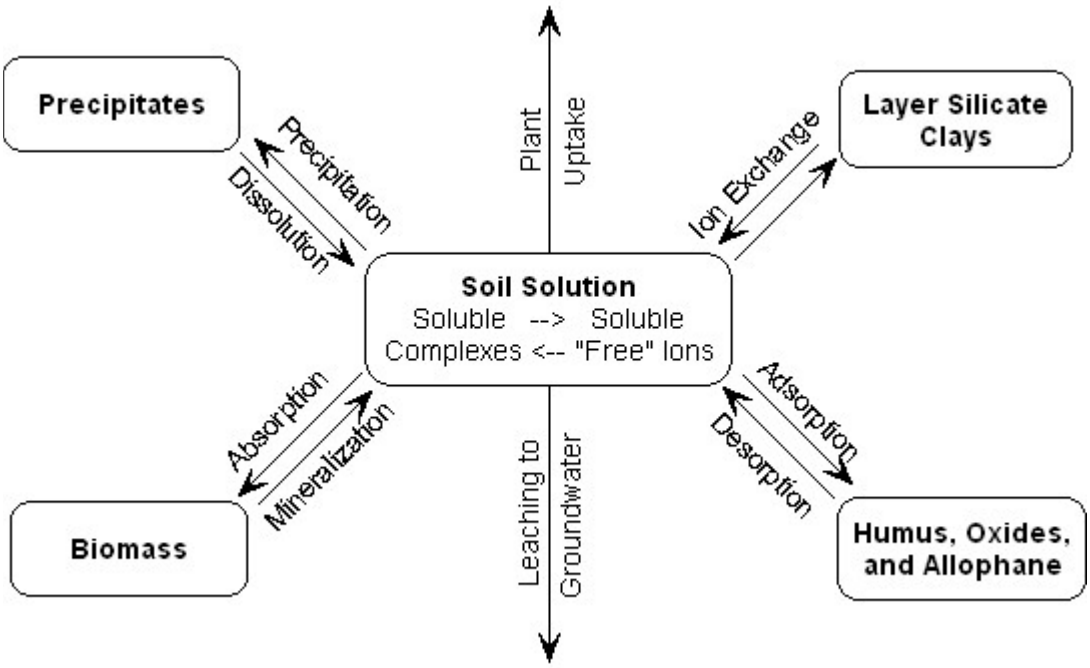


Figure 1-1. Interactions between soil constituents and heavy metals (from Adriano, 2001b).

Table 1-4. Chemical species of trace metals with regard to their bioavailability and potential toxicity to organisms

Metal	Dominant chemical species[a]		Most toxic species[b]
	Soil	Water	
Cu	Cu^{2+}	Cu^{2+}-fulvate	Cu^{2+}
Zn	Zn^{2+}	Zn^{2+}	Zn^{2+}
Cd	Cd^{2+}	Cd^{2+}	Cd^{2+}
Pb	Pb^{2+}	$Pb(OH)^{+}$	Pb^{2+}
Hg	Hg^{2+}; Hg^{2+}-fulvate	$Hg(OH)_2$; $HgCl_2$; CH_3Hg	CH_3Hg

[a] Does not account for ion-pairs or complex-ion species; [b] Considers degree of bioavailability
Source: Adapted from Adriano, 2001a.

Table 1-5. Summary of soil remediation techniques

Technique	Description	Applicability	Comments	Reference
Excavation	Excavation and burial of the contaminated soils at a hazardous waste site	Organics/ inorganics	Expensive, only transfers problems from one location to another	McGowen et al. (2001); Maenpaa et al. (2002)
Containment	Use physical barriers to retain, immobilize, or isolate contaminated soil (e.g. slurry walls and landfills)	Organics / inorganics	Require extensive preparation of the site and/or pretreatment of the waste	Mulligan et al. (2001a); US EPA (1997)
Soil washing	*Ex situ,* aqueous-based technique to extract and separate contaminants from soils	Organics/ inorganics	Not very effective for soils containing high silt and clay content or contaminated with high concentrations of mineralized metals or hydrophobic organics	Mulligan et al. (2001a); US EPA (1997); Andrade et al. (2007); Isoyama and Wada (2007); Ehsan et al. (2006a); Ehsan et al. (2006b)
Soil flushing	*In situ* technique based on injection or infiltration of chemical solutions through soils to extract contaminants, similar to soil washing.	Organics / inorganics	Not very effective for soils with high clay and organic matter. Large-scale treatment is limited to metals	Mulligan et al. (2001a); US EPA (1997); Park and Bielefeldt (2005); Tsang et al. (2007); Zhu et al. (2005)
Thermal treatment	"Use direct or indirect heat exchanges to desorb, vaporize, or separate contaminants from soils"	Volatile / Semi-volatile organics	Can be highly cost intensive due to high moisture content	US EPA (1997); Harjanto et al. (2002); Kasai et al. (2000); Merino and Bucalá (2007)

Table 1-5. Continued

Technique	Description	Applicability	Comments	Reference
Incineration	*Ex situ* remediation technique, which is also known as controlled-flame combustion or calcinations	Organics	Very expensive to treat wastes with very high moisture content or low organic content	US EPA (1997); Leuser et al. (1990); Anthony and Wang (2006)
Vitrification	*In situ* technique based on heating the soil electrically around 1600-2000°C, then allow the molten volume to solidify as it cools	Organics/ inorganics	Expensive technique, high clay and moisture contents can affect the efficiency	Mulligan et al. (2001a); Hamby (1996); Spalding (1994)
Electrokinetic remediation	*In situ* or *Ex situ* technique based on by passing electric current in contaminated soils	Organics / inorganics	Mainly applicable for saturated soils with low groundwater flow rates and fine-grained soils of low hydraulic permeability	Mulligan et al. (2001a) ; Reddy et al. (2003) ; Kim et al. (2002); Altin and Degirmenci (2005); Ottosen et al. (1997); Page and Page (2002)
Phytoremediation	*In situ* technique using plants to make soil contaminants less harmful or non-toxic	Organics / inorganics	An environment friendly technique but requires a very long treatment period, disposal of contaminated crop is also a concern	Chaney et al. (1997); Banuelos et al. (1997); Barocsi et al. (2003); Begonia et al. (2003); Garbisu and Alkorta (2001);
Chemical immobilization	A technique by introducing chemicals into the soil to reduce the solubility, mobility or availability of contaminants	Inorganics	Less expensive than excavation and landfilling and may provide a long-term remediation solution	Hamby (1996); Yang et al. (2001); Basta and McGowen (2004); Illera et al. (2004)

Table 1-5. Continued

Technique	Description	Applicability	Comments	Reference
Vapor Extraction	*In situ* or *ex situ* technique based on using vacuum pump or blower to induce air flow through the waste to extract contaminants	Volatile / semi-volatile organics	Contaminants must have low water solubility and above the water table, soil moisture content must be low and the soil must be sufficiently permeable	Hamby (1996); US EPA (1997); Upreti et al. (2007); Travis and Macinnis (1992); Poulsen et al. (1998); Park et al. (2005)
Bioremediation	*In situ* or *ex situ* technique based on using microbial species to degrade and transform contaminants	Organics / inorganics	Release of unpleasant odors, added nutrients may be carried to surface water or leach to groundwater	US EPA (1997); Wilson and Jones (1993); White et al. (1998); Rojas-Avelizapa et al. (2007); Van Dillewijn et al. 2007)

Table 1-6. US EPA categories of treatments potentially applicable to sediments

Technique	Description	Applicability
In situ treatment		
Capping	Underwater covering of contaminated sediments with less contaminated sediments with or without lateral walls	Organics/ inorganics
Solidification/stabilization	Add chemicals or cements to encapsulate sediments and/or make them less soluble, mobile or toxic	Organics/ inorganics
Biological treatment	Add microorganisms and/or chemicals to initiate or enhance bioremediation	Organics
Chemical treatment	Treat sediments by neutralization, precipitation, oxidation, and chemical dechlorination	Organics/ inorganics
Ground freezing	Form a wall of frozen sediment by placing refrigeration probes	Organics/ inorganics
Ex situ treatment		
Biological treatment	Use microorganisms to breakdown organic contaminants	Organics
Dechlorination	Potentially effective in detoxifying specific types of aromatic organic contaminants	Aromatic organic compounds
Solvent extraction	Volume reduction technique leaches contaminants with organic solvents	Organics
Soil washing	Water-based process to mechanically scrub excavated sediments	Organics/ inorganics
Thermal desorption	Remove contaminants by heating the sediment at a temperature below combustion	Volatile/ semi-volatile organics
Solidification/ stabilization	Add materials such as fly ash to reduce mobility or solubility of waste constituents	Most effective on inorganics and metals, not effective on volatile organics
Incineration	A thermal treatment to destroy organic compounds	Organics

Source: US EPA 1998a; 1993

Table1-7. Applications of magnetic separation

Fields of application	Purpose	References
Chemical and allied industries; food, drink and tobacco manufacturing; coal processing; metals industries	Remove tramp metal to protect machine	Parker (1977)
Industrial raw materials processing	Extract ferrous contamination	Parker (1977)
Mineral dressing industries	Extract/enrich magnetic ores	Parker (1977)
Clinical	Separation of red cells from whole blood	Melville et al. (1975); Melville at al. (1982); Takayasu et al. (2000)
Food industry	Remove contaminants from milk	Kaminski et al. (2000)
Environmental remediation	Oil spill remediation Industrial sludge treatment Water purification Wastewater treatment Nuclear waste treatment	Chun et al. (2001); Yanagisawa et al. (1981); Petrakis and Ahner (1976); Navratil and Tsair (2002); Chiba et al. (2002); DeLatour (1973); Bitton et al. (1974, 1975); Gokon et al. (2002); Ebner et al. (1999)

CHAPTER 2
TOXICOLOGICAL APPROACH FOR ASSESSING THE HEAVY METAL BINDING CAPACITY OF SOILS

2.1 Introduction

Soils are widely used as sinks for organic and inorganic contaminants such as metals. They are useful in mitigating the impact of contaminants on groundwater resources and surface waters. Soils are impacted by several types of toxic wastes, including industrial wastes, biosolids, mining, and construction and demolition wastes. Toxic metals in the applied wastes have attracted the attention of regulatory agencies because they can be transported to groundwater or taken up by agricultural crops, leading to concerns over human and animal health. Toxic metals tend to bind to soils, thus becoming less available to the biota and to roots of agricultural crops (Adriano, 2001a; Salomons, 1995). Metal phytoavailability (i.e., availability to plants) is controlled by several factors, including metal speciation, soil characteristics (e.g., pH, clay type and content, organic matter content, moisture content) and contact time between soil and metals (Naidu et al., 2003; Weng et al., 2002). Moreover, bioavailability of metals in soils also depends on the type of clay and organic matter (Lock and Janssens, 2001). Soil amendment with clay minerals (e.g., bentonite, zeolite), iron oxides (e.g., goethite, hematite), and phosphate fertilizers has been reported to be effective in reducing metal availability to wheat (*Triticum aestivum*) (Usman et al., 2005).

Metal binding and immobilization in soils involves several mechanisms, such as adsorption, ion exchange, complexation by humic substances, and precipitation reactions (Weng et al., 2002). Sequential extraction procedures provide a good indication of metal partitioning in soils. They involve a range of chemical reagents that extract different metal fractions (soluble, exchangeable, carbonate-bound, oxide/hydroxide-bound, organic matter-bound, and residual fractions) in soils (Balasoiu et al., 2001; Smith et al., 1999; Tack and Verloo, 1995; Tessier et al.,

1979; Yong et al., 2001). The soluble and, potentially, the exchangeable fractions are generally believed to be available to plants and the biota, and the total concentration of metals in soils does not indicate their availability to plants (Adriano, 2001a). Phytoavailability may vary among different soils contaminated with the same total metal concentration, suggesting that the soil matrix plays an important role in phytoavailability and, ultimately, phytotoxicity (Naidu et al., 2003).

The metal fractionation chemical procedures need, however, to be complemented with toxicity testing to obtain information about the biological activity of metals in soils. Some investigators have used both chemical and toxicological approaches to assess the bioavailability of metals in solid matrices (Kong and Bitton, 2003; Schultz et al., 2004; De Vevey et al., 1993).

In this chapter, we developed a relatively rapid test to assess the heavy metal binding capacity (HMBC) of five soils. The test is based on the use of MetPLATE^{TM}, a bioassay that responds specifically to heavy metal toxicity (Bitton et al., 1994). The test compares the relative toxicity of a metal in a given soil to metal toxicity in a reference soil (Ottawa sand).

2.2 Material and Methods

2.1.1 Soils Used

Three soil types were used to assess their capacity to bind metals, such as copper, zinc and mercury. Two sandy soils were collected from the top 4 feet at two different sites and were chosen because they are representatives of the soils prevailing in North Central Florida. An organic rich soil (organic soil 1) was collected from the first few top inches along Hogtown Creek in Gainesville, FL. The second organic rich soil (organic soil 2) was a top soil purchased from a local landscaping store. A clay rich soil (Georgia clay soil, top 20 cm) was collected in Atlanta, Georgia. Table 2-1 shows some characteristics of the soils under study. The soil pH was measured according to the U.S. EPA method 9045D (US EPA, 2004). Soil redox potential (Eh)

was measured by Fisher Scientidic Accument Model 15 pH/mV meter. Particle size distribution was determined according to the USDA Soil Survey Lab Method (USDA, 1992). The Walkley & Black Method (Walkey and Black, 1934) was used to measure the soil organic carbon content. Soil effective cation exchange capacity (CEC) was determined according to a method developed by Sumner and Miller (1996). Ottawa Sand, due to its low ability to bind metals, was selected as a reference soil.

2.2.2 Methodology for Assessing Soil Heavy Metal Binding Capacity (SHMBC)

Briefly, as shown in Figure 2-1, the test consists of adding metal-laden solutions to soils under study, allowing the mixtures to reach equilibrium, separating the solid phase from the pore water by centrifugation, and assaying for metal toxicity of the soil extracts. A similar methodology was used for the Ottawa sand, which serves as a reference soil.

Soils were first air-dried, screened (sieve # 16; 1.19 mm particles), and homogenized. Subsequently, serial dilutions of metal-spiked solutions were prepared in moderately hard water (60 mg/L Ca, 60 mg/L Mg, pH = 7.4-7.8) for soil spiking. The solutions were labeled "A", "B", "C", "D", and "E". A sixth solution labeled "F", was not spiked with metals and served as the negative control for the soil. Five other metal-spiked solutions were added to Ottawa sand (reference soil) and were labeled "A_0", "B_0", "C_0", "D_0", and "E_0". A sixth solution, labeled "F_0", was prepared without any metal added. Twenty milliliter of each solution was added to 5 g of soil or Ottawa sand in 50-mL Erlenmeyer flasks. The flasks were covered with parafilm and placed on a shaker at 300 rpm for 4 hours. After shaking, the soils were centrifuged at 10,000 rpm for 15 minutes. The metal toxicity of the soil extracts was assayed with MetPLATE^{TM}, a microbial test which responds specifically to heavy metal toxicity. The MetPLATE^{TM} assay was carried out according to Bitton et al. (1994). Following rehydration of the MetPLATE^{TM} bacterial reagent, 0.1 mL of bacterial suspension was added to 0.9 mL of the soil extracts in

small culture tubes. The tubes were vortexed and placed in an incubator at 35°C for 90 minutes. Following incubation, 0.2 mL of the content from each tube was transferred to a 96-well microplate. 0.1 mL of rehydrated MetPLATE™ chromogenic substrate was added to each well. The plate was shaken gently and returned to the incubator until a purple color developed (after about 1 hr) in the negative controls (solutions "F" and "F_o"). The absorbance was determined with a microplate spectrophotometer (Maxline Microplate Readers, Molecular Devices, Sunnyvale, CA) at 570 nm. All HMBC tests were run in triplicate and three MetPLATE™ toxicity tests were run for each HMBC test.

Regression analysis was used to determine the EC_{50} for both the soil under study and Ottawa sand, the reference soil. The soil heavy metal binding capacity (SHMBC) was determined by dividing the EC_{50} for the soil sample by the EC_{50} for the metal in the reference soil, Ottawa Sand. The SHMBC was obtained as followed:

$$\text{SHMBC} = \frac{EC_{50} \text{ of field soil spiked with a given metal}}{EC_{50} \text{ of Ottawa sand spiked with the same metal}} \qquad (2\text{-}1)$$

The SHMBC methodology, summarized in Figure 2-1, was used to determine the Cu, Hg and Zn binding capacity of the five soils. A detailed procedure for the MetPLATE™ assay along with an example of the EC_{50} calculation was included in Appendix A.

2.3 Results and Discussion

Soil heavy metal binding capacity (SHMBC) was determined for five soils sampled in Florida and Georgia. Three metals (Cu, Zn, and Hg) were tested for their binding to the soils. Table 2-2 shows the EC_{50}s (expressed as metal added in mg/kg soil) of the three metals in the different soils under study. The Ottawa sand (reference soil) extracts were quite toxic, with EC_{50}s of 1.1 mg/kg for Cu, 0.9 mg/kg for Zn, and 1.5 mg/kg for Hg, indicating that the Ottawa

sand displayed a relatively low binding capacity for metals, thus justifying its selection as a reference soil.

It is worth mentioning that the higher the EC_{50}, the lower the toxicity of the soil extracts, indicating that the metal was bound by the soil and, thus, unavailable to the test organisms. The Georgia clay soil displayed the highest EC_{50}s (i.e., lowest toxicity to MetPLATE) for the three metals tested. Among the five soils under study, the EC_{50}s, expressed as metal added to soils in mg/kg, varied between 19.1 and 416 for Cu, between 14.3 and 296.9 for Zn, and between 8.4 and 448.4 for Hg (Table 2-2).

The SHMBC for the three metals and five soils tested is shown in Figure 2-2. As regarding their binding capacity towards the three metals, the soils were classified in the following order:

Georgia clay rich soil > organic rich soils > sandy soils

The organic (e.g., humic substances) and inorganic (e.g., clay minerals) colloidal particles in soils generally play a significant role in binding metals. Georgia clay rich soil contains 21% clay and thus displayed the highest binding capacity towards all three metals. Similarly, the organic rich soils (1 & 2) showed much higher SHMBCs than the sandy soils, due to their much higher organic matter content (12.2-18.8%) (Figure 2-2). Thus, the higher the SHMBC, the higher is metal binding to the soils.

The HMBC concept, as reviewed by Bitton et al. (2005), was previously used to assay metal bioavailability in surface waters (Huang et al., 1999) and municipal landfill leachates (Ward et al., 2005). The present research shows the first application to date of this bioassay to soils. It is a relatively rapid methodology to assess metal bioavailability in soils. This methodology is based on toxicity testing of soil extracts with MetPLATE, a test specific for

heavy metal toxicity (Bitton et al., 1994). The ability of this test to predict metal uptake and subsequent toxicity to plants is discussed in Chapter 7. Culture studies with plants show that metal uptake by plants varies with the type of soil, with the uptake being higher in sandy than in clay soils which generally display a higher metal binding capacity (Naidu et al., 2003). For example, terrestrial plants should be protected from Cu toxicity at the benchmark concentration of 100 mg/kg (Will and Suter, 1995). However, no phytotoxicity was found in Australian orchard soils with a range of copper concentrations of 11 to 320 mg/kg (Merry et al., 1983). Cu and Cr toxicity to barley was lower in a spiked natural forest soil (82.8 sand, 9.2% silt, 8.0% clay, 3.8% organic matter content) than in an artificial sandy soil (100% sand; pH 7.80, 0.27% organic matter) (Ali et al., 2004). Around a Peruvian copper mine, phytoxicity was found to be higher in soils with low organic matter (Bech et al., 1997). These findings confirm that total soil metal concentrations do not give an indication of metal bioavailability and phytotoxicity, with the soil matrix playing an important role in metal toxicity. Our findings, using a bacterial toxicity test, confirm that, at least for Cu, Zn and Hg, the SHMBC for clay and organic soils is much higher than for sandy soils.

2.4 Conclusions

This new technique, based on a toxicological bioassay, shows a novel approach to evaluate heavy metal binding to soils and, hence, bioavailability. We have shown that the soil metal binding capacity (SHMBC) varies with the type of soil, with clay rich and organic rich soils displaying a higher metal binding than sandy soils, which was used in latter experiments to determine the heavy metal concentrations in spiked soils. This relatively rapid test could be used in a number of applications, mainly ecological risk assessment. The SHMBC test could also be used to assess the suitability of soils to receive metallic wastes. The test could simulate more realistic conditions by determining the SHMBC of metal-spiked soils which have been

subsequently “aged” for a few weeks or a few months. Moreover, it would be valuable to run SHMBC tests in parallel with metal uptake by terrestrial plants to determine their application in assessing phytoavailability and potential for phytotoxicity.

Table 2-1. Soils characteristics

Characteristic	Red sandy soil	Sandy soil	Organic soil 1	Organic soil 2	Georgia clay soil
pH	6.2	5.7	5.7	5.3	5.7
Eh (mV)	485.0	422.0	403.0	337.0	324.0
% Organic carbon	0.1	0.5	6.4	4.1	0.6
% Organic matter	0.3	1.6	18.8	12.2	1.8
% Sand	90.7	96.92	93.1	92.6	56.4
% Silt	3.2	0.02	2.0	0.8	22.6
% Clay	6.1	3.06	4.8	6.6	21.0
CEC ($cmol_c/kg$)	14.4	14.1	230.1	107.8	79.6

Table 2-2. EC_{50}s (metal added in mg/kg soil), as determined by MetPLATE™, of water extracts from five soils and Ottawa sand

Soil type	Heavy metal	EC50 (mg/kg soil)
Ottawa sand (reference soil)	Cu	1.1 ± 0.2*
	Zn	0.9 ± 0.1
	Hg	1.5 ± 0.3
Red sandy soil	Cu	19.1 ± 1.3
	Zn	14.3 ± 2.1
	Hg	8.4 ± 0.2
Sandy soil	Cu	54.3 ± 3.1
	Zn	55.0 ± 2.5
	Hg	88.4 ± 8.0
Organic soil 1	Cu	86.4 ± 2.7
	Zn	153.3 ± 36.0
	Hg	268.9 ± 53.1
Organic soil 2	Cu	128.4 ± 3.3
	Zn	129.3 ± 4.4
	Hg	300.7 ± 22.5
Georgia clay soil	Cu	416.0 ± 16.2
	Zn	296.9 ± 21.5
	Hg	448.4 ± 22.3

*mean of 3 replicates ± one standard deviation

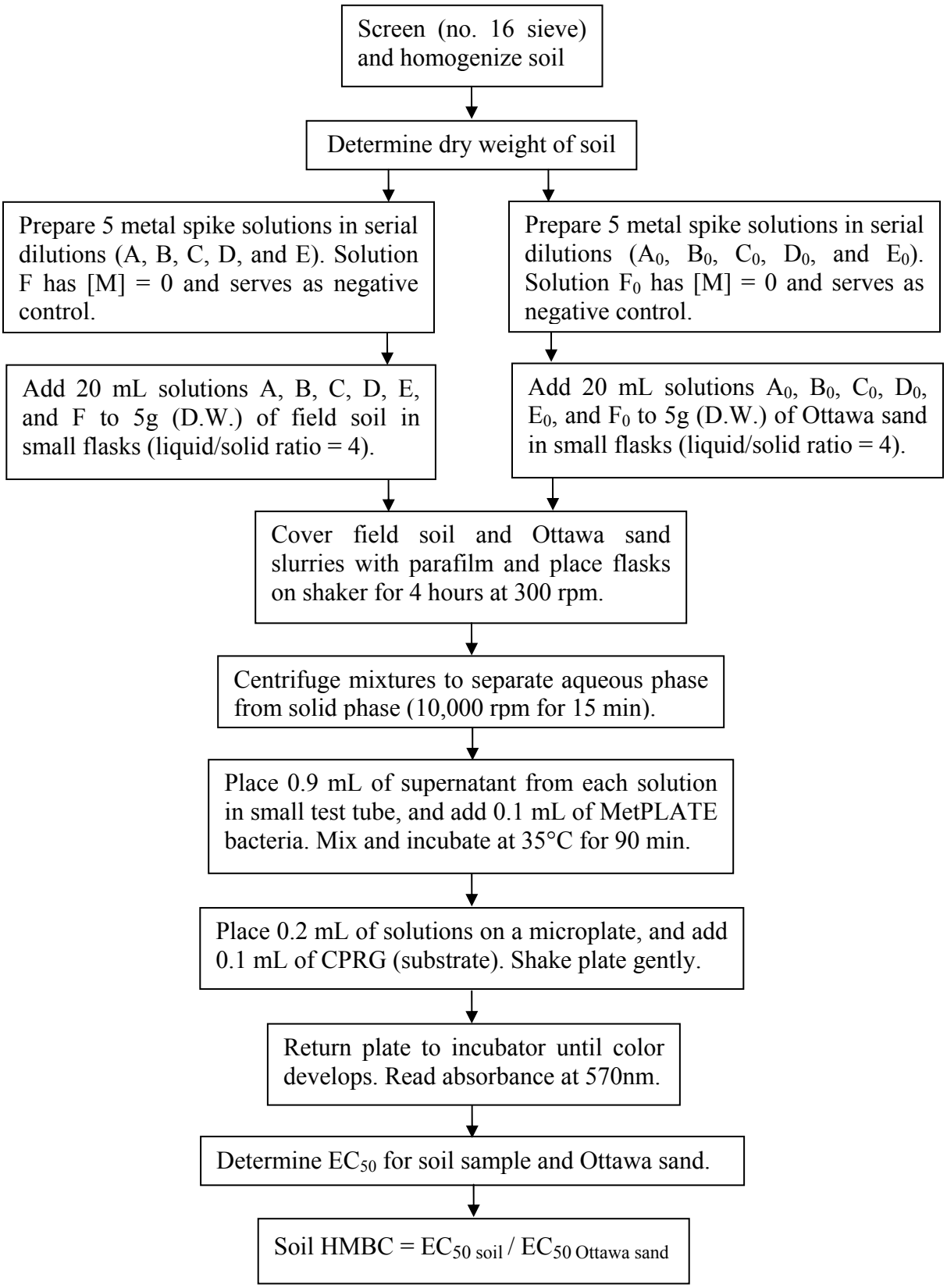


Figure 2-1. Soil HMBC (SHMBC) methodology

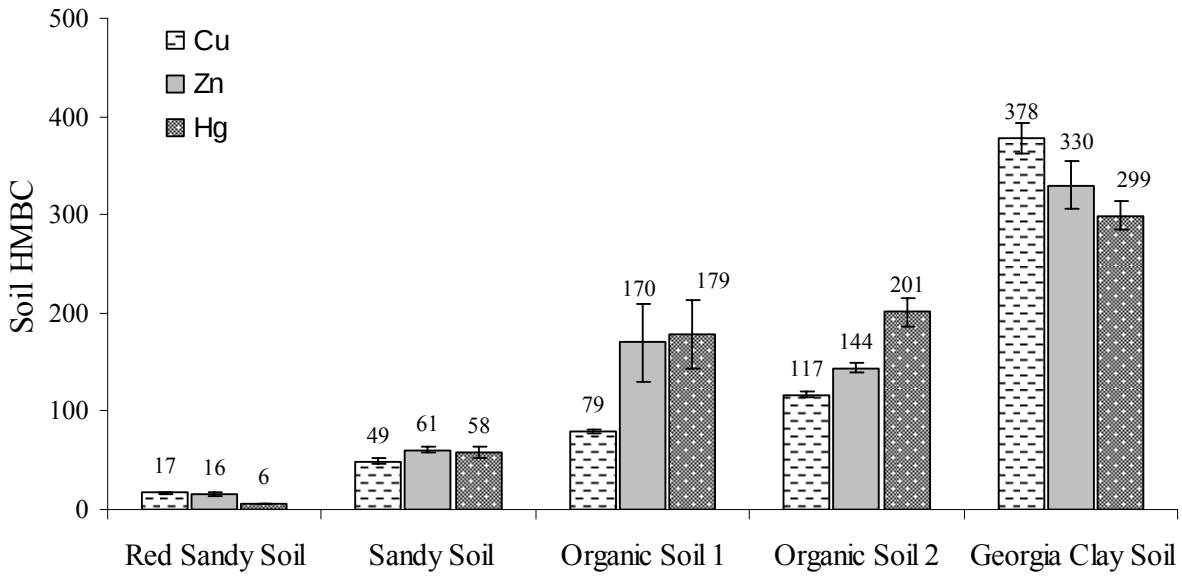


Figure 2-2. SHMBC for three metals (Cu, Zn, Hg) and five soils.

CHAPTER 3
HEAVY METAL REMOVAL FROM SOILS USING MAGNETIC SEPARATION

3.1 Introduction

Heavy metal contamination of soils and sediments is a worldwide problem that has been attracting considerable attention over the past decades. Heavy metals in soils frequently accumulate in the upper horizons and can adversely impact soil microbial activities, crop productivity, and the food chain (O'Connor et al., 2003). Moreover, toxic metals are highly persistent in the environment, with a residence time of hundreds of years or even more (Mulligan and Wang, 2006; Sas-Nowosielska et al., 2004). In response to these adverse effects, a variety of decontamination strategies, including *in situ* and *ex situ* techniques, have been developed or are under ongoing development. Soil excavation and burial at landfills is the most traditional method, but it is very costly and only transfers the heavy metal problem from one location to another (Maenpaa et al., 2002; McGowen et al, 2001). Soil washing with acid or chelating agents such as EDTA is another frequently used technique; however, it can decrease soil productivity and change the chemical and physical structure of soils (Reed et al., 1996). The recovery of heavy metal-EDTA complexes is also difficult (Hong et al., 2002). Therefore, a large number of alternative options have been investigated which are considered less intrusive and more cost effective. One of the alternatives that has received a considerable amount of attention is heavy metal immobilization in soils via addition of various of amendments (Gray et al., 2006). Examples of these amendments include lime, phosphate-based materials (Basta and McGowen, 2004), organic matter (biosolids) (Brown et al., 2003, 2004; Farfel et al., 2005), as well as various industrial products, such as zeolites (Boularbah et al., 1996; Edwards et al., 1999; Friesl et al., 2003; Oste et al., 2002). Immobilization is less expensive than excavation and may provide a long-term remediation solution (McGowen et al., 2001). Phytoremediation and

electroremediation are other emerging heavy metal treatment techniques. The goal of phytoremediation is to extract metals by using living plants and it is increasingly being regarded as a cost-effective and environmentally friendly alternative (Garbisu and Alkorta, 2001; Wu et al., 2004). However, phytoremediation technology is still a new field and holds some drawbacks and limitations that require further investigation. Electroremediation consists of passing a low intensity electric current between appropriately distributed electrodes imbedded in the contaminated soil (Mulligan et al., 2001a; Page and Page, 2002). The process can be applied *in situ* or *ex situ*. This method is more effective for clay soils as compared to soil washing, however, the demonstrations of this technology are limited so far (Mulligan et al., 2001a).

In this chapter, experiments were performed to evaluate the effectiveness of removal of heavy metals (Cu^{2+}, Zn^{2+}, and Cd^{2+}) from four soils using iron filings followed by a magnetic separation step. Iron (hydr)oxides are known to bind heavy metals by adsorption or coprecipitation to decrease heavy metal mobility in soils (Crawford et al., 1993; Hartley et al., 2004; Mckenzie, 1980; Mench et al., 1994), and have been used to remove heavy metals from wastewater and liquid hazardous wastes (Benjamin et al., 1996; Yeager, 1998). Magnetic separation has been used extensively in industries, such as in the processing of minerals. In recent years, the application of magnetic separation technology to environmental problems has received considerable attention (Orbell et al., 1997). As reported by Kochen and Navratil (1997), magnetic polymer resins were able to remove actinides and heavy metals efficiently from contaminated water. Studies have also proven that high gradient magnetic separation (HGMS) can also be used for the removal of non-magnetic water pollutants, such as suspended solid particles (DeLatour, 1975), phosphorus (Bitton et al., 1974; Franzreb and Höll, 2000; Karapinar et al., 2004), organic compounds (Sakai et al., 1997), and algae (Bitton et al., 1975). Little is

known about the application of magnetic separation methods to soil remediation. Macášek et al. (2002) demonstrated the possibility of removal of cesium-137, strontium-85, and europium-152 from artificially contaminated soils by a magnetic sorbent.

After the determination of best treatment conditions, the soil extracts were tested by MetPLATE^{TM} assay, 48-h Ceriodaphni*a dubia* acute toxicity test, and 96-h *Selenastrum capricornutum* chronic toxicity test to assess the reduction of heavy metal toxicity in soils. Chemical analysis and mass balance studies were also performed to investigate heavy metal distribution in the soil matrix and extracts. In addition, a sequential extraction procedure was employed to assess the fractionations of the added heavy metals in a sandy soil and an organic rich soil after magnetic treatment. The regeneration of iron filings was also investigated, and the retrieval of Cu, Cd and Zn by iron filings was further examined, using energy dispersive X-ray spectroscopy (EDS).

3.2 Material and Methods

3.2.1 Soils Used

Four soils were used to investigate the effectiveness of the proposed magnetic treatment method. A sandy soil was sampled from the top 4 feet at the McCarty Woods on the University of Florida campus. A red sandy soil was sampled from Perdido Landfill in Cantonment, FL. An organic rich soil (organic soil) was collected from the first few top inches along Hogtown Creek in Gainesville, FL. A clay rich soil (Georgia clay soil, top 20 cm) was collected in Atlanta, Georgia. Table 3-1 shows the main characteristics of the soils under study. All soils samples were first air-dried, screened (sieve # 10; 2.0 mm particles), and homogenized prior to use.

3.2.2 Chemicals Used

Three heavy metal solutions ($Cu^{2+,}$ Cd^{2+}, and Zn^{2+}) and a heavy metal mixture (a solution consisting of equal concentrations of Cu^{2+}, Cd^{2+}, and Zn^{2+}) were used. Copper solution was

prepared from copper sulfate ($CuSO_4 \cdot H_2O$, Sigma®, St. Louis, OM). Cadmium solution was prepared from cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) purchased from Fisher (Pittsburgh, PA). Zinc solution was prepared from zinc chloride ($ZnCl_2$, Sigma®). Iron filings (Fisher, 40 mesh) were placed in 1 M NaOH for 72 hours to increase the adsorption capacity and then washed thoroughly with distilled water before use (Yeager, 1998).

3.2.3 Recovery of Iron Filings from Soils

The recovery of iron filings from the soils under study, using a Ferrimag rectangular magnet (Scientifics®, 152 ×102×25mm, 3.4 megagauss oersteds) was investigated. Iron filings removal was studied under three different soil conditions (air-dried, field capacity, and water-saturated) and three concentrations of iron filings (1%, 2%, and 5%, w/w). Under each soil condition, dry iron filings were added to 50 g of soils and were retrieved with the magnet following 2-hr incubation. The retrieved iron filings were washed thoroughly with distilled water and dried at 70°C overnight and then weighed to determine the percent retrieval. All samples were run in triplicate.

3.2.4 Determination of Iron Filings Concentration

Fifty gram of sandy soil, weighed in a 250 ml centrifuge tube, was spiked with 40 mL of a heavy metal mixture containing 50 mg/L Cu^{2+}, 50 mg/L Cd^{2+}, and 50 mg/L Zn^{2+} (resulting in 40 mg/kg Cu^{2+}, 40 mg/kg Cd^{2+}, and 40 mg/kg Zn^{2+}). The soil slurry was shaken for 1 hour. Then, three concentrations (2.5%, 5%, and 10%, w/w) of iron filings were added and the system was shaken for 6 hours. The mixture was then transfered into a plastic container (34 cm × 20cm × 10cm), and the iron filings were magnetically retrieved by moving the magnet back and forth above the surface of the soil slurry. The soil slurry was then transfered back to the centrifuge tube, and 60 mL of distilled water were added to the system to bring the total solution volume to 100 mL. The soil slurry was centrifuged at 10,000 rpm for 15 minutes. The supernatant (soil

extract) was removed with a pipet. Each soil sample was run in triplicate. Soil without iron treatment served as the control.

3.2.5 Determination of the Contact Time between Iron Filings and Soil Matrix

We studied the effect of the contact time between the metal-spiked soils and iron filings. Metal removal was determined following contact times of 1.5 hr, 3 hrs and 6 hrs.

3.2.6 Magnetic Separation of Heavy Metals from Four Metal-Spiked Soils

Fifty gram of sandy soil, red sandy soil, organic rich soil, and Georgia clay rich soil were spiked with 40 mL of Cu^{2+}, Cd^{2+} or Zn^{2+} solutions. Three concentrations (250 mg/L, 500 mg/L, and 1000 mg/L) of heavy metal solutions were used, resulting in 200 mg-metal/kg soil, 400 mg-metal/kg soil, and 800 mg-metal/kg soil. The soil slurry was shaken for 1 hour at room temperature. Metal separation efficiency was studied under the following conditions derived from the previous experiments: addition of iron filings at 5% (w/w) concentration; contact time of 3 hrs. Then, the iron filings were magnetically retrieved and the soil slurry was centrifuged at 10,000 rpm for 15 minutes and the supernatant (soil extract) was removed with a pipet. The retrieved iron filings and the soil matrix after centrifugation were dried at 70°C overnight. Soil without iron treatment served as the control. Toxicity tests were undertaken for all soil extracts before and after magnetic treatment. Chemical analysis was performed for all factions, including soil extracts, retrieved iron filings, and soil matrix. Each soil sample was run in triplicate.

3.2.7 Toxicity of Soil Extracts

Three toxicity assays, MetPLATE^{TM}, the 48-h *Ceriodaphnia dubia* acute bioassay, and the 96-h *Selenastrum capricornutum* chronic toxicity test were used to assess the toxicity of the soil extracts before and after iron treatment. To determine the EC_{50} for the soil extracts, 4 to 5 dilutions of the soil extracts were prepared, and a regression analysis was used to calculate the EC_{50}s (See Appendix A for details). All samples were run in triplicate.

Toxicity unit (TU) (Bitton, 1998), defined in the Equation 3-1, was used as an expression of metal toxicity. A higher TU value indicates higher toxicity.

$$TU = \frac{100}{EC_{50}} \qquad (3\text{-}1)$$

3.2.7.1 MetPLATE™ protocol

The MetPLATE™ assay (as described in Section 2.2.2, also see Appendix A for further details), a microbial test which is specific for heavy metal toxicity and does not respond to organic toxicity (Bitton et al., 1994), was used to determine the toxicity of the soil extracts. Moderately hard water (60 mg/L Ca, 60 mg/L Mg, pH = 7.4-7.8) was used as the negative control.

3.2.7.2 48-hour *Ceriodaphnia dubia* acute bioassay

The 48-h acute *Ceriodaphnia dubia* bioassay was carried out according to the U.S. EPA's standard method (US EPA, 2002a). Neonate daphnids (first instar *Ceriodaphnia* less than 24 hours old) were used for testing and were fed 2 hours before the test started. Five neonates were exposed in each plastic cup containing 20 mL of the sample. Moderately hard water (60 mg/L Ca, 60 mg/L Mg, pH = 7.4-7.8) was used as the negative control. The test temperature was 25^{o}C. After 48 hours, the number of motile and dead daphnids was counted (see Appendix A for details).

3.2.7.3 96-hour *Selenastrum capricornutum* chronic toxicity test.

The 96-h chronic *Selenastrum capricornutum* test was carried out according to the U.S. EPA's standard method (US EPA, 2002a). The preliminary algal assay procedure (PAAP) medium was used as the negative control. When test began, 1 mL of the algal seed, with a cell density of 5×10^5 cells/mL, was spiked into each test flask containing 50 mL of the sample. Then all flasks were immediately foam-stoppered and placed under continuous light condition (400 ±

40ft-c). The test temperature was 25^{o}C. The test flasks were shaken and rearranged randomly at least once per day. After 96 hours, growth inhibition was measured by counting the cell density in each sample and negative control by a hemacytometer (Hausser Scientific, Horsham, PA) under microscope (see Appendix A for details).

3.2.8 Chemical Analysis

Chemical analysis was undertaken for all fractions, including the soil matrix, soil extracts and iron filings, before and after magnetic treatment. Soil extracts were digested according to the U.S. EPA method 3010A (US EPA, 1992), and soils and iron filings were digested according to the U.S. EPA method 3050B (US EPA, 1996). All digested samples were analyzed for metals using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Mass balance studies were also performed for the Cu^{2+}, Zn^{2+}, and Cd^{2+} spiked soils. All samples were run in triplicate. Detailed digestion procedures are included in Appendix B.

3.2.9 Sequential Extraction of Metals from Soils

A sequential extraction procedure adapted from Tessier et al. (1979) and Ma and Rao (1997) was used to assess the fractionations of Cu, Zn, and Cd in spiked soils before and after magnetic treatment. The sandy soil and organic rich soil were spiked with 200 mg/kg Cu^{2+}, 200 mg/kg Zn^{2+}, or 200 mg/kg Cd^{2+}, respectively. The spiked soils were allowed to dry at room temperature, followed by adding distilled water to reached saturation, mixing very well, and letting them dry again at room temperature (3 days for sandy soil, and 5 days for organic rich soil). After four wet-dry cycles, a portion of each spiked soil was treated by magnetic separation according to the following procedure: 100 mL of distilled water was added to 50 g of soil, and the soil slurry was shaken for 1 hour at room temperature. Then, 5% (w/w, 2.5 g) of iron filings were added, and the system was shaken for 3 hours followed by magnetic retrieval of the iron

filings. The soil slurry after treatment was dried at 70°C overnight. Both treated and non-treated soils were used for sequential extraction according to the procedure described below:

Fraction 1: Exchangeable. One gram of soil was extracted at room temperature for 1 hour with 8 mL of 1 M sodium acetate (pH = 8.2) with continuous agitation.

Fraction 2: Bound to carbonates. The residue from faction 1 was extracted at room temperature for 5 hours with 8 mL of 1M sodium acetate (pH adjusted to 5.0 with acetate) with continuous agitation.

Fraction 3: Bound to Fe-Mn oxides. The residue from faction 2 was extracted with 20 mL of 0.04 M hydroxylamine hydrochloride in 25% (v/v) acetate for 6 hours at 96 ± 3°C with occasional agitation.

Fraction 4: Bound to organic matter. Three milliliter of 0.02 M nitric acid and 5 mL of 30% hydrogen peroxide (pH adjusted to 2 with nitric acid) were added to the residue from fraction 3, and the mixture was heated to 85 ± 2°C for 3 hours with intermittent agitation. After cooling, 5 mL of 3.2 M ammonium acetate in 20% (v/v) nitric acid was added and the sample was diluted to 20 mL and agitated continuously for 30 min.

Fraction 5: Residual. The residual fraction was a theoretical value that was calculated by subtracting the sum of the previous four factions from the total.

To minimize losses of solid material, the extractions were conducted in 50mL polypropylene centrifuge tubes. Between each successive extraction, separation was performed by centrifuging at 10,000 rpm for 30 min, and the supernatant was removed with a pipet and filtered through a 0.2 μm membrane filter. The residue was washed with 8mL of deionized water by vigorous hand shaking, and after centrifugation for 30 min, this second supernatant was

discarded. Metal concentrations in each fraction were determined by ICP-AES. All extractions were conducted in triplicate.

3.2.10 Energy Dispersive X-ray Spectroscopy of Retrieved Iron Filings

Energy dispersive X-ray spectroscopy (EDS), measured by SEM JEOL JSM 6400, was also employed to further examine both unused and used iron filings following magnetic retrieval from 800 mg/kg Cu, Zn and Cd-spiked sandy soils. Since three magnetic treatments were required for 800 mg/kg Zn- and Cd- spiked soils, the iron filings retrieved from each single treatment were combined and mixed very well for spectroscopy analysis.

3.2.11 Regeneration of Iron Filings

Nitric acid (1 N) was used to treat the magnetically retrieved iron filings. First of all, the effect of the contact time between HNO_3 and iron filings on the recovery of iron filings was studied. Two point five gram of unused iron filings was treated with 10 ml 1 N HNO_3 for 24 hrs, 2 hrs, and 1hr, and then washed thoroughly with distilled water and dried at 70°C overnight and then weighed to determine metal recovery. Each sample was run in triplicate.

Once the optimal regeneration time (i.e., 1 hour) was determined, the effectiveness of the regenerated iron filings on the adsorption of Cu, Zn, and Cd from a spiked sandy soil was tested. Fifty gram of sandy soil was spiked with 40 mL of Cu, Zn, or Cd solution to reach a final metal concentration of 400 mg/kg. The soil slurry was shaken at room temperature for 1 hour followed by a magnetic treatment using 5% (w/w, 2.5 g) of fresh iron filings according to the procedure described in Section 3.2.5. After treatment, the retrieved iron filings were regenerated in 1 N HNO_3 for 1 hour and retreated in 1 M NaOH for 72 hours, and then washed thoroughly by distilled water and dried at 70°C overnight. The effectiveness of the iron filings after the first regeneration were tested by treating the same spiked sandy soil, and the retrieved iron filings from this treatment were regenerated (i.e., second regeneration) and tested again. The toxicity of

the soil extracts after treatment was determined by both MetPLATE™ and the 48-h acute *Ceriodaphia dubia* assay, and a comparison of the toxicity of the soil extracts treated by fresh iron filings and regenerated iron filings was made. All experiments were conducted in triplicate.

3.3 Results and Discussion

3.3.1 Recovery of Iron Filings from Soils

The iron filings introduced in soils to trap metals must be retrieved efficiently to achieve a good removal of metals. Thus, iron filings were added at different concentrations to a sandy soil, red sandy soil, organic rich soil, and Georgia clay rich soil, and their recovery from the soil matrix was determined. The recovery of iron filings from these four soils by magnetic separation under three different conditions (dry soil, soil at field capacity, and saturated soil) is shown in Figure 3-1. With regard to the sandy soil, under all of the three conditions, the iron filings recovery was quite high and varied from 92.6% to 98.6%, and both iron filing concentrations and degree of soil water saturation did not significantly influence the recovery. In the case of the other three soils, the recovery of iron filings under air-dried and water saturated conditions was very high, varied from 93.4% to 99.2%, and was not significantly affected by neither soil type nor iron filing concentrations. However, magnetic separation from red sandy soil, organic rich soil, and Georgia clay rich soil at field capacity showed somewhat lower iron recoveries (from 62.0% to 86.1%) than under the other two conditions (air-dried and water saturated soil). Therefore, all following experiments were carried out under water-saturated conditions.

3.3.2 Effect of Iron Filings Concentration and Contact Time on Metal Removal from Soils

Heavy metal removal was investigated using increasing iron filing concentrations and different contact time periods between iron filings and a sandy soil spiked with a mixture of metals (Cu^{2+}, Cd^{2+}, and Zn^{2+}). The metal removal was assessed, using the MetPLATE™ toxicity test. The effect of iron filings concentration is shown in Table 3-2. The addition of iron filings at

a final concentration of 2.5% (w/w) resulted in an increase of the EC_{50} of the soil extract from 3% before treatment to 6.8% after treatment, thus showing a decrease in toxicity of the soil extract (i.e., toxicity decreases as EC_{50} increases). In the presence of 5% (w/w) and 10% (w/w) iron filings, the EC_{50}s of the soil extracts were 25.5% and 37.5%, respectively, showing a substantial drop in soil toxicity. The EC_{50}s were converted to toxicity units (TU, see Equation 3-1) to calculate the percent removal of heavy metal toxicity from a sandy soil following the magnetic iron filings (MIF) treatment. At 2.5% iron filing concentration, the toxicity removal was 55.5%, as compared to 87.9% and 92.0% at iron concentrations of 5% and 10%, respectively. In subsequent experiments we used 5% iron filings as a best cost-effective treatment concentration. Equation 3-2 shows the calculation of the % toxicity removal.

$$\% \text{ Toxicity removal (TR)} = \frac{TU_{\text{before treatment}} - TU_{\text{after treatment}}}{TU_{\text{before treatment}}} \times 100\% \qquad (3\text{-}2)$$

Regarding the contact time between iron filings and the soil matrix, Table 3-2 shows that, after 1.5-hr contact time, the EC_{50} of the soil extract increased from 3.8% to 8.4% whereas after 3- and 6-hr contact time, the EC_{50}s were 44% and 36.4%, respectively, suggesting a more substantial decrease in soil toxicity. Following conversion of EC_{50}s to TUs, the percent toxicity removal was 54.8% after 1.5-hr contact time, as compared to 92.2% and 92.1% after 3-hr and 6-hr contact times, respectively. A 3-hr contact time was chosen in subsequent experiments.

3.3.3 Evaluation of Magnetic Separation of Cu, Zn and Cd from Spiked Soils, Using MetPLATE™

The magnetic removal of individual metals (Cu^{2+}, Zn^{2+}, Cd^{2+}) from spiked sandy soil, red sandy soil, organic rich soil, and Georgia clay rich soil were investigated. Tables 3-3, 3-4, 3-5, and 3-6 show that the toxicity removal efficiency from the spiked soils varied with the type of metal and soil. For the three metals under study, we tested the toxicity of the soil extracts by

determining the EC_{50}s which were then converted into toxicity units (TUs). The percent toxicity removal was calculated according to Equation 3-2.

Table 3-3 shows that the TUs in the sandy soil extracts varied from 21.9 TUs at 200 mg Cu/kg soil to 1661 TUs at 800 mg Cu^{2+}/kg. The percent toxicity removal from the sandy soil was generally higher than 95%. With regard to the Zn-spiked sandy soil, 96.1% of the Zn toxicity was removed at the input concentration of 200 mg Zn^{2+}/kg soil (Table 3-3). However, at 400 mg/kg and 800 mg Zn^{2+}/kg soil, only 70.0% and 49.6% of the toxicity was removed by magnetic treatment. Therefore, additional treatments were necessary to achieve toxicity removal higher than 90%. As shown in Table 3-3, at 400 mg Zn^{2+}/kg soil, 98.7% toxicity removal was achieved after two successive treatments whereas at 800 mg Zn^{2+}/kg soil, 90.1% removal was obtained after three successive treatments. In the case of Cd-spiked sandy soil, a single magnetic treatment removed 51.1% of the Cd toxicity when the spiked Cd concentration was 200 mg/kg soil. Cd removal increased to 92.6% following a second magnetic treatment (Table 3-3). At 400 mg Cd/kg soil the removal increased from 27.5% after one treatment to 91.9% after a second treatment. At 800 mg Cd/kg soil, metal removal reached 90.4% only after 3 magnetic treatments (Table 3-3).

With regard to the Cu-spiked red sandy soil and Georgia clay rich soil, the percent toxicity removal was generally higher than 97% at all of the three Cu concentrations (200 mg/kg, 400 mg/kg, and 800 mg/kg) (Tables 3-4 and 3-6). In the case of Zn-spiked red sandy soil and Georgia clay rich soil, 97.6% and 97.4% of the toxicity was removed respectively from the soils when the spiked Zn concentration was 200 mg/kg (Tables 3-4 and 3-6). However, as the spiked Zn concentration increased to 400 mg/kg and 800 mg/kg, additional treatments were required to reach a higher toxicity removal. Table 3-4 shows that at 400 mg Zn^{2+}/kg red sandy soil, the

toxicity removal increased from 68.5% to 98.5% after a second magnetic treatment, whereas at 800 mg Zn^{2+}/kg red sandy soil, 87.5% removal was achieved after three successive treatments. Similar results were obtained toward Zn-spiked Georgia clay rich soil (Table 3-6), when Zn concentration was 400 mg/kg soil, 91.8% toxicity removal was achieved after two treatments; at 800 mg Zn^{2+}/kg soil, 90.3% removal was obtained after three successive treatments.

After a single treatment of the Cu-spiked organic rich soil, the TUs of the soil extracts varied from 1.1 to 2.4 at Cu input concentrations from 200 mg/kg to 800 mg/kg, which result in a toxicity removal from 55.4% to 90.1% (Table 3-5). As discussed in Chapter 2, organic rich soil has a higher metal binding capacity than sandy soils; therefore, compared to sandy soils, the spiked organic rich soil showed lower TUs before magnetic treatment, which could lead to a lower toxicity removal. Besides, as shown in Table 3-5, at 200 mg Zn^{2+}/kg soil, the soil extract was not toxic after one treatment, at 400mg Zn^{2+}/kg soil, 87.85 of the toxicity was removed after two successive treatments whereas at 800mg Zn^{2+}/kg soil, 85.2% removal was obtained after three successive treatments.

Cd-spiked soils showed higher toxicity than Zn- and Cu-spiked soils (Tables 3-3 through 3-6). In the case of Cd-spiked red sandy soil, organic rich soil, and Georgia clay rich soil, even at 200 mg Cd^{2+} /kg soil, two successive treatments were required to obtain a toxicity removal higher than 80.1%, and three magnetic treatments were necessary for 400 mg/kg and 800 mg/kg Cd-spiked soils to achieve a toxicity removal between 80.1% and 95.4% (Tables 3-4, 3-5, and 3-6).

In all, comparing the three heavy metals used, at equal concentration, Cd resulted in the highest toxicity (or highest TU values) in the soil extracts than Zn and Cu either before or after treatment, while Cu produced the lowest toxicity. Moreover, for each metal at the same

concentration, the toxicity they produced in different soils increased as follows: sandy soils> Georgia clay rich soil > organic rich soil, which is associated with the soil heavy metal binding capacity (SHMBC; see Chapter 2). Moreover, the magnetic treatment method worked best on Cu-spiked soils, followed by the Zn-spiked soils and the Cd-spiked soils. In a study of adsorption of divalent metals on iron (III) oxide, Tamura et al. (1994) found similar results and reported that the affinity for metals increased in the following order: $Zn^{2+}<Cu^{2+}<Pb^{2+}$. In another study, heavy metal adsorption by hydrous iron was as follows: $Cd^{2+} < Zn^{2+} < Pb^{2+}$ (Rao and Laitinen, 1974). Although Pb^{2+} was not addressed in this study, our work shows that the affinity of heavy metals for iron filings followed the order: $Cd^{2+} < Zn^{2+} < Cu^{2+}$. In addition, considering the effect of soil characteristics, the effectiveness of this magnetic treatment was not significantly affected by the type of soil in Cu-spiked soils. However, with regard to the Zn- and Cd-spiked soils, this treatment worked better in sandy soils than in organic rich soil and Georgia clay rich soil.

In our study, although more than one treatment were sometimes required for Zn and Cd removal from spiked soils when the metal concentrations exceeded a certain level, the toxicity reductions were very high after the final treatment (varied from 80.1% to 98.7%), and the whole experiment could still be finished within 12 hours. Our proposed magnetic separation method is relatively much faster than phytoremediation which is defined as the use of plants to remove pollutants from soils (Cunningham et al., 1995; Raskin et al., 1997). In addition, other limitations of phytoremediation have been addressed by researchers, such as selectively (some plants can only take up a given metal but not others which may not be appropriate for a mixture of metals), depth of remediation, (Abramovitch et al., 2003; Mulligan et al., 2001a), and safety concerns following the disposal of contaminated crop materials (Sas-Nowosielska et al., 2004). Moreover, soil texture, pH, salinity, and metal concentrations must be within the limits of plant tolerance,

and potential problems may be encountered when highly water soluble contaminants leach out from the root zone (Cunningham et al., 1995). However, the magnetic treatment method proposed in our study can potentially be applied *ex situ* even on a soil contaminated with mixed metals.

3.3.4 Evaluation of Magnetic Separation of Cu, Zn and Cd from Spiked Soils, Using *Ceriodaphnia dubia* Acute Toxicity Test

Metal toxicity removal from soils following magnetic treatment was also evaluated with the 48-hr *Ceriodaphnia dubia* acute toxicity test. The soils spiked with the highest metal concentration (800 mg/kg soil) used in the previous experiments were tested in this section. The results are shown in Table 3-7. The 48-h *C. dubia* test showed higher TU values than the MetPLATE^{TM} assay, due to the higher sensitivity of the daphnid test. For Cu, the toxicity removal from the four soils varied from 90.1% to 99.9% by MetPLATE^{TM}, and from 81.4% to 99.9% by *C. dubia* test. For Zn, after three treatments, the toxicity removal from the soils ranged from 85.2% to 90.3% by MetPLATE^{TM}, and from 82.7% to 89.2% by *C. dubia* test. As regards Cd-spiked soils, the toxicity removal percentages after three treatments varied from 80.1% to 95.4% and from 84.8% to 96.2% as shown by MetPLATE^{TM} and *C. dubia*, respectively. The two tests showed, however, a similar trend as regards toxicity removal of Cu, Zn or Cd from soils.

3.3.5 Evaluation of Magnetic Separation of Cu, Zn and Cd from Spiked Sandy Soils, Using *Selenastrum capricornutum* Chronic Toxicity Test

The soil extracts tested by the 48-h *C. dubia* assay were also evaluated with the 96-h *Selenastrum capricornutum* test. As shown in Table 3-8, the 96-h algae test showed the highest TU values among the three toxicity tests. In other words, the 96-h algae test displayed the highest sensitivity toward the three metals used. The three tests, however, showed a similar trend as regards toxicity removal of Cu, Zn or Cd from the soils. For Cu, the toxicity removal from the

four soils varied from 90.0% to 99.9% as shown by the algae test. As regards Zn- and Cd- spiked soils, after three treatments, the toxicity removal percentages varied from 90.2% to 98.7% and from 94.9% to 96.0%, respectively. Comparing with the results discussed in Section 3.3.4, the metal toxicity removal shown by algae test was slightly higher than that indicated by MetPLATE^{TM} and *C. dubia*.

3.3.6 Assessment of Metal Removal Efficiency Using Chemical Analysis

We have used toxicity tests to assess soil toxicity following the proposed magnetic treatment. We then used chemical analysis to study Cu, Zn, and Cd distributions in the soil matrix and extracts and to demonstrate that the metals were indeed adsorbed and concentrated on the iron filings. As shown in Tables 3-9, 3-10, and 3-11, for each heavy metal, in the same soil, metal removal from the soil extract was always higher than that from the soil matrix.

Cu removal. The sandy soil was spiked with copper to reach final concentrations of 200 mg/kg, 400 mg/kg and 800 mg/kg. Cu removal from the soil matrix ranged from 48.7% at 200 mg Cu/kg and 80.7% at 800 mg Cu/kg. Cu removal from the soil extracts ranged from 96.9% at 200 mg Cu/kg and 99.5% at 800 mg Cu/kg. Table 3-9 also shows that Cu was adsorbed to the iron filings and reached Cu concentrations ranging from 2022 mg/kg filings at soil concentration of 200 mg Cu/kg and 12,186 mg/kg at soil concentration of 800 mg Cu/kg. Chemical analysis for the red sandy soil, organic rich soil and Georgia clay rich soil spiked with 800mg Cu/kg soil was also performed. As shown in Table 3-9, the retrieved iron filings following magnetic treatment reached Cu concentrations of 10,140 mg/kg, 6,262 mg/kg, and 11,400 mg/kg for red sandy soil, organic rich soil, and Georgia clay rich soil, respectively. The metal removal percentages from soil extracts were 98.6% for red sandy soil and 99.9% for Georgia clay rich soil, whereas the metal removal percentages from soil matrices were lower, which were 53.9% for red sandy soil and 85.2% for Georgia clay rich soil. In the case of 800 mg/kg Cu spiked

organic rich soil, the metal removal percentages from both soil extract (83.9%) and soil matrix (49.1%) were lower than those from sandy soils and Georgia clay rich soil.

Zn and Cd removal. The soils (sandy, red sandy, organic rich, and Georgia clay rich) spiked with the highest Zn and Cd concentration (800 mg/kg soil) were chemically analyzed. Since three successive treatments were required for these spiked soils, the chemical analysis was performed on the soil matrices and soil extracts after the final treatment, and a mixture of the iron filings retrieved form each treatment.

As regards Zn removal (Table 3-10), no significant difference was observed among the four types of soil extracts (sandy soil extract, 83.4%; red sandy soil extract, 72.0%; organic rich soil extract, 78.8%; Georgia clay rich soil extract, 71.1%). However, the removal of Zn from the soil matrices showed somewhat larger difference, varied from 4% in Georgia clay rich soil to 67.3% in sandy soil, with red sandy soil (14.6%) and organic rich soil (18.5%) in between. The concentrations of Zn adsorbed to the retrieved iron filings, varied from 559.2 mg/kg (from Georgia clay rich soil) to 3,371.9 mg/kg (from sandy soil), also demonstrated that the metal was indeed adsorbed and concentrated on the iron filings (Table 3-10).

In the case of Cd removal, Table 3-11 indicates that the percentage removal from the soil extracts, ranged from 49.9% to 60.6%, was not significantly affected by the type of soil. Whereas the removal of Cd from the soil matrices followed this order: organic rich soil (5.2%) ≈ red sandy soil (5.7%) < Georgia clay rich soil (19.7%) < sandy soil (49.5%). The retrieved iron filings reached Cd concentrations form 117.6 mg/kg (from organic rich soil) to 2,386.9 mg/kg (from sandy soil).

In all, comparing the three heavy metals (Cu, Zn, and Cd) studied, the metal removal percentages from both the soil extract and soil matrix followed this order in sandy soil, red sandy

soil, and organic rich soil: Cu > Zn > Cd. However, in the Georgia clay rich soil, the same trend was found for metal removal from soil extracts, while a different trend (Cu> Cd> Zn) was shown for metal removal from soil matrix.

The chemical analysis discussed above suggested that Cu, Zn, and Cd were removed from both the soil extracts and the soil matrix. As regards chemical immobilization, it is a remediation technique that uses chemical amendments to decrease the concentration of dissolved contaminants by sorption or precipitation (Basta and McGowen, 2004). Numerous studies have been carried out to investigate the effectiveness of lime and phosphate-based materials on immobilizing heavy metals in soils (Eighmy et al., 1997; Hettiarachchi et al., 2000; Illera et al., 2004; Maenpaa et al., 2002; McGowen et al., 2001; Raicevic et al., 2005; Yoon et al., 2007). Ma et al. (1995) reported that phosphate rocks reduced water-soluble Pb from a contaminated soil by 56.8-100%. Chen et al. (1997) used mineral apatite to stabilize a Pb, Cd, and Zn contaminated soil, and they found that the removal of Cd and Zn by the apatite was pH dependent, whereas removal of Pb was not. The removals were 0.729 mmol of Pb, 0.489-1.317 mmol of Cd, and 0.596-2.187 mmol of Zn/g of apatite. Kumpiene et al. (2007) also evaluated the effectiveness of coal fly ash and natural organic matter (peat) in reducing soil Cu and Pb mobility. Their results indicated that the amount of leached Cu and Pb decreased by 74.5% and 61.0% after the addition of 5% organic matter, and by 91.1% and 87.1% after the addition of 5% coal fly ash. In another study carried out by Shanableh and Kharabsheh (1996), they studied the stabilization of Cd, Ni, and Pb in a contaminated soil using a natural zeolite. At 500mg metal/kg soil, Pb leaching was reduced by more than 97% using a minimum of 25% zeolite. Using up to 50% zeolite, Ni and Cd leaching was reduced by a maximum of 50% and 60%, respectively. However, no matter what amendment is used, the aim of chemical immobilization is to reduce the metal solubility,

mobility and toxicity, but the metal itself is still retained in the soil which may require further monitoring. Compared with the immobilization approach, our magnetic separation method results in both immobization of metals as well as their physical removal from the soil matrix.

3.3.7 Mass Balance of Metals in Soils

Tables 3-12, 3-13, and 3-14 display the mass balance of Cu, Zn, and Cd in the spiked soils (sandy, red sandy, organic rich, Georgia clay rich) before and after magnetic treatment. As shown in these tables, a large portion of the metals was immobilized in the soil matrix before treatment, but the metals associated with the soil matrix and in the soil extracts were both reduced following magnetic treatment. The total recovery of metals was not significantly affected by the soil type. Before treatment (i.e., no iron filings added), the total recovery of added Cu (81.3%-91.3%), Zn (83.2%- 87.4%) and Cd (80.8%-91.3%) in the four soils were very close. After magnetic treatment, 82.2% to 95% of Cu, 80.8% to 88.3% of Zn, and 79.7% to 88.4% of Cd was recovered from the soils under study.

3.3.8 Sequential Extraction of Metals in Soils

A sequential extraction procedure was performed to show the distribution of Cu, Zn, and Cd in a sandy soil and an organic rich soil before and after magnetic treatment. The sandy soil and organic rich soil were spiked with Cu, Zn, or Cd to reach a final metal concentration of 800 mg/kg. The results are shown in Figures 3-2, 3-3 and Table 3-15.

Metal fractionations in sandy soil. Many factors, such as soil organic matter, cation exchange capacity, soil pH, and metal properties, can affect the distribution of heavy metals (Buanuam et al, 2005; Ma and Rao, 1997). The most important sinks for metals are the Fe-Mn oxides, organic matter, sulfides, and carbonates (Adriano, 2001c). Figure 3-2 displays the distribution of Cu, Zn, and Cd in treated and non-treated sandy soil. Before treatment, a large portion of Cu was bound to the carbonate phase (52.6%), and much lower proportions of Cu

were extracted from the exchangeable (6.7%), Fe-Mn oxide bound (19%), organic bound (4.6%), and residual phases (17.1%). The percentage distribution of Zn in non-treated sandy soil was different from Cu, as 35.6% and 30.9% of Zn were associated with the carbonate and Fe-Mn oxide phase, respectively. Other researchers have also found Zn to be associated with carbonates and Fe-Mn oxides (Chao et al., 2006; Ma and Rao, 1997; Ramos et al., 1994; Šurija and Branica, 1995). The exchangeable and residual Zn was 18.8% and 14.1%, respectively, whereas insignificant amount of Zn (0.6%) was bound to organic matter due to the very low organic content of the sandy soil. The distribution of Cd in non-residual fractions followed this order: exchangeable (54.9%) > carbonate-bound (20.6%) > Fe/Mn oxide-bound (2.5%) > organic-bound (1.2%). Maiz et al., (2000) showed a similar Cd distribution in a polluted soil, which is exchangeable + carbonate phase (51%) > Fe-Mn oxide phase (29%) > organic phase (5%). As shown in Table 3-1, under a soil pH of 5.7, metals extracted by sodium acetate probably came from the soil fractions that are similar to carbonates. After magnetic treatment, as shown in Table 3-15, metal removal from the spiked sandy soil was the highest in the exchangeable phase (79.9% for Cu, 79.2% for Zn, and 65.8% for Cd), followed by the carbonate phase (46% for Cu, 44.0% for Zn, and 36.1% for Cd). However, Cu was not removed from the Fe-Mn oxide and the organic fractions. The percentage removal of Zn from the Fe-Mn oxide and organic factions were much lower, which were 24.4% and 8.1%, respectively. 29.2% of Cd was removed from the organic bound phase, whereas no Cd removal was observed in the Fe-Mn oxide fraction.

Metal fractionations in organic soil. The distribution of Cu, Zn, and Cd factions in spiked organic rich soil was different from that in sandy soil (Figure 3-3). Before magnetic treatment, the largest portion of Cu was bound to the organic matter (49.6%), followed by carbonate-bound (20.7%) and Fe-Mn oxide bound (20.3%). Much lower amount of Cu was

retained in the exchangeable phase (1.9%) and residual phase (7.5%). The major association of Cu with the organic fraction is probably due to the high sorption capacity of organic matter for Cu and the stability of organic-Cu complexes (Adriano, 2001c). Some other fractionation studies have also shown that appreciable amounts of Cu are bound to organic matter (Kishk et al., 1973; Kuo et al., 1983; Ma and Rao, 1997). As regards Zn-spiked organic rich soil, only 2.1% of Zn was bound to the organic matter, which is the lowest fraction among the five phases. Much higher proportions of Zn were distributed in the carbonate phase (33.7%), Fe-Mn oxide phase (34.4%), and residual phase (24.6%), and a small amount of Zn was found in the exchangeable fraction (5.2%). Figure 3-3 also illustrates the distribution of Cd in the spiked organic rich soil, which followed the order: Carbonate (44.3%) > Exchangeable (26.9%) > Fe-Mn oxide (21.8%) > residual (5.5%) > organic (1.5%). After magnetic treatment, unlike in sandy soil, the removal of Cu, Zn, and Cd from the organic rich soil was observed in all fractions as shown in Table 3-15. The greatest removal was from the organic-bound phase, which was 45.1% for Cu, 48.3% for Zn, and 53.3% for Cd. The second highest removal was from the exchangeable phase for Cu (32.4%) and the Fe-Mn oxide phase for Zn (34.8%) and Cd (45.5%). Cu removal from the carbonate and Fe-Mn oxide factions were somewhat lower, which were 22.3% and 29.5%, respectively. The removal of Zn and Cd from the exchangeable and carbonate phases was very close, varied from 16.1% to 21.5%.

3.3.9 Energy Dispersive X-ray Spectroscopy of Retrieved Iron Filings

Energy dispersive X-ray analysis is a nondestructive method for the microanalysis of element composition (Heckmann et al., 2007). Numerous researchers have employed this technique to detect, measure, and determine the location of chemical elements within samples (Choel et al., 2005; Heckmann et al., 2007; Helsen and Van den Bulck, 1998; Lewis et al., 2000; Otulakowska and Nicholson, 2006; Petry et al., 2006). Al-Asheh and Duvnjak (1999) examined

the mechanisms of metal biosorption by moss from solutions using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopies (EDS), and they found that metal ions were sorbed mainly at the cell wall of the moss and only a small amount of ions diffused into the cytoplasm. Liu et al. (2007) also used SEM and EDS to investigate the localization of Cd in the root tissue of *Allium cepa* L. In our study, the comparison of EDS between unused and used iron filings retrieved from Cu, Zn, and Cd-spiked sandy soils confirmed that Cu, Zn, and Cd were indeed adsorbed onto the iron filings (Figure 3-4). The aluminum detected on the retrieved iron filings probably came from the sandy soil matrix. The silica peaks shown in the EDS of both unused and used iron filings can be considered as impurity in the iron filings.

3.3.10 Regeneration of Iron Filings

Yeager (1998) had treated the used iron filings with concentrated HNO_3 for 10 minutes with shaking and then washed to remove heavy metals from the surface; however, this regeneration method was ineffective as displayed by MetPLATE^{TM} toxicity test results. Therefore, we investigated longer contact times (i.e. 1-hr, 2-hr, and 24-hr) between HNO_3 and iron filings. Table 3-16 shows the recovery of iron filings from 1N HNO_3 after three different regeneration periods. The 24-hr contact time recovered only 43.2% of iron filings. Thus, shorter regeneration periods (2-hr and 1-hr) were used, and the recovery of iron filings after two successive regenerations was studied for both of the two regeneration times. As regards 2-hr contact time, the recovery of iron filings reached 73.9% after the first regeneration; however, much lower recovery (48.4%) was obtained following the second regeneration. In the case of 1-hr regeneration, 86.2% and 85.3% of iron filings was recovered respectively following the first and second regeneration. Therefore, the 1-hr regeneration period was used for later experiments.

To compare the effectiveness of magnetic treatments using fresh and regenerated iron filings for the removal of Cu, Zn, and Cd from a spiked sandy soil, two toxicity tests,

MetPLATE™ (Table 3-17) and the 48-h *C. dubia* (Table 3-18) were used to assess the toxicity of the soil extracts after treatment. The sandy soil was spiked with metal solutions to reach a Cu, Zn or Cd concentration of 400 mg/kg soil. As shown in Table 3-17, for Cu-spiked sandy soil, the TUs of treated soil extracts were 1.1 TUs, 1.1TUs, and less than 1 TU by using fresh iron, iron regenerated once, and iron regenerated twice, respectively. For Zn-spiked sandy soil, 45.9 TUs, 25.8 TUs, and 18.5 TUs were produced respectively by the soil extracts treated with fresh iron, iron regenerated once, and iron regenerated twice. With regard to Cd-spiked sandy soil, the TUs of fresh iron treated soil extract was 251.5 TUs, which were reduced to 161.2 TUs and 124.7 TUs by using iron filings regenerated once and twice, respectively.

The results obtained by using the 48-h *C. dubia* assay were quite similar (Table 3-18). After being treated with fresh iron filings, Cu-, Zn-, and Cd- spiked soils extracts generated 24.8 TUs, 590.6 TUs, and 719.2 TUs, respectively. However, by using iron filings regenerated once and twice, the TUs were respectively reduced to 15.5 TUs and 6.4 TUs for Cu-spiked soil extracts, 174.6 TUs and 107.8 TUs for Zn-spiked soil extracts, and 389.2 TUs and 291.3 TUs for Cd-spiked soil extracts. Therefore, the adsorption capacity of regenerated iron filings was completely restored and even slightly higher than that of the fresh iron filings.

Other regeneration methods for iron (hydr)oxides have also been evaluated by researchers. Peng et al. (2006) used montmorillonite-Cu(II)/Fe(III)oxides magnetic material as an adsorbent to successfully remove humic acid from solutions and they also found that the magnetic material can be thermally regenerated at 300°C for 3 hrs. The regenerated adsorbent was still magnetic and had as good adsorption capacity as the unused material. In another study performed by Kornmuller et al. (2002), granulated iron hydroxide was used as a sorbent to remove reactive dye in textile wastewater. They reported that the spent iron hydroxide could be regenerated by

hydrogen peroxide at room temperature, and a regeneration time of 3.5-hr was necessary for decolorization and 6-hr was required to restore the adsorption capacity completely.

3.4 Conclusions

The feasibility of decontamination of heavy metal-contaminated soils by magnetic separation was demonstrated in laboratory experiments with a sandy soil, red sandy soil, organic rich soil, and Georgia clay rich soil artificially spiked with Cu, Cd, and Zn. The results of this study showed a significant reduction of toxicity generated by Cu, Cd or Zn in soil extracts after one to three magnetic treatments. As regards toxicity reduction in soil extracts, this magnetic treatment method worked best on Cu-spiked soils, followed by Zn-spiked soils and Cd-spiked soils. The speciation of Cu, Zn, and Cd determined by sequential extractions was found to depend on the soil characteristics as well as the metal type. The comparison of metal fractionations in spiked soils before and after treatment suggested that, in the spiked sandy soil, the removal of Cu, Zn, and Cd was the greatest from the exchangeable (65.8%-79.7%) fraction, followed by the carbonate fraction (36.1%-46.0%). Cu was not removed from both the Fe-Mn oxide and organic fractions, and Cd was not removed from the organic phase. In the spiked organic rich soil, the removal of Cu, Zn, and Cd was found in all fractions, in which the greatest removal was from the organic-bound phase (45.1%-53.3%), and the second highest removal was from the exchangeable phase for Cu (32.4%) and the Fe-Mn oxide phase for Zn (34.8%) and Cd (45.5%). In addition, the energy dispersive X-ray spectroscopy (EDS) of magnetically retrieved iron filings confirmed the adsorption of Cu, Zn, and Cd on iron filings.

Chemical analysis by ICP-AES suggested that all the three metals were removed from both the soil matrix and the soil extracts. However, metal removal from the soil matrix was lower than removal from the soil extracts.

Finally, the regeneration of used iron filings was also investigated, and the results indicated that the adsorption capacity of the iron filings for Cu, Zn, and Cd was completely restored by regenerating in 1N HNO_3 for 1 hr and then in 1M NaOH for 72 hrs.

In all, we conclude that this magnetic treatment method shows great potential as a rapid *ex situ* remediation technology for heavy metal-contaminated soils, and the retrieved iron filings could be regenerated and reused.

Table 3-1. Soils characteristics

Characteristic	Red sandy soil	Sandy soil	Organic soil	Georgia clay soil
pH	6.2	5.7	5.7	5.7
Eh (mV)	485.0	422.0	403.0	487.5
% Organic carbon	0.1	0.5	6.4	0.06
% Organic matter	0.3	1.6	18.8	0.1
% Sand	90.7	96.92	93.2	41.0
% Silt	3.2	0.02	2.0	43.8
% Clay	6.1	3.06	4.8	15.2
CEC ($cmol_c/kg$)	14.4	14.1	230.1	27.6

Table 3-2. Effect of iron filings concentration and contact time between iron filings and soil matrix on the removal of heavy metals from a spiked sandy soil, as determined by the MetPLATE™ toxicity test

Spiked heavy metal mixture[a] (mg/kg)	Study factors		EC_{50}[c] of soil extract (% soil extract)		Toxicity units[d] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Cu^{2+}+Cd^{2+}+Zn^{2+} (40mg/kg for each)	Iron filings conc.[b] (%)	2.5	3.0±0.2%	6.8±0.7%*	33.5±2.1	14.9±1.5*	55.5±4.6%*
		5	3.0±0.2%	25.5±5.0%**	33.5±2.1	4.0±0.8**	87.9±2.4%**
		10	3.0±0.2%	37.4±3.8%***	33.5±2.1	2.7±0.3***	92.0±0.8%***
	Iron filings[e] and soil contact time (h)	1.5	3.8±0.2%	8.4±1.4%*	26.9±1.1	12.2±1.9*	54.8±7.2%*
		3	3.5±0.6%	44.0±1.2%**	29.1±4.9	2.3±0.1**	92.2±0.2%**
		6	2.9±0.3%	36.4±4.7%***	35.0±3.9	2.8±0.4**	92.1±1.0%**

[a] 50 g of sandy soil was spiked with 40 mL of combined heavy metal solution containing 50 mg/L Cu^{2+}, 50 mg/L Cd^{2+}, and 50 mg/L Zn^{2+}; [b] Iron filings and soil contact time was 6 hours; [c] Mean of 3 replicates ± 1 standard deviation; [d] Toxicity units were expressed as $100/EC_{50}$; [e] 5% (2.5g) iron filings were added to 50 g of spiked sandy soil; [f] Means followed by the same number of asterisks within the same column are not significantly different at the 5% level.

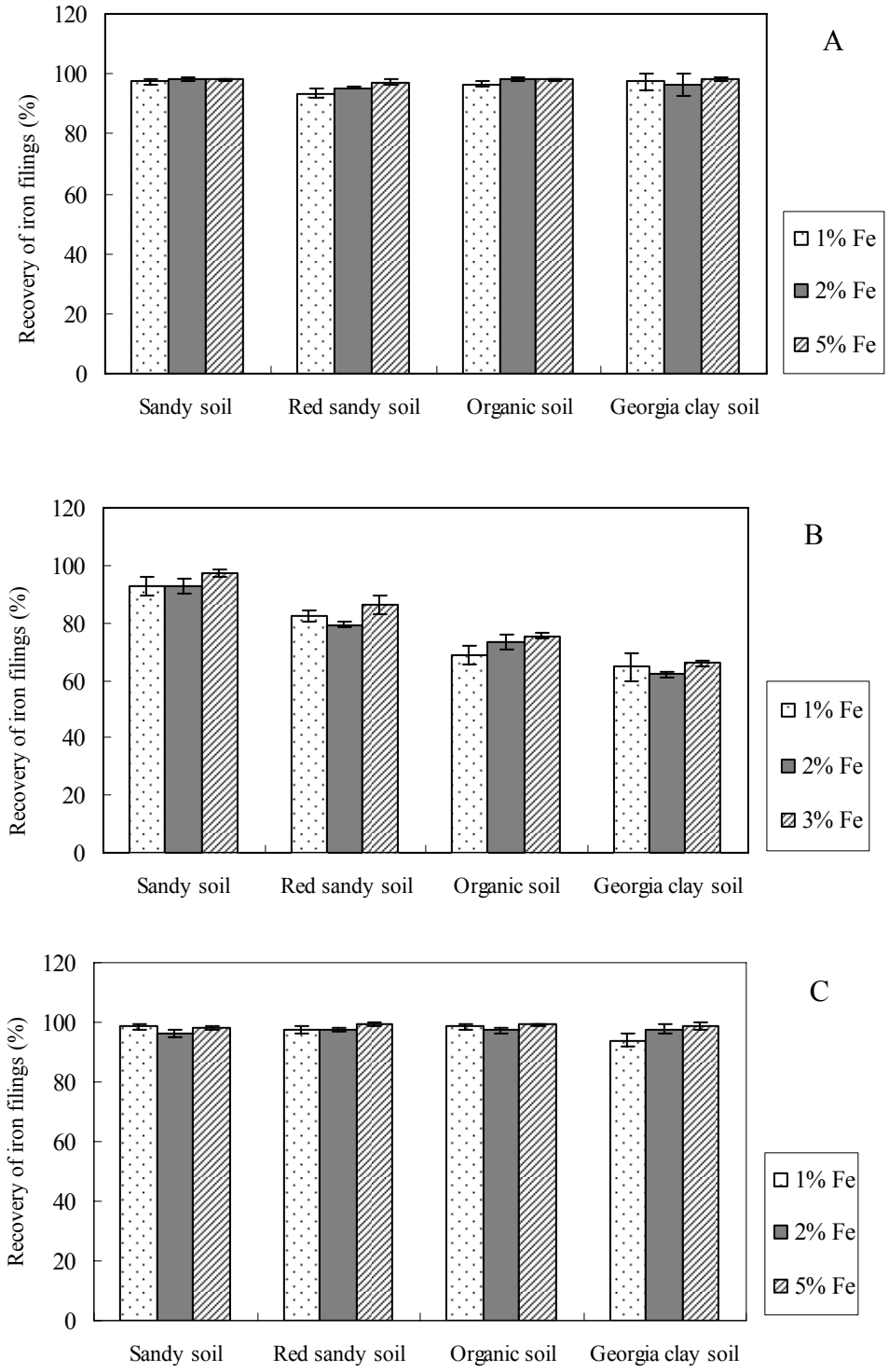


Figure 3-1. Recovery of iron filings from soils under different condition. A) Air-dried condition. B) Field capacity condition. C) Water saturated condition. (Field capacities of sandy, red sandy, organic rich, and Georgia clay rich soil were 0.2 mL/g, 0.4 mL/g, 0.5 mL/g, and 0.7 mL/g, respectively. Error bars represent standard deviation of three replicates).

Table 3-3. Copper, zinc and cadmium toxicity removal from a sandy soil by magnetic treatment, as determined by MetPLATE™.

Heavy metal type	Heavy metal concentration in spiked soil (mg/kg)	Number of treatments[a]	EC_{50}[b] of soil extract (% soil extract)		Toxicity units[c] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Cu[d]	200	1	4.60 ± 0.6%	>100%	21.9 ± 2.8	<1[g]	>95.4%
	400	1	0.20 ± 0.03%	96.2 ± 4.0%	481.0 ± 57.2	1.04 ± 0.04	99.8 ± 0.03%
	800	1	0.06 ± 0.0%	51.3 ± 4.9%	1661.2 ± 96.1	2.0 ± 0.2	99.9 ± 0.0%
Zn[e]	200	1	3.20 ± 0.2%	86.8 ± 7.6%	30.9 ± 1.8	1.2 ± 0.2	96.1 ± 1.0%
	400	1	0.60 ± 0.03%	2.10 ± 0.3%	164.2 ± 8.1	49.3 ± 7.3	70.0 ± 4.2%
		2	0.50 ± 0.06%	40.7 ± 5.6%	200.6 ± 23.6	2.5 ± 0.3	98.7 ± 0.3%
	800	1	0.25 ± 0.1%	0.50 ± 0.01%	411.3 ± 94.3	198.7 ± 2.3	49.6 ± 13.5%
		2	0.26 ± 0.02%	0.53 ± 0.05%	386.1 ± 29.8	188.6 ± 17.6	51.2 ± 1.3%
		3	0.20 ± 0.03%	2.10 ± 0.2%	499.7 ± 79.4	48.1 ± 4.7	90.1 ± 2.3%
Cd[f]	200	1	1.50 ± 0.1%	3.40 ± 1.0%	66.5 ± 4.1	31.6 ± 8.4	51.9 ± 14.8%
		2	0.20 ± 0.03%	3.30 ± 1.0%	438.9 ± 53.6	32.6 ± 10.0	92.6 ± 1.5%
	400	1	0.30 ± 0.01%	0.40 ± 0.05%	357.1 ± 11.9	250.6 ± 32.3	27.5 ± 7.8%
		2	0.09 ± 0.01%	1.10 ± 0.1%	1121.8 ± 64.0	91.1 ± 5.6	91.9 ± 0.2%
	800	1	0.10 ± 0.00%	0.13 ± 0.01%	1013.7 ± 15.8	772.3 ± 59.6	23.8 ± 6.5%
		2	0.03 ± 0.01%	0.10 ± 0.01%	3962.4 ± 1246.8	1006.0 ± 95.0	72.5 ± 10.7%
		3	0.01 ± 0.00%	0.08 ± 0.01%	12632.3 ± 1591.4	1205.3 ± 146.6	90.4 ± 1.0%

[a] 5% (2.5g) iron filings were added to the spiked sandy soil , and the contact time was 3 hours; [b] Mean of 3 replicates ± 1 standard deviation; [c] Toxicity units = $100/EC_{50}$; [d] 50 g of sandy soil was spiked with 40 mL of Cu solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cu^{2+}respectively; [e] 50 g of sandy soil was spiked with 40 mL of Zn solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Zn^{2+} respectively; [f] 50 g of sandy soil was spiked with 40 mL of Cd solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cd^{2+}respectively; [g] Non-toxic.

Table 3-4. Copper, zinc and cadmium toxicity removal from a red sandy soil by magnetic treatment, as determined by MetPLATE™.

Heavy metal type	Heavy metal concentration in spiked soil (mg/kg)	Number of treatments[a]	EC_{50}[b] of soil extract (% soil extract)		Toxicity units[c] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Cu[d]	200	1	1.1 ± 0.1%	44.9 ± 8.4 %	94.1 ± 7.1	2.3 ± 0.5	97.6 ± 1.0%
	400	1	0.2 ± 0.03%	36.1 ± 3.4%	552.2 ± 74.5	2.8 ± 0.3	99.5 ± 0.1%
	800	1	0.05 ± 0.01%	7.3 ± 2.2%	2055.6 ± 419.6	14.5 ± 3.7	99.3 ± 0.3%
Zn[e]	200	1	0.8 ± 0.03%	34.8 ± 6.7%	125.2 ± 5.3	2.9 ± 0.5	97.6 ± 0.5%
	400	1	0.36 ± 0.06%	1.2 ± 0.1%	280.6 ± 48.4	87.8 ± 10.7	68.5 ± 1.9%
		2	0.34 ± 0.04%	23.7 ± 5.9%	299.8 ± 35.2	4.4 ± 1.3	98.5 ± 0.6%
	800	1	0.12 ± 0.02%	0.21 ± 0.02%	867.5 ± 119.1	485.7 ± 36.8	43.4 ± 8.1%
		2	0.12 ± 0.01%	0.40 ± 0.01%	812.0 ± 37.0	252.1 ± 3.7	68.9 ± 1.9%
		3	0.12 ± 0.01%	0.94 ± 0.01%	858.6 ± 43.7	106.8 ± 0.7	87.5 ± 0.7%
Cd[f]	200	1	0.25 ± 0.05%	0.77 ± 0.07%	417.9 ± 94.1	131.1 ± 12.0	67.4 ± 8.4%
		2	0.20 ± 0.02%	2.3 ± 0.4%	495.4 ± 53.2	44.7 ± 7.2	90.8 ± 2.3%
	400	1	0.09 ± 0.01%	0.17 ± 0.03%	1120.4 ± 125.3	589.3 ± 100.1	47.5 ± 6.6%
		2	0.07 ± 0.01%	0.36 ± 0.04%	1527.8 ± 240.6	279.6 ± 27.0	81.2 ± 4.8%
		3	0.01 ± 0.00%	0.20 ± 0.04%	11111.1 ± 0.00	516.4 ± 77.2	95.4 ± 0.7%
	800	1	0.04 ± 0.01%	0.09 ± 0.02%	2777.8 ± 481.1	1179.9 ± 222.4	56.5 ± 12.3%
		2	0.02 ± 0.01%	0.08 ± 0.01%	4444.4 ± 962.3	1216.9 ± 183.3	72.0 ± 5.6%
		3	0.003 ± 0.00%	0.07 ± 0.02%	30555.6 ± 4811.3	1402.1 ± 278.7	95.4 ± 0.4%

[a] 5% (2.5g) iron filings were added to the spiked red sandy soil , and the contact time was 3 hours; [b] Mean of 3 replicates ± 1 standard deviation; [c] Toxicity units = $100/EC_{50}$; [d] 50 g of red sandy soil was spiked with 40 mL of Cu solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cu^{2+}respectively; [e] 50 g of red sandy soil was spiked with 40 mL of Zn solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Zn^{2+} respectively; [f] 50 g of red sandy soil was spiked with 40 mL of Cd solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cd^{2+}respectively.

Table 3-5. Copper, zinc and cadmium toxicity removal from an organic rich soil by magnetic treatment, as determined by MetPLATE™.

Heavy metal type	Heavy metal concentration in spiked soil (mg/kg)	Number of treatments[a]	EC_{50}[b] of soil extract (% soil extract)		Toxicity units[c] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Cu[d]	200	1	40.5 ± 2.1%	90.8 ± 3.5 %	2.5 ± 0.1	1.1 ± 0.04	55.4 ± 0.7%
	400	1	13.9 ± 4.8%	75.8 ± 6.2%	7.7 ± 2.3	1.3 ± 0.1	81.7 ± 5.7%
	800	1	4.0 ± 0.3%	41.2 ± 4.0%	24.9 ± 2.1	2.4 ± 0.2	90.1 ± 1.6%
Zn[e]	200	1	33.7 ± 7.9%	> 100%	3.1 ± 0.8	< 1[g]	> 66.3%
	400	1	3.8 ± 0.3%	11.6 ± 1.6%	26.3 ± 2.0	8.7 ± 1.2	67.0 ± 2.3%
		2	3.7 ± 0.3%	31.0 ± 5.8%	27.4 ± 2.5	3.3 ± 0.6	87.8 ± 3.0%
	800	1	1.8 ± 0.1%	4.9 ± 0.5%	54.6 ± 1.7	20.6 ± 2.1	62.4 ± 2.8%
		2	1.8 ± 0.3%	6.3 ± 0.4%	56.6 ± 9.6	16.0 ± 0.9	71.4 ± 3.3%
		3	2.2 ± 0.03%	15.0 ± 1.0%	45.1 ± 0.6	6.7 ± 0.4	85.2 ± 0.8%
Cd[f]	200	1	28.1 ± 1.3%	47.2 ± 3.4%	3.6 ± 0.2	2.1 ± 0.1	40.5 ± 1.9%
		2	19.9 ± 1.7%	> 100%	5.0 ± 0.4	< 1[g]	> 80.1%
	400	1	8.2 ± 0.6%	20.0 ± 4.7%	12.2 ± 0.9	5.2 ± 1.1	57.0 ± 11.3%
		2	4.7 ± 0.5%	19.7 ± 2.5%	21.7 ± 2.3	5.1 ± 0.7	76.3 ± 2.3%
		3	4.1 ± 0.1%	27.1 ± 0.6%	24.6 ± 0.8	3.7 ± 0.1	84.9 ± 0.8%
	800	1	2.3 ± 0.1%	3.4 ± 0.2%	42.9 ± 2.2	29.7 ± 1.4	30.7 ± 1.1%
		2	1.0 ± 0.03%	2.7 ± 0.02%	96.2 ± 2.4	37.6 ± 0.3	60.9 ± 0.7%
		3	0.8 ± 0.02%	3.9 ± 0.1%	129.3 ± 2.5	25.3 ± 0.6	80.4 ± 0.3%

[a] 5% (2.5g) iron filings were added to the spiked organic rich soil , and the contact time was 3 hours; [b] Mean of 3 replicates ± 1 standard deviation; [c] Toxicity units = $100/EC_{50}$; [d] 50 g of organic rich soil was spiked with 40 mL of Cu solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cu^{2+}respectively; [e] 50 g of organic rich soil was spiked with 40 mL of Zn solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Zn^{2+} respectively; [f] 50 g of organic rich soil was spiked with 40 mL of Cd solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cd^{2+}respectively; [g] Non-toxic.

Table 3-6. Copper, zinc and cadmium toxicity removal from a Georgia clay rich soil by magnetic treatment, as determined by MetPLATE™.

Heavy metal type	Heavy metal concentration in spiked soil (mg/kg)	Number of treatments[a]	EC_{50}[b] of soil extract (% soil extract)		Toxicity units[c] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Cu[d]	200	1	2.8 ± 0.4%	> 100 %	35.8 ± 4.8	< 1[g]	> 97.2%
	400	1	0.4 ± 0.1%	85.9 ± 3.3%	234.3 ± 35.2	1.2 ± 0.1	99.5 ± 0.1%
	800	1	0.16 ± 0.01%	53.2 ± 3.2%	640.5 ± 45.3	1.9 ± 0.1	99.7 ± 0.04%
Zn[e]	200	1	2.1 ± 0.2%	81.4 ± 13.2%	47.9 ± 4.8	1.2 ± 0.2	97.4 ± 0.2%
	400	1	0.96 ± 0.03%	3.9 ± 0.6%	104.9 ± 7.0	25.9 ± 3.7	75.2 ± 5.2%
		2	0.43 ± 0.05%	5.2 ± 0.6%	233.9 ± 2.7	19.3 ± 2.2	91.8 ± 0.8%
	800	1	0.23 ± 0.03%	0.49 ± 0.02%	438.1 ± 53.9	206.4 ± 9.0	52.4 ± 7.9%
		2	0.16 ± 0.01%	0.52 ± 0.03%	627.5 ± 55.5	192.6 ± 10.5	69.3 ± 1.0%
		3	0.11 ± 0.02%	1.2 ± 0.07%	903.2 ± 136.9	87.1 ± 5.4	90.3 ± 0.9%
Cd[f]	200	1	0.38 ± 0.05%	1.2 ± 0.3%	264.7 ± 36.8	89.5 ± 25.5	66.5 ± 5.0%
		2	0.44 ± 0.07%	3.7 ± 0.7%	230.2 ± 37.0	27.3 ± 5.0	88.2 ± 0.3%
	400	1	0.11 ± 0.02%	0.2 ± 0.04%	933.8 ± 142.1	485.0 ± 92.4	48.1 ± 2.0%
		2	0.11 ± 0.02%	0.6 ± 0.1%	972.2 ± 196.4	183.3 ± 41.7	80.3 ± 8.3%
		3	0.11 ± 0.01%	0.7 ± 0.1%	954.5 ± 64.3	139.6 ± 13.7	85.4 ± 0.5%
	800	1	0.07 ± 0.02%	0.12 ± 0.01%	1594.5 ± 464.7	871.2 ± 53.6	43.4 ± 13.1%
		2	0.06 ± 0.001%	0.13 ± 0.01%	1724.7 ± 42.4	801.3 ± 45.3	53.6 ± 1.5%
		3	0.04 ± 0.01%	0.18 ± 0.01%	2916.7 ± 589.3	571.9 ± 23.1	80.1 ± 3.2%

[a] 5% (2.5g) iron filings were added to the spiked Georgia clay rich soil , and the contact time was 3 hours; [b] Mean of 3 replicates ± 1 standard deviation; [c] Toxicity units = $100/EC_{50}$; [d] 50 g of Georgia clay rich soil was spiked with 40 mL of Cu solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cu^{2+}respectively; [e] 50 g of Georgia clay rich soil was spiked with 40 mL of Zn solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Zn^{2+} respectively; [f] 50g of Georgia clay soil was spiked with 40mL of Cd solution containing 250 mg/L, 500 mg/L, and 1000 mg/L Cd^{2+}respectively; [g] Non-toxic

Table 3-7. Effect of magnetic treatment on the removal of Cu, Zn, and Cd from four soils as determined by the 48-h acute *Ceriodaphnia dubia* toxicity test.

Soil[a] type	Metal conc. in spiked soil[a] (mg/kg)	Number of treatments[b]	EC_{50}[c] of soil extract (% soil extract)		Toxicity units[d] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Sandy	Cu^{2+} (800mg/kg)	1	0.005 ± 0.001%	5.3 ± 1.2%	22148.6 ± 5142.0	19.8 ± 5.2	99.9 ± 0.01%
	Zn^{2+} (800mg/kg)	1	0.016 ± 0.0003%	0.03 ± 0.003%	6062.7 ± 125.3	3473.2 ± 380.5	42.7 ± 6.0%
		2	0.017 ± 0.001%	0.04 ± 0.005%	5859.6 ± 343.9	2308.8 ± 309.9	60.7 ± 3.2%
		3	0.016 ± 0.002%	0.1 ± 0.01%	6164.4 ± 600.0	979.9 ± 113.9	83.9 ± 3.0%
	Cd^{2+} (800mg/kg)	1	0.014 ± 0.002%	0.03 ± 0.01%	7333.3 ± 1154.8	3614.2 ± 1262.1	51.8 ± 10.6%
		2	0.011 ± 0.003%	0.03 ± 0.02%	9792.0 ± 2102.2	3426.7 ± 1535.6	66.3 ± 9.1%
		3	0.009 ± 0.0005%	0.07 ± 0.001%	10759.0 ± 588.8	1477.3 ± 32.1	85.8 ± 0.5%
Red sandy	Cu^{2+} (800mg/kg)	1	0.005 ± 0.001%	0.6 ± 0.1%	20988.6 ± 2669.8	173.6 ± 37.6	99.2 ± 0.3%
	Zn^{2+} (800mg/kg)	1	0.017 ± 0.001%	0.02 ± 0.002%	5896.0 ± 347.4	4444.4 ± 481.1	24.7 ± 4.6%
		2	0.024 ± 0.002%	0.05 ± 0.007%	4181.8 ± 314.9	1866.7 ± 230.9	54.9 ± 8.5%
		3	0.022 ± 0.004%	0.1 ± 0.006%	4753.1 ± 950.3	1036.7 ± 59.5	77.4 ± 6.3%
	Cd^{2+} (800mg/kg)	1	0.015 ± 0.002%	0.03 ± 0.002%	6869.7 ± 742.3	3387.3 ± 293.1	50.2 ± 8.1%
		2	0.008 ± 0.001%	0.04 ± 0.002%	13112.8 ± 1197.6	2504.2 ± 125.4	80.8 ± 2.5%
		3	0.008 ± 0.001%	0.05 ± 0.004%	13461.5 ± 1665.4	2111.1 ± 157.1	84.8 ± 1.1%

[a] 50 g of soil was spiked with 40 mL of heavy metal solution containing 1000 mg/L Cu^{2+}, 1000 mg/L Zn^{2+}, and 1000 mg/L Cd^{2+} respectively; [b] 5% (2.5 g) iron filings were added to the spiked soil , and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Toxicity units = $100/EC_{50}$.

Table 3-7. Continued.

Soil[a] type	Metal conc. in spiked soil[a] (mg/kg)	Number of treatments[b]	EC_{50}[c] of soil extract (% soil extract)		Toxicity units[d] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Organic rich	Cu^{2+} (800mg/kg)	1	0.9 ± 0.2%	4.9 ± 0.6%	112.4 ± 18.6	20.8 ± 2.4	81.4 ± 1.3%
	Zn^{2+} (800mg/kg)	1	0.5 ± 0.1%	1.3 ± 0.2%	196.7 ± 41.3	76.3 ± 8.8	59.4 ± 13.5%
		2	0.5 ± 0.1%	1.6 ± 0.2%	211.8 ± 42.1	62.1 ± 6.1	69.8 ± 7.2%
		3	0.6±0.1%	5.9 ± 0.4%	158.2 ± 16.0	17.0 ± 1.2	89.2 ± 1.7%
	Cd^{2+} (800mg/kg)	1	0.7 ± 0.1%	0.9 ± 0.1%	151.0 ± 23.7	107.2 ± 12.7	28.7 ± 3.1%
		2	0.4 ± 0.1%	0.8 ± 0.05%	247.7 ± 65.0	121.4 ± 8.1	49.4 ± 9.6%
		3	0.3 ± 0.07%	2.2 ± 0.5%	386.8 ± 87.5	51.2 ±5.8	85.8 ±3.0%
Georgia clay rich	Cu^{2+} (800mg/kg)	1	0.07±0.003%	4.4 ± 0.1%[e]	1481.5±59.5	22.9 ± 0.8	98.5 ± 0.01%
	Zn^{2+} (800mg/kg)	1	0.08±0.004%	0.1 ± 0.003%	1180.1±52.7	831.5 ± 18.8	29.4 ± 4.7%
		2	0.08±0.004%	0.14 ± 0.004%	1282.1±72.5	737.2 ± 22.7	42.5 ± 1.5%
		3	0.07±0.007%	0.4 ± 0.04%	1547.6±168.4	265.2 ± 30.1	82.7 ± 3.8%
	Cd^{2+} (800mg/kg)	1	0.02±0.001%	0.04±0.004%	4591.6±363.1	2418.4±206.0	51.2 ± 0.6%
		2	0.02±0.001%	0.06 ± 0.02%	5558.3±174.7	1669.0±412.6	69.8 ± 8.4%
		3	0.004±0.0005%	0.1 ± 0.01%	25731.2±3113.8	983.8 ±81.8	96.2 ± 0.1%

[a] 50 g of soil was spiked with 40 mL of heavy metal solution containing 1000 mg/L Cu^{2+}, 1000 mg/L Zn^{2+}, and 1000 mg/L Cd^{2+} respectively; [b] 5% (2.5 g) iron filings were added to the spiked soil , and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Toxicity units = $100/EC_{50}$.

Table 3-8. Effect of magnetic treatment on the removal of Cu, Zn, and Cd from four soils as determined by the 96-h chronic *Selenastrum capricornutum* toxicity test.

Soil[a] type	Metal conc. in spiked soil[a] (mg/kg)	Number of treatments[b]	EC_{50}[c] of soil extract (% soil extract)		Toxicity units[d] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Sandy	Cu^{2+} (800mg/kg)	1	0.003 ± 0.000%	2.3 ± 0.4%	35744.7 ± 1277.4	45.5 ± 9.3	99.9 ± 0.02%
	Zn^{2+} (800mg/kg)	1	0.007 ± 0.001%	0.03 ± 0.006%	13639.0 ± 1164.1	3848.3 ± 714.1	71.4 ± 7.4%
		2	0.006 ± 0.001%	0.04 ± 0.008%	17725.9 ± 2693.5	2573.6 ± 565.4	84.9 ± 5.9%
		3	0.006 ± 0.002%	0.07 ± 0.005%	17474.3 ± 6062.8	1475.5 ± 106.1	90.9 ± 2.9%
	Cd^{2+} (800mg/kg)	1	0.003 ± 0.0004%	0.008 ± 0.001%	28912.1 ± 3498.1	12584.6 ± 1067.0	56.2 ± 5.5%
		2	0.003 ± 0.0004%	0.02 ± 0.001%	31100.5 ± 4850.8	5179.5 ± 310.5	83.2 ± 1.7%
		3	0.003 ± 0.0006%	0.05 ± 0.001%	41483.1 ± 9259.6	2105.5 ± 31.3	95.5 ± 0.1%
Red sandy	Cu^{2+} (800mg/kg)	1	0.003 ± 0.001%	0.6 ± 0.1%	31846.2 ± 6832.4	181.6 ± 29.5	99.4 ± 0.2%
	Zn^{2+} (800mg/kg)	1	0.007 ± 0.001%	0.03 ± 0.001%	12823.6 ± 1785.0	2904.3 ± 100.8	77.1 ± 2.3%
		2	0.005 ± 0.001%	0.08 ± 0.004%	18856.7 ± 2893.7	1196.7 ± 54.2	93.6 ± 1.1%
		3	0.004 ± 0.001%	0.15 ± 0.002%	28170.4 ± 8165.6	688.4 ± 10.7	97.5 ± 0.7%
	Cd^{2+} (800mg/kg)	1	0.001 ± 0.0002%	0.014 ± 0.001%	70277.7 ± 8825.3	7192.6 ± 325.8	89.7 ± 1.1%
		2	0.001 ± 0.0001%	0.02 ± 0.004%	76907.7 ± 6313.0	4703.8 ± 973.8	93.9 ± 0.8%
		3	0.001 ± 0.0002%	0.03 ± 0.006%	69444.0 ± 7750.4	3516.8 ± 801.9	94.9 ± 0.9%

[a] 50 g of soil was spiked with 40 mL of heavy metal solution containing 1000 mg/L Cu^{2+}, 1000 mg/L Zn^{2+}, and 1000 mg/L Cd^{2+} respectively; [b] 5% (2.5 g) iron filings were added to the spiked soil , and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Toxicity units = $100/EC_{50}$.

Table 3-8. Continued.

Soil[a] type	Metal conc. in spiked soil[a] (mg/kg)	Number of treatments[b]	EC_{50}[c] of soil extract (% soil extract)		Toxicity units[d] of soil extract		Toxicity removal (%)
			No Treatment	Magnetic Treatment	No Treatment	Magnetic Treatment	
Organic	Cu^{2+} (800mg/kg)	1	0.4 ± 0.04%	4.4 ± 0.4%	231.2 ± 19.3	23.0 ± 2.2	90.0 ± 1.4%
	Zn^{2+} (800mg/kg)	1	0.2 ± 0.02%	0.5 ± 0.09%	439.9 ± 46.3	188.9 ± 30.7	56.3 ± 11.1%
		2	0.2 ± 0.01%	0.7 ± 0.04%	510.4 ± 18.6	138.2 ± 7.6	72.9 ± 1.7%
		3	0.2 ± 0.01%	3.2 ± 0.2%	423.3 ± 15.9	30.2 ± 0.4	92.7 ± 0.2%
	Cd^{2+} (800mg/kg)	1	0.07 ± 0.001%	0.2 ± 0.01%	1433.6 ± 10.5	527.0 ± 34.2	63.4 ± 2.2%
		2	0.05 ± 0.004%	0.5 ± 0.1%	1888.8 ± 133.6	191.0 ± 37.6	89.8 ± 2.6%
		3	0.04 ± 0.004%	1.1 ± 0.04%	2375.1 ± 222.0	94.7 ± 4.1	96.0 ± 0.4%
Georgia clay	Cu^{2+} (800mg/kg)	1	0.003 ± 0.0006%	2.8 ± 0.1%	30921.3 ± 5341.3	36.3 ± 0.7	99.9 ±0.02%
	Zn^{2+} (800mg/kg)	1	0.004 ± 0.0001%	0.09 ± 0.002%	22554.3 ± 719.0	1148.9 ± 21.0	94.9 ± 0.1%
		2	0.003 ± 0.001%	0.12 ± 0.02%	29081.1 ± 4640.5	870.0 ± 157.0	97.0 ± 0.1%
		3	0.003 ± 0.0002%	0.21 ± 0.02%	36377.4 ± 2408.9	482.1 ± 39.7	98.7 ± 0.02%
	Cd^{2+} (800mg/kg)	1	0.002 ± 0.000%	0.02 ± 0.003%	52265.7 ± 753.2	5890.5 ± 996.4	88.7 ± 1.7%
		2	0.002 ± 0.000%	0.03 ± 0.0003%	60243.9 ± 3356.7	3701.3 ± 41.6	93.8 ± 0.4%
		3	0.002 ± 0.000%	0.04 ± 0.003%	57939.9 ± 7624.5	2578.0 ± 196.1	95.5 ± 0.9%

[a] 50 g of soil was spiked with 40 mL of heavy metal solution containing 1000 mg/L Cu^{2+}, 1000 mg/L Zn^{2+}, and 1000 mg/L Cd^{2+} respectively; [b] 5% (2.5 g) iron filings were added to the spiked soil , and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Toxicity units = $100/EC_{50}$.

Table 3-9. Effect of magnetic treatment on the removal of Cu^{2+} from spiked soils, as determined by chemical analysis.

Soil type	Initial Cu conc. in spiked soil (mg/kg)[a]		Cu conc.[c] in soil matrix, extracts, and iron filings[d]			Cu removal from soil fractions	
			In soil matrix (mg/kg)	In soil extract (mg/L)	Adsorbed on iron filings (mg/kg)	From soil matrix (%)	From soil extract (%)
Sandy	200	No treatment	171.9 ± 3.1[e]	3.8 ± 0.6	No iron filings added	48.7±6.4%	96.9±0.3%
		Magnetic treatment[b]	88.0 ± 9.9	0.1 ± 0.04	2022.4±66.9		
	400	No treatment	279.8±30.7	39.4 ± 1.1	No iron filings added	74.2±4.2%	99.1±0.2%
		Magnetic treatment	71.3 ± 5.6	0.4 ± 0.07	5583.4±424.3		
	800	No treatment	362.0±4.6	144.2 ± 1.0	No iron filings added	80.7±0.8%	99.5±0.05%
		Magnetic treatment	69.8±2.0	0.8 ± 0.07	12186.7±200.7		
Red sandy	800	No treatment	453.1 ± 21.1	277.6 ± 7.8	No iron filings added	53.9±2.8%	98.6±0.1%
		Magnetic treatment	209.0± 19.1	3.8 ± 0.4	10140.0±402.9		
Organic rich	800	No treatment	705.8 ± 21.1	2.6 ± 0.3	No iron filings added	49.1 ± 3.8%	83.9 ± 1.5%
		Magnetic treatment	358.9 ± 20.1	0.4 ± 0.02	6261.7 ± 124.4		
Georgia clay rich	800	No treatment	591.5 ± 34.4	110.3 ± 3.8	No iron filings added	85.2 ± 3.6%	99.9 ± 0.04%
		Magnetic treatment	87.3 ± 19.0	0.1 ± 0.04	11400 ± 542.9		

[a] 50 g of soil was spiked with 40 mL of Cu solution containing 250 mg/L, or 500 mg/L, or 1000 mg/L Cu^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil, and the contact time was 3 hours; [c] Detected by ICP-AES; [d] Iron filings had a Cu background value of 2503.3 mg/kg. This concentration was subtracted from the Cu concentration in filings after treatment; [e] Mean of 3 replicates ± one standard deviation.

Table 3-10. Effect of magnetic treatment on the removal of Zn^{2+} from spiked soils, as determined by chemical analysis.

Soil type	Initial Zn conc. in spiked soil (mg/kg)[a]		Zn conc.[c] in soil matrix, extracts, and iron filings[d]			Zn removal from soil fractions	
			In soil matrix (mg/kg)	In soil extract (mg/L)	Adsorbed on iron filings (mg/kg)	From soil matrix (%)	From soil extract (%)
Sandy	800	No treatment	423.4±11.3[e]	242.6 ± 4.4	No iron filings added	67.3±5.6%	83.4±3.2%
		Magnetic treatment[b]	142.4 ± 19.7	40.5 ± 8.6	3371.9±170.6		
Red sandy	800	No treatment	383.4±10.3	288.2 ± 8.3	No iron filings added	14.6±3.0%	72.0±1.8%
		Magnetic treatment	327.4 ± 5.5	80.5 ± 4.6	1590.6±41.9		
Organic rich	800	No treatment	694.4 ± 19.5	5.8 ± 0.5	No iron filings added	18.5 ± 5.2%	78.8 ± 3.8%
		Magnetic treatment	565.5 ± 20.4	1.2 ± 0.3	559.2 ± 20.5		
Georgia clay rich	800	No treatment	477.5 ± 4.1	187.9 ± 3.6	No iron filings added	4.0 ± 1.7%	71.1 ± 0.7%
		Magnetic treatment	458.6 ± 12.1	54.2 ± 0.4	1291.9 ± 36.8		

[a] 50 g of soil was spiked with 40 mL of Zn solution containing 1000 mg/L Zn^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil, and the contact time was 3 hours; [c] Detected by ICP-AES; [d] Iron filings had a Zn background value of 13.1 mg/kg. This concentration was subtracted from the Zn concentration in filings after treatment; [e] Mean of 3 replicates ± one standard deviation.

Table 3-11. Effect of magnetic treatment on the removal of Cd^{2+} from spiked soils, as determined by chemical analysis.

Soil type	Initial Cd conc. in spiked soil (mg/kg)[a]		Cd conc.[c] in soil matrix, extracts, and iron filings[d]			Cd removal from soil fractions	
			In soil matrix (mg/kg)	In soil extract (mg/L)	Adsorbed on iron filings (mg/kg)	From soil matrix (%)	From soil extract (%)
Sandy	800	No treatment	434.7±38.5[e]	235.8 ± 5.9	No iron filings added	49.5±6.4%	59.7±0.8%
		Magnetic treatment[b]	217.8±10.2	95.0 ± 1.3	2386.9±102.9		
Red sandy	800	No treatment	384.7±7.5	261.6 ± 4.1	No iron filings added	5.7±2.8%	54.0±1.0%
		Magnetic treatment	362.7±9.1	120.3 ± 0.9	1084.9±33.3		
Organic	800	No treatment	726.2 ± 38.1	3.9 ± 1.2	No iron filings added	5.2 ± 1.8%	60.6 ± 7.7%
		Magnetic treatment	688.2 ± 22.8	1.5 ± 0.2	117.6 ± 9.7		
Georgia clay	800	No treatment	507.6 ± 22.8	152.6 ± 2.4	No iron filings added	19.7 ± 7.4%	49.9 ± 0.6%
		Magnetic treatment	407.0 ± 19.2	81.0 ± 0.4	955.9 ± 66.0		

[a] 50 g of soil was spiked with 40 mL of Cd solution containing 1000 mg/L Cd^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil, and the contact time was 3 hours; [c] Detected by ICP-AES; [d] Iron filings had a Cd background value of 108.4 mg/kg. This concentration was subtracted from the Cd concentration in filings after treatment; [e] Mean of 3 replicates ± one standard deviation.

Table 3-12. Mass balance of Cu in spiked soils before and after magnetic treatment.

Soil[a] type	Initial Cu mass (mg)		Cu in soil matrix (mg)	Cu in soil extract (mg)	Cu adsorbed on iron filings[c] (mg)	Total recovered Cu mass (mg)	Total recovery of Cu (%)
	10	Before treatment[b]	8.6±0.2	0.4±0.1	No iron filings added	9.0±0.3[d]	90.0±3%
		After treatment	4.4±0.5	0.01±0.004	5.1±0.2	9.5±0.7	95.0±7%
Sandy	20	Before treatment	14.0±1.5	3.9±0.1	No iron filings added	17.9±1.6	89.5±8%
		After treatment	3.6±0.3	0.04±0.01	14.0±1.1	17.6±1.4	88.0±7%
	40	Before treatment	18.1±0.2	14.4±0.1	No iron filings added	32.5±0.3	81.3±0.8%
		After treatment	3.5±0.1	0.1±0.01	30.5±0.5	34.1±0.6	85.3±1.5%
Red sandy	40	Before treatment	22.7±1.1	13.9±0.4	No iron filings added	36.5±0.9	91.3±2.3%
		After treatment	10.5±1.0	0.2±0.02	25.4±1.0	36.0±0.6	90.0±1.5%
Organic rich	40	Before treatment	35.3±1.1	0.1±0.01	No iron filings added	35.4±1.1	88.5±2.6%
		After treatment	17.9±1.0	0.02±0.00	15.7±0.3	33.6±0.7	84.1±1.8%
Georgia clay rich	40	Before treatment	29.6±1.7	5.5± 0.2	No iron filings added	35.1±1.6	87.7±3.9%
		After treatment	4.4±1.0	0.01±0.002	28.5±1.4	32.9±1.3	82.2±3.1%

[a] 50 g of soil was spiked with 40 mL of Cu solution containing 250 mg/L, or 500 mg/L, or 1000 mg/L Cu^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil for 3 hours; [c] The amount of background Cu mass in iron filings was subtracted from the Cu mass in filings after treatment; [d] Mean of 3 replicates ± one standard deviation.

Table 3-13. Mass balance of Zn in spiked soils before and after magnetic treatment.

Soil[a] type	Initial Zn mass (mg)		Zn in soil matrix (mg)	Zn in soil extract (mg)	Zn adsorbed on iron filings[c] (mg)	Total recovered Zn mass (mg)	Total recovery of Zn (%)
Sandy	40	Before treatment	21.2±0.6	12.1±0.2	No iron filings added	33.3±0.8[d]	83.3±1.9%
		After treatment	7.1±1.0	2.0±0.4	25.3±1.3	34.4±1.1	86.1±2.8%
Red sandy	40	Before treatment	19.2±0.5	14.4±0.4	No iron filings added	33.6±0.8	84.0±1.9%
		After treatment	16.4±0.3	4.0±0.2	11.9±0.3	32.3±0.7	80.8±1.7%
Organic rich	40	Before treatment	34.7±1.0	0.2±0.02	No iron filings added	35.0±1.0	87.4±2.5%
		After treatment	28.3±1.0	0.05±0.01	4.2±0.2	32.5±0.9	81.3±2.3%
Georgia clay rich	40	Before treatment	23.9±0.2	9.4±0.2	No iron filings added	33.3±0.4	83.2±1.0%
		After treatment	22.9±0.6	2.7±0.02	9.7±0.3	35.3±0.9	88.3±2.2%

[a] 50 g of soil was spiked with 40 mL of Zn solution containing 1000 mg/L Zn^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil for 3 hours; [c] The amount of background Zn mass in iron filings was subtracted from the Zn mass in filings after treatment; [d] Mean of 3 replicates ± one standard deviation.

Table 3-14. Mass balance of Cd in spiked soils before and after magnetic treatment.

Soil[a] type	Initial Cd mass (mg)		Cd in soil matrix (mg)	Cd in soil extract (mg)	Cd adsorbed on iron filings[c] (mg)	Total recovered Cd mass (mg)	Total recovery of Cd (%)
Sandy	40	Before treatment	21.7±1.9	11.8±0.3	No iron filings added	33.5±1.7[d]	83.8±4.2%
		After treatment	10.9±0.5	4.8±0.1	17.9±0.8	33.5±0.5	83.9±1.2%
Red sandy	40	Before treatment	19.2±0.4	13.1±0.2	No iron filings added	32.3±0.2	80.8±0.6%
		After treatment	18.1±0.4	6.0±0.1	8.1±0.3	32.3±0.6	80.7±1.4%
Organic rich	40	Before treatment	36.3±1.9	0.2±0.06	No iron filings added	36.5±2.0	91.3±4.9%
		After treatment	34.4±1.1	0.07±0.01	0.88±0.07	35.4±1.2	88.4±3.0%
Georgia clay rich	40	Before treatment	25.4±1.1	7.6±0.1	No iron filings added	33.0±1.3	82.5±3.2%
		After treatment	20.4±1.0	4.1±0.02	7.5±0.5	31.9±0.5	79.7±1.1%

[a] 50 g of soil was spiked with 40 mL of Cd solution containing 1000 mg/L Cd^{2+}; [b] 5% (2.5 g) iron filings were added to the spiked soil for 3 hours; [c] The amount of background Cd mass in iron filings was subtracted from the Cd mass in filings after treatment; [d] Mean of 3 replicates ± one standard deviation.

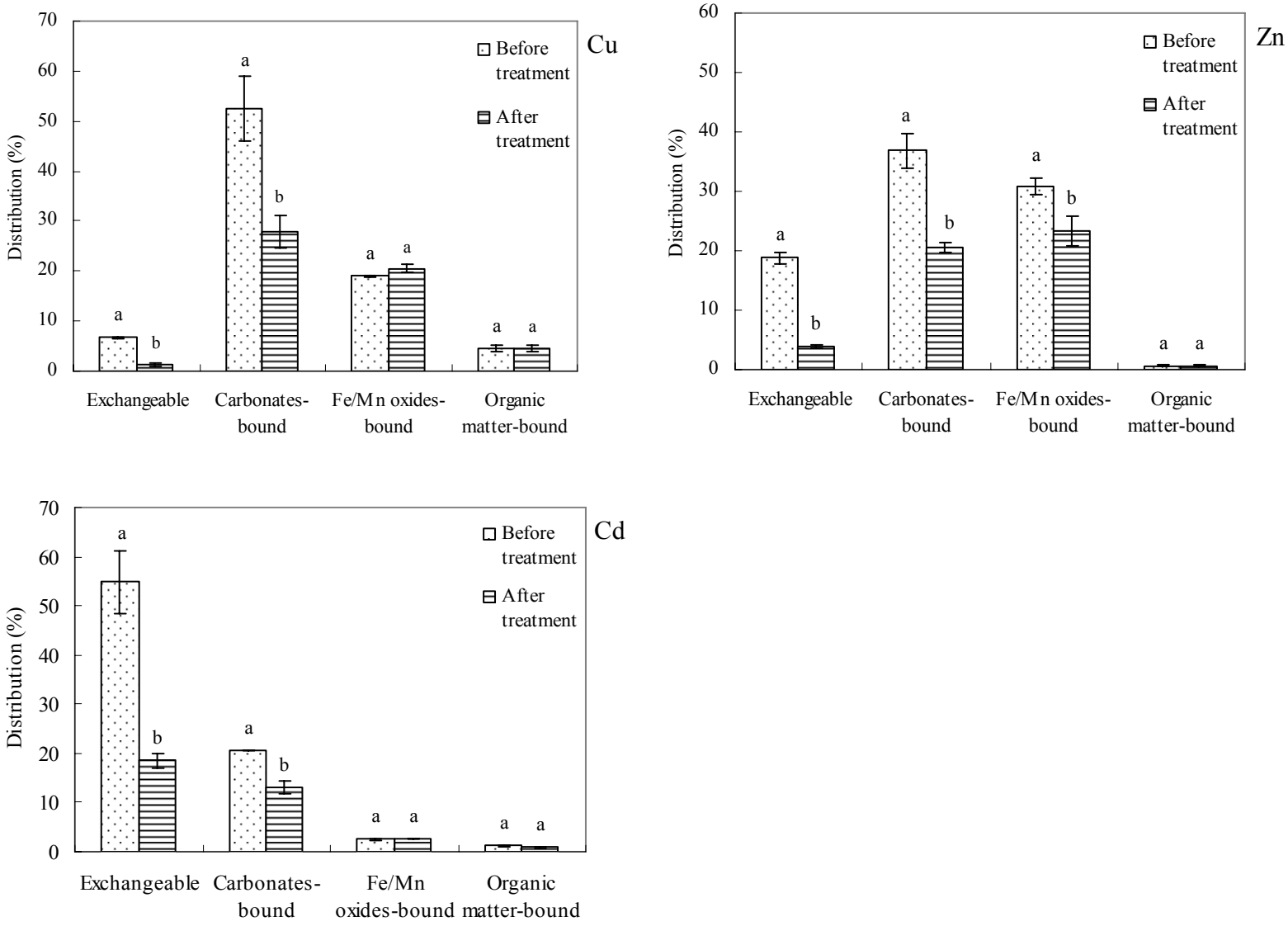


Figure 3-2. Distribution of Cu, Zn, and Cd fractions in a spiked sandy soil. (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Student's *t*-test)

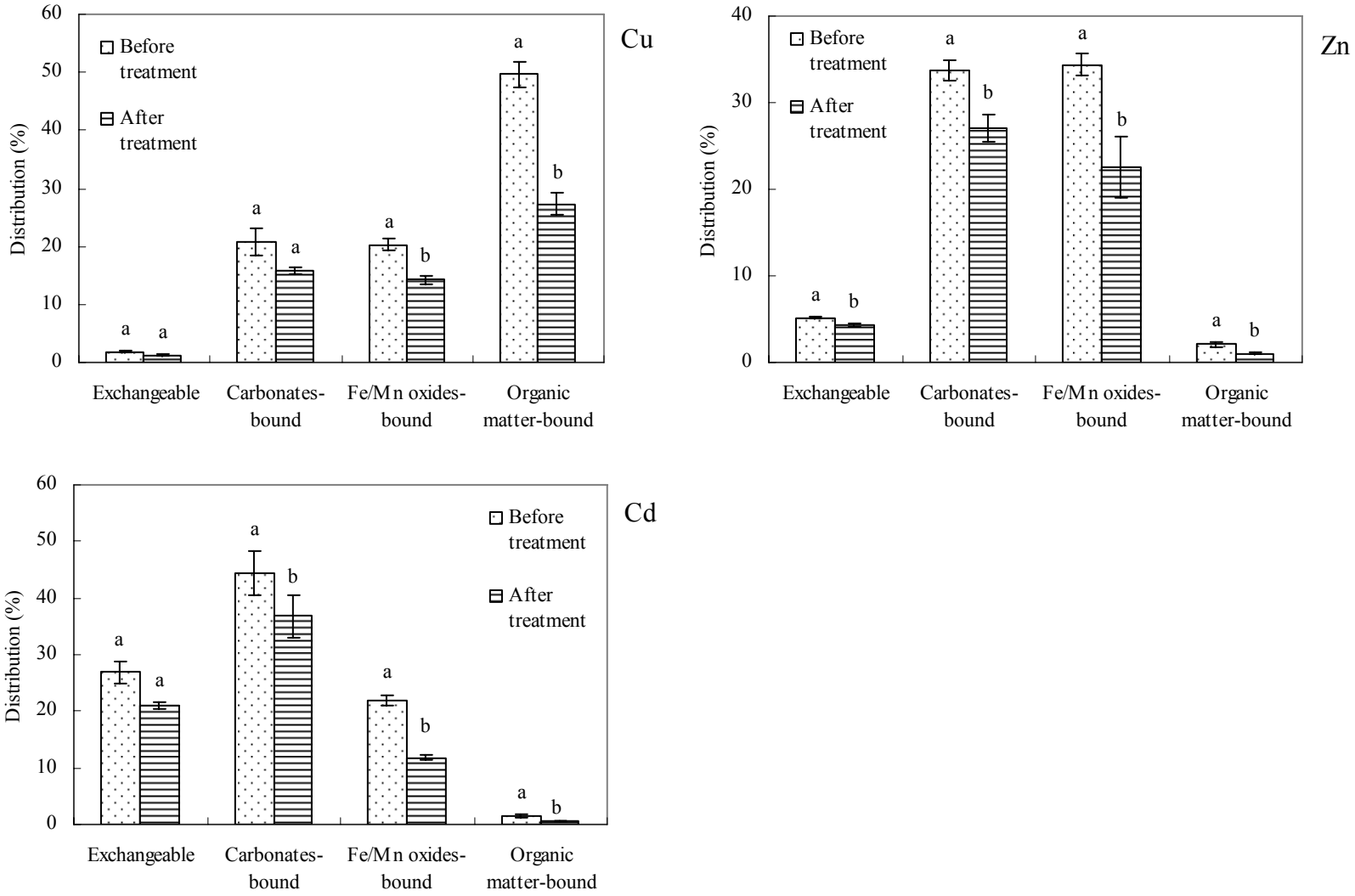


Figure 3-3. Distribution of Cu, Zn, and Cd fractions in a spiked organic rich soil. (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Student's *t*-test).

Table 3-15. Effect of magnetic treatment on the removal of Cu, Zn, and Cd from each soil fraction, as determined by sequential extraction.

Soil[a] type	Metal type	Metal removal from each fraction after treatment[b] (%)			
		Exchangeable	Carbonates-bound	Fe/Mn oxides-bound	Organic matter-bound
Sandy	Cu	79.9 ± 3.3%[c]	46.0 ± 12.4%	-7.6 ± 4.4	0 ± 0%
	Zn	79.2 ± 1.7%	44.0 ± 2.3%	24.4 ± 4.5%	8.1 ± 3.3%
	Cd	65.8 ± 6.2%	36.1 ± 5.9%	0 ± 0%	29.2 ± 6.4%
Organic	Cu	32.4 ± 8.6%	22.3 ± 11.6%	29.5 ± 6.1%	45.1 ± 2.1%
	Zn	16.1 ± 5.1%	19.6 ± 3.4%	34.8 ± 8.0%	48.3 ± 5.5%
	Cd	21.5 ± 5.0%	17.0 ± 2.1%	45.5 ± 2.8%	53.3 ± 11.7%

[a] Soil was spiked with 200 mg/kg of Cu, Zn, and Cd; [b] 5% (2.5 g) iron filings were added to the spiked soil for 3 hours; [c] Mean of 3 replicates ± one standard deviation.

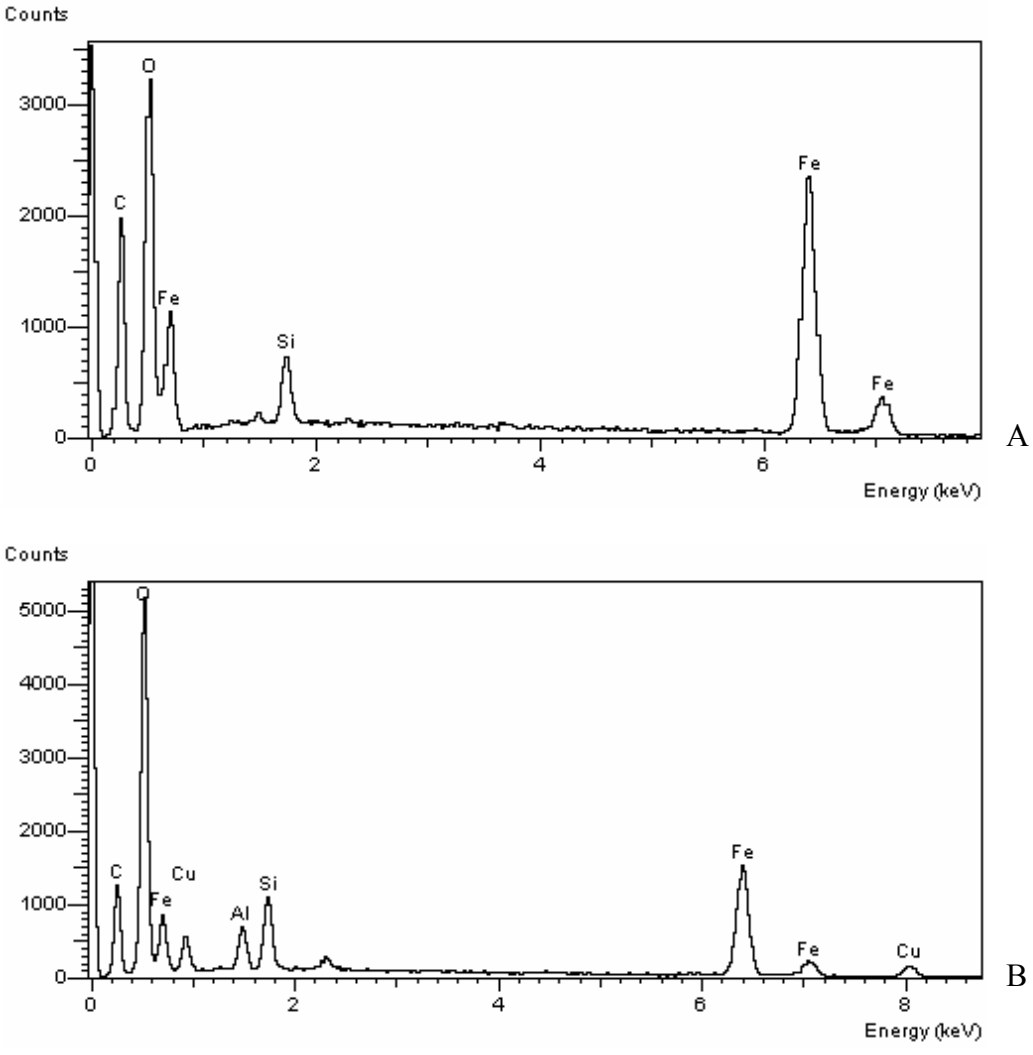
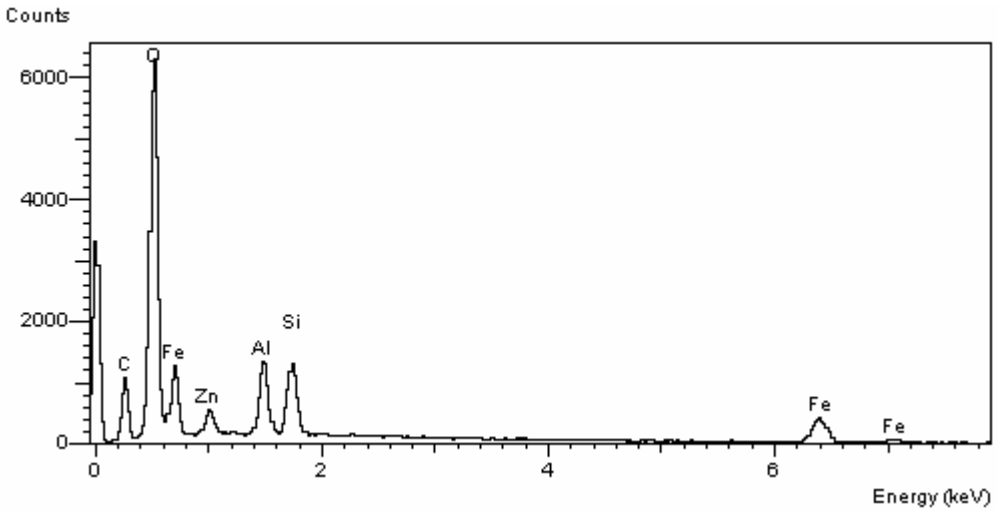
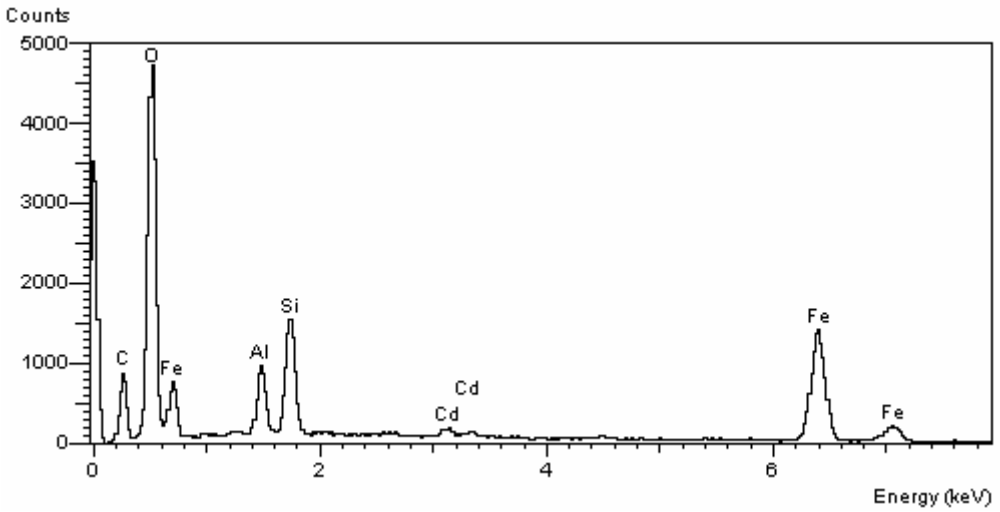


Figure 3-4. Energy dispersive x-ray spectroscopy (EDS) of iron filings. A) was unused Fe. B) was retrieved from 800 mg/kg Cu-spiked sandy soil, C) was retrieved from 800 mg/kg Zn-spiked sandy soil, and D) was retrieved from 800 mg/kg Cd-spiked sandy soil.



C



D

Figure 3-4. Continued.

Table 3-16. Effect of contact time between iron filings and 1 N HNO_3 on the recovery of fresh iron filings.

	Contact time between iron filings and 1N HNO_3				
	24-hr	2-hr		1-hr	
Number of regenerations	1st regeneration	1st regeneration	2nd regeneration	1st regeneration	2nd regeneration
Recovery of iron filings (%)	43.2 ± 1.3%	73.9±1.6%	48.4 ± 2.5%	86.2 ± 0.9%	85.3 ± 0.4%

[a] Mean of 3 replicates ± one standard deviation

Table 3-17. Comparison of the toxicity of sandy soil extracts treated with fresh iron filings and regenerated iron filings, as determined by MetPLATE™.

Heavy metal in spiked soil[a]	Fresh Fe		After 1st regeneration		After 2nd regeneration	
	EC_{50} of treated soil extract (% soil extract)	TUs[b] of treated soil extract	EC_{50} of treated soil extract (% soil extract)	TUs of treated soil extract	EC_{50} of treated soil extract (% soil extract)	TUs of treated soil extract
Cu	87.0±1.6%[c]	1.1±0.02	91.6±9.0%	1.1±0.1	>100%	<1[d]
Zn	2.2±0.1%	45.9±2.3	4.1±1.3%	25.8±8.0	5.4±0.5%	18.5±1.8
Cd	0.4±0.02%	251.5±12.2	0.6±0.1%	161.2±14.9	0.8±0.02%	124.7±3.4

[a] 50 g of sandy soil was spiked with 40 mL of Cu, Zn, or Cd solution containing 500 mg/L Cu^{2+}, Zn^{2+}, and Cd^{2+}, respectively; [b] Toxicity unit (TU) = $100/EC_{50}$; [c] Mean of 3 replicates ± one standard deviation; [d] Non-toxic.

Table 3-18. Comparison of the toxicity of sandy soil extracts treated by fresh iron filings and regenerated iron filings, as determined by the 48-h acute *Ceriodaphnia dubia* toxicity test.

Heavy metal in spiked soil[a]	Fresh Fe		After 1st regeneration		After 2nd regeneration	
	EC_{50} of treated soil extract (% soil extract)	TUs[b] of treated soil extract	EC_{50} of treated soil extract (% soil extract)	TUs of treated soil extract	EC_{50} of treated soil extract (% soil extract)	TUs of treated soil extract
Cu	4.0±0.2%[c]	24.8±1.4	6.7±2.0%	15.5±4.5	15.6±0.2%	6.4±0.1
Zn	0.2±0.003%	590.6±10.6	0.6±0.3%	174.6±18.0	1.0±0.2%	107.8±25.3
Cd	0.14±0.01%	719.2±41.0	0.26±0.04%	389.2±61.2	0.34±0.02%	291.3±19.0

[a] 50 g of sandy soil was spiked with 40 mL of Cu, Zn, or Cd solution containing 500 mg/L Cu^{2+}, Zn^{2+}, and Cd^{2+}, respectively; [b] Toxicity unit (TU) = $100/EC_{50}$; [c] Mean of 3 replicates ± one standard deviation.

CHAPTER 4
EFFECT OF AGING OF METAL-SPIKED SOILS ON METAL TOXICITY AND REMOVAL USING MAGNETIC SEPARATION

4.1 Introduction

After soluble metals are added to soil, several reactions may occur, which can change the partitioning of metals between the aqueous and solid phases in soil and thereby their mobility and bioavailability to organisms (Adriano, 2001b; Ma et al., 2006a). Sorption is considered the most important process controlling the partitioning of metals in soils, which may represent the combined effects of ion exchange, specific adsorption, precipitation, and complexation (Adriano, 2001b). Factors influencing metal behavior in soils include soil characteristics, metal species, and the contact time between soil and metals (Adriano, 2001b; Naidu et al., 2003a; Weng et al., 2002). Soil pH is a main factor that controls metal partitioning, followed by organic matter which is a time-sensitive parameter (Daoust et al., 2006) and subject to long-term transformations (e.g. chemical or biological processes) (Martínez et al., 2003). It has been shown that an increase of soil pH and organic matter generally decrease the mobility and, thereby, the (phyto)toxicity of metals (Adriano, 2001b; Alva et al., 2000; Sauvé et al., 2000).

The term "aging" refers to the process during which the availability of certain compounds changes as the compounds stay in soil for some time (Alexander, 1995). Some studies have revealed that as the residence time increases, the binding of metals to soils tends to increase, which could result in lower toxicity and availability of metals in aged contaminated soils than in freshly spiked soils (Alexander, 1995; Amorim et al., 2005; Smit and Van Gestel, 1998; Ma et al., 2006a, b; Song et al., 2006; Oorts et al., 2007; Stewart et al., 2003). Therefore, the bioavailability of metals derived from laboratory experiments using freshly spiked soils may be overestimated (Van Straalen and Denneman 1989), and the addition of metal salts to soils may

result in a different metal speciation than in the field (Pedersen et al., 2000). The decrease of metal mobility with time could be generally due to micropore diffusion, cavity entrapment, occlusion in soil phase, or surface precipitation (Ma et al., 2006a).

Although long-term aging is a very important process which can change metal availability and toxicity overtime, it is usually not considered in the risk assessment of metals in field soils, and very limited research on this subject has been done so far (Ma et al., 2006a). Ma et al. (2006a) have studied the aging of Cu added to 19 European soils based on a two-year period. The results indicated that the total Cu in soil pore water from leached soils decreased rapidly initially followed by further decrease at slower rates. They concluded that soil pH was a vital factor affecting the aging rate of Cu. Moreover, by employing a semi-mechanistic model, they found that when the soil pH was below 5, only slow processes (likely diffusion) occurred. Another study conducted by Arias-Estéves et al. (2007) has investigated the influence of aging on Cu fractionation in an acid soil. After 500 days of incubation, the change of soil pH was negligible. However, Cu in the exchangeable and organically-bound factions decreased with increasing incubation time, whereas Cu concentration in the residual faction increased. Ma and Uren (2006) also studied the effect of aging on the phytoavailability of Zn in a slightly calcareous soil. This two- year green house study provided biological evidence that long-term aging decreases the bioavailability of Zn to corn. In addition, an investigation of the transport of Cd in an aged sandy soil was carried out by Seuntjens et al. (2001). No conclusive evidence was found that aging affects Cd transport.

In our study, we examined the effect of aging on the change of Cu and Zn toxicity in spiked soils. Besides, the aging effect of contaminants should also be considered when performing remediation in field soils. In other words, remediation strategies that show promising

results in laboratory experiments using freshly spiked soils may not work as well in aged soils under field conditions. Thus, we also examined the effectiveness of the proposed magnetic treatment on decontaminating aged Cu- and Zn-spiked soils.

4.2 Material and Methods

4.2.1 Soils Used

The sandy soil and organic rich soil used in Chapter 2 were further tested in this section of study. Table 3-1 shows the main characteristics of the soils under study. All soils samples were first air-dried, screened (sieve # 10; 2.0 mm particles) and homogenized.

4.2.2 Chemicals Used

Two heavy metal solutions (Cu^{2+} and Zn^{2+}) were used. Copper solution was prepared from copper sulfate ($CuSO_4 \bullet H_2O$, Sigma®), and zinc solution was prepared from zinc chloride ($ZnCl_2$, Sigma®). Iron filings (Fisher, 40 mesh) were placed in 1M NaOH for 72 hours to increase the adsorption capacity and then washed thoroughly with distilled water before use (Yeager, 1998).

4.2.3 Soil Aging without Wet/Dry Cycle

Three kilogram of sandy soil was spiked with 1L of 600 mg/L Cu^{2+} or Zn^{2+} solution to reach a final soil metal concentration of 200 mg/kg. Three kilogram of organic rich soil was spiked with 2 L of 1200 mg/L Cu^{2+} or Zn^{2+} solution, resulting in 800 mg-metal /kg soil. These metal concentrations were based on the toxic levels determined in Chapter 2. The volume of the heavy metal solutions was above the soil field capacity to ensure a sufficient contact between the metal ions and the soil particles. The spiked soil slurries were mixed very well and dried at room temperature. A portion of each dried soil was regularly tested for toxicity and treated by magnetic separation over a 4-month period, with 1 month interval. Freshly spiked soils served as controls and all tests were run in triplicate.

4.2.4 Soil Aging with Wet/Dry Cycle

After 4-month aging as discussed above, a wet-dry procedure (3 days for sandy soil, 5 days for organic rich soil) was performed to the spiked sandy soil and organic rich soil to simulate the field conditions in Florida during the rainy season. The dried soils were wetted with distilled water to reached saturation, and then the soil slurries were mixed very well and allowed to dry again at room temperature. After four wet-dry cycles, a portion of dried soil was tested for toxicity and treated by magnetic separation method. The entire aging experiment lasted approximately 7 months, including the initial 4-month aging and 20 wet-dry cycles. All tests were run in triplicate.

4.2.5 Toxicity of Aged Soil Extracts

One hundred milliliter of distilled water was added to 50 g of spike soil, and the soil mixture was shaken for 4 hours. Then the soil slurry was centrifuged at 10,000 rpm for 15 minutes and the supernatant (soil extract) was removed with a pipet and was used for toxicity tests after overnight settling in the refrigerator. Two toxicity assays, MetPLATE^{TM} and the 48-h *Ceriodaphnia dubia* acute bioassay (as discussed in Section 3.2.6, also see Appendix A for detailed procedures) were used to assess the toxicity of the aged soil extracts. To determine the EC_{50}s for the soil extracts, a regression analysis was performed. The toxicity units (TU) were then calculated according to Equation 3-1 (see Chapter 3). All samples were run in triplicate.

4.2.6 Magnetic Treatment of Aged Soils

One hundred milliliter of distilled water was added to 50 g of spiked soil, and the soil slurry was shaken for 1 hour at room temperature. Then, 5% (2.5 g) of iron filings were added and the system was shaken for 3 hours followed by magnetic retrieval of the iron filings. The soil extracts were then separated from the solid phase by centrifuging at 10,000 rpm for 15 mins, and the supernatant (soil extract) was removed with a pipet. All experiments were run in triplicate.

4.3 Results and Discussion

4.3.1 Effect of Aging on Cu and Zn Toxicity

4.3.1.1 Toxicity of Cu and Zn in aged sandy soil

Figure 4-1 represents the change of Cu and Zn toxicity in spiked sandy soil over a 4-month period and 20 wet-dry cycles. In 200 mg/kg Cu- spiked sandy soil, the toxicity of Cu in soil extracts determined by both MetPLATE™ and the 48-hr *C. dubia* test did not show significant ($\alpha = 0.05$) overall change over the 4-month dry aging period, with TU values of 43.3 by MetPLATE™ and 443.7 TUs by *C .dubia* at time 0, and 40.9 TUs by MetPLATE™ and 461.4 TUs by *C.dubia* at the 4th month. However, after subjecting the sandy soil to 20 wet-dry cycles, Cu toxicity decreased significantly ($\alpha = 0.05$) and gradually with time. After 20 wet-dry cycles, the toxicity of the sandy soil extracts decreased to 11.8 TUs when using MetPLATE™ and to 148.6 TUs when using the *C. dubia* test.

The change of Zn toxicity in aged sandy soil is also displayed in Figure 4-1. After 1-month aging, the toxicity of Zn did not change significantly as shown by neither MetPLATE™ nor *C. dubia* test. However, after aging for 2 months, Zn toxicity in the sandy soil started to decrease significantly ($\alpha = 0.05$) as shown by both toxicity tests. When using MetPLATE™ toxicity test, the TU values of the soil extracts decreased from 268.7 TUs at month 1 to 182.6 TUs at month 4, and then continued decreasing to 88.0 after the 20th wet-dry cycle. The 48-h *C .dubia* test showed similar trends as MetPLATE™. Over the entire aging period (4-month dry aging plus 20 wet-dry cycles), the Zn toxicity in soil extracts dropped from a initial value of 520.7 TUs to 174.4 TUs, during which a very dramatic decrease was observed after the 12th wet-dry cycle (Figure 4-1).

These findings generally agreed with those reported by other researchers (Arias-Estéves et al., 2007; Lock and Janssens, 2003; Ma et al., 2006a; Ma and Uren, 2006; Oorts et al., 2007; Song et al., 2006; Stewart et al., 2003; Tye et al., 2003). Pedersen and van Gestel (2001)

compared the toxicity of copper to the collembolan *Folsomia fimetaria* L. in a spiked soil and in a soil from old Cu-contaminated field site. Large differences in effects were found between spiked soil and field soil when concentrations were expressed on the basis of total soil Cu concentration. The EC_{10} and EC_{50} values for reproduction of *Folsomia fimetaria* in spiked soil were 700 and 1400 mg Cu/kg soil, whereas no effects were found in field soil at Cu concentrations up to 2500mg/kg. Ma et al. (2006a) studied the long-term (2 years) effect of aging on Cu in 19 soil samples. They found that upon the addition of water soluble Cu to the soils, free Cu^{2+} activity in pore water in the soils with low pH (<5.5) and high organic matter content (>12.94%) was not significantly affected by incubation time. However, for the other soils, the free Cu^{2+} activity in soil pore water decreased with incubation time. Tye et al. (2003) examined the changes of Cd and Zn concentrations in soil pore water in 23 soils over an 818-day period. They reported that the changes in labile Cd and Zn were dependent on both time and pH. At low pH values, only a small decrease in metal concentrations was found over the aging period, whereas at high soil pH levels, greater decrease with time was observed. In addition, both Zn^{2+} and Cd^{2+} activities in soil pore water decreased with time. They also pointed out that the changes in metal concentrations and activities were probably due to time-dependent "fixation" of added metals and the readjustment of soil pH. In another study performed by Ma and Uren (2006), the effect of aging on the availability of Zn in a slightly calcareous soil was investigated. Their results showed that uptake of Zn by corn decreased over a 2-year aging period. Meanwhile, they also studied the effect of aging on the speciation and extractability of Zn added to the soil by a sequential extraction procedure. They concluded that when water-soluble Zn was added to a soil, it changed gradually into less available forms, mostly into the forms associated with Fe (Al) oxides and minerals.

4.3.1.2 Toxicity of Cu and Zn in aged organic rich soil

Soil organic matter is a critical component for the retention of heavy metals in soils; however, both the character and stability of the organic materials can influence the partitioning, speciation and environmental fate of metals (Martínez et al., 2003).

In our study, as shown in Figure 4-1, in 800 mg/kg Cu-spiked organic rich soil, the toxicity of Cu did not significantly ($\alpha = 0.05$) change during the entire aging period (4-month dry aging plus 20 wet-dry cycles) as determined by *C. dubia*, and TU values ranged from 18.1 to 25.7. When using MetPLATE^{TM} toxicity test, the toxicity of Cu was slightly increased from 4.6 TUs at time 0 to 5.5 TUs at month 4 and after the 20th wet-dry cycle, the TU value further increased to 7.6. Long-term chemical or biological transformation of soil organic matter may result in the release of dissolved organic mater and a potential increase in heavy metal mobility and bioavailability (Martínez et al., 2003). Martínez and his colleagues (2003) studied the combined effect of time and temperature on metal release and speciation from a metal contaminated soil (120 mg/kg Pb, 39.3 mg/kg Cd, 420 mg/kg Cu, 1480 mg/kg Zn, 100 mg/kg Ni, 800 mg/kg Mn, and 13 mg/kg Mo; 12.3% organic carbon) over a two-month period. Their results indicated that dissolved organic carbon (DOC) increased both with temperature and incubation time, and in general, total soluble metal release measured by inductively coupled plasma spectroscopy (ICP) paralleled the behavior of DOC.

As regards the change of Zn toxicity in spiked organic rich soil (Figure 4-1), when using the MetPLATE^{TM} toxicity test, from month 2 to the 12th wet-dry cycle, Zn toxicity slightly increased from 45.4TUs to 57.2TUs. However, after the 12th cycle, Zn toxicity started dropping, and the TU value after the 20th cycle decreased to 40.1, which was significantly ($\alpha = 0.05$) lower than the TU value at time 0. When using *C.dubia* test, from time 0 to the 12th wet-dry cycle, no significant change of Zn toxicity was observed, whereas from the 12th to the 20th cycle, the

toxicity of Zn decreased significantly ($\alpha = 0.05$) from 108.6 TUs to 63.7 TUs. Lock and Janssen (2003) studied the effect of aging on Zn toxicity and bioavailability in 20 soils (organic matter content ranged from 1.5% to 12%), and they reported that in freshly spiked soils, Zn partitioning is mainly determined by pH and cation exchange capacity, whereas only pH determines metal availability in aged soil. They also found that Zn availability was the highest in soils with low pH values, and aging has little effect on Zn bioavailability in these soils.

4.3.2 Effect of Aging on Magnetic Treatment

The effect of aging on magnetic separation of Cu and Zn from soils was also investigated. A comparison of treating freshly contaminated soils and aged soils is shown in Figure 4-2. After testing the toxicity of the treated soil extracts by both MetPLATE™ and *C. dubia*, it is found that soil aging had no adverse effect on magnetic separation of Cu from spiked sandy soil and organic rich soil. Throughout the entire aging period, after magnetic treatment, the TU values of all Cu-spiked sandy soil extracts were less than 1 as determined by MetPLATE™, and decreased from 6.2 TUs at time 0 to 2.8 TUs after the 20th wet-dry cycle, as determined by the 48-h *C .dubia* test. In the case of Cu-spiked organic rich soil, the toxicity of all treated soil extracts did not change much over the aging period, varying from 1.8 TUs to 2.2 TUs when using MetPLATE™, and decreased slightly from 5.4 TUs at time 0 to 2.8 TUs at the end when using *C. dubia* test.

In the case of Zn removal from the aged sandy soil, after magnetic treatment, the TU values of freshly spiked soil extract were 1.2 by MetPLATE™ and 5.0 by *C. dubia*, and the TU values of aged soil extracts varied from 1.1 to 1.7 by MetPLATE™, and from 3.5 to 5.3 by *C. dubia*. With regard to Zn removal from organic rich soil, compared to the TU values of treated soil extract at time 0 (30.1 by MetPLATE™ and 30.8 by *C. dubia*), the toxicity of treated soil extracts after different aging periods varied from 21.0 TUs to 27.9 TUs by MetPLATE™, and

from 24.8 TUs to 32.0 TUs by *C. dubia*. In conclusion, the aged soils extracts showed less or very close TU values to the freshly spiked soil extracts after magnetic treatment, which indicated that aging had no significant adverse effect on magnetic separation of Zn from sandy soil and organic rich soil.

It has been recognized that, in most cases, the chemical state and bioavailability of freshly spiked metals is very different from that of the same material in aged environmental medium (Sauvé, 2003). Therefore, ignoring changes in metal bioavailability due to the effect of aging may lead to under- or overestimation in risk assessment of contaminated soils (Lock and Janssen, 2003). However, it has also been reported that aging has little effect on metal availability in soils with low pH values; therefore, it should not be assumed that aging will eventually resolve all problems associated with metal contaminated soils (Lock and Janssen, 2003). Moreover, the effects of aging also raise questions about the possible effectiveness of remediation of sites containing compounds that may have aged and the application of existing models to predict the fate of chemicals at real field sites (Hatzinger and Alexander, 1995). Therefore, caution should be taken when relating results derived from bench scale laboratory tests using spiked soils to remediation of aged contaminated soils under field conditions. Ottosen et al. (2006) compared the effectiveness of electrodialytic removal of Cu from spiked kaolinite, spiked soil and industrially polluted soils under the same operational conditions. The results showed that the removal rate was higher in kaolinite than in both spiked soil and industrially polluted soils. The duration of spiking was also important in simulation remediation of industrially polluted soils. It was found that spiking for 2 days was too short; however, after 30 days the spiked soil showed a pattern similar to that of industrially polluted soils both regarding sequential extraction and remediation result, though the remediation still progressed slightly faster in the spiked soil. In

our study, as regards the effect of aging on magnetic separation of Cu and Zn from sandy soil and organic soil, we did not find significant adverse effect over the 7-month aging period, which indicated the potential of utilizing this magnetic separation method to remediate contaminated soils under field conditions.

4.4 Conclusions

In this study, the change of Cu and Zn toxicity in aged soils and the effect of aging on magnetic separation of Cu and Zn from soils were investigated. During the initial 4-month aging, as determined by either MetPLATE^{TM} or the 48-h *C. dubia* test, the toxicity of Cu in sandy soil did not show significant change. However, after 20 wet-dry cycles, Cu toxicity decreased significantly and gradually with time. Zn toxicity in aged sandy soil gradually decreased after 2-month aging until the 20th wet-day cycle as shown by both toxicity tests. Compared to freshly-spiked organic rich soil, Cu toxicity did not change significantly as determined by *C. dubia* test, whereas MetPLATE^{TM} showed a slight increase in Cu toxicity. Zn toxicity in aged organic rich soil only showed some decrease after the 12th wet-dry cycle as shown by both toxicity tests. As regards the effect of aging on magnetic separation of Cu and Zn from aged soils, no significant adverse effect was observed. Further investigation using longer aging periods would be necessary to evaluate the significance of this study.

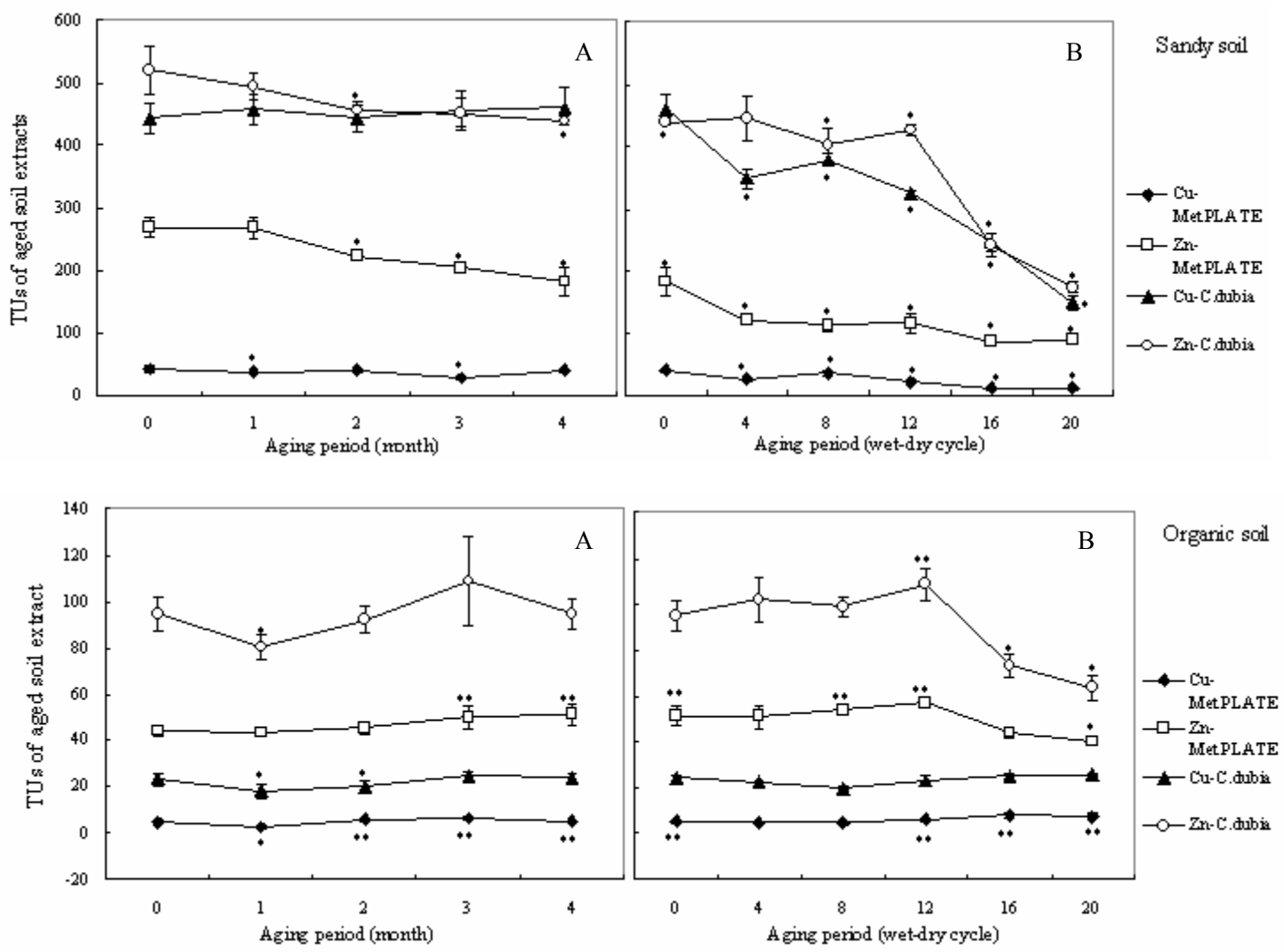


Figure 4-1. Toxicity of Cu and Zn in an aged sandy soil (A) dry aging (B) wet-dry aging, and an organic rich soil (A) day aging (B) wet-dry aging over a 4-month period and 20 wet-dry cycles, as determined by MetPLATE™ and the 48-h acute *Ceriodaphia dubia* assay (TU value indicated by one asterisk was significantly lower at the 5% level than the TU value at time 0; TU value indicated by two asterisks was significantly higher at the 5% level than the TU value at time 0. Error bars represent standard deviation of three replicates).

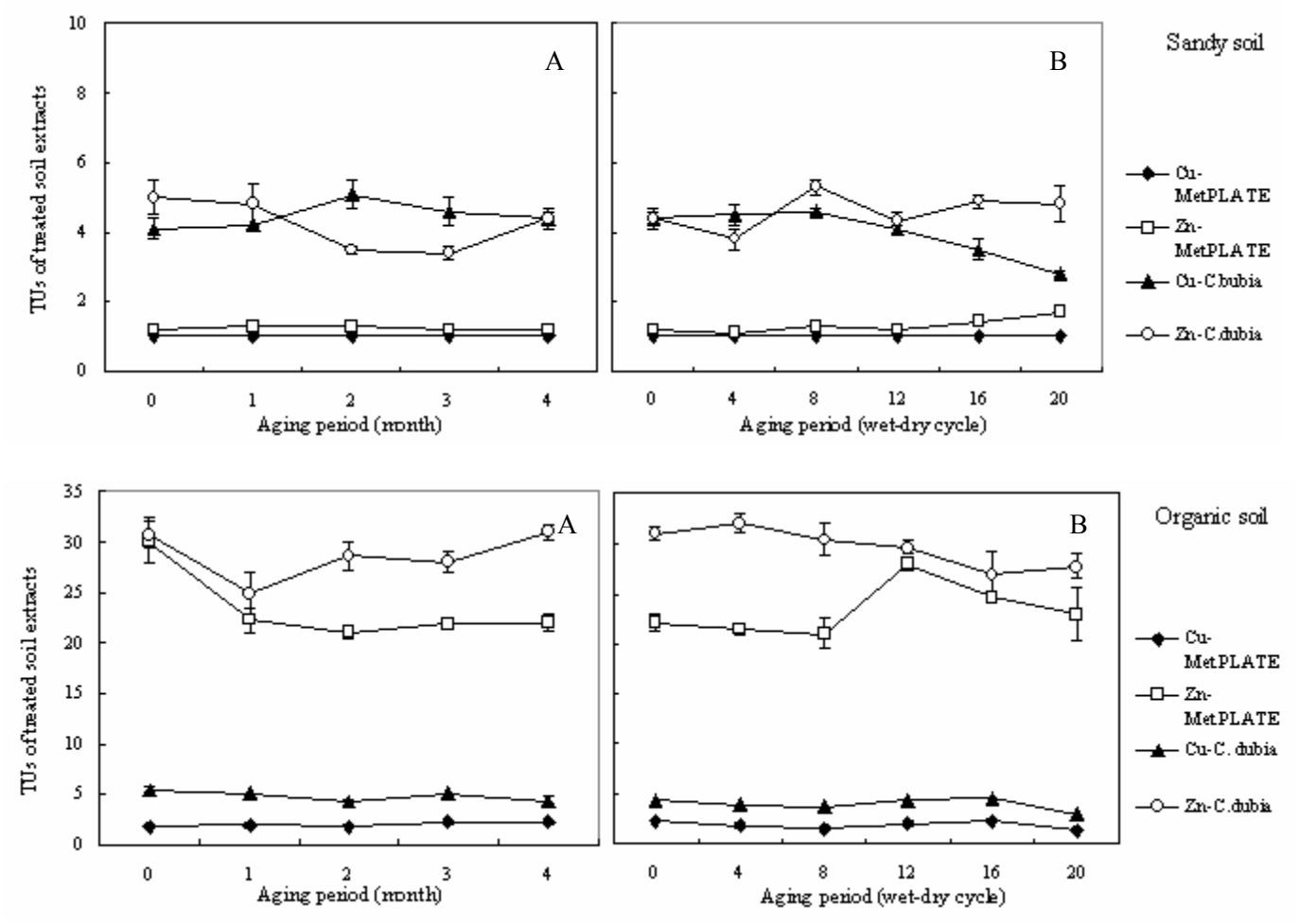


Figure 4-2. Effect of soil aging on magnetic treatment of spiked sandy soil (A) dry aging (B) wet-dry aging, and organic rich soil (A) dry aging (B) wet-dry aging over a 4-month period and 20 wet-dry cycles (Toxicity of treated soil extracts were measured by MetPLATE^{TM} and the 48-h acute *Ceriodaphnia dubia* assay. All Cu-spiked sandy soil extracts showed less than 1 TU after treatment, as determined by MetPLATE^{TM}. Error bars represent standard deviation of three replicates).

CHAPTER 5
LEAD REMOVAL FROM SHOOTING RANGE SOILS USING MAGNETIC SEPARATION

5.1 Introduction

Lead (Pb), as a well known toxic heavy metal, has been attracting much attention due to its widespread distribution and potential adverse effect on the environment (Zeng et al., 2007). Pb-contaminated soils can be found in both urban and rural environments (Chen et al., 2003). Humans can be exposed to Pb via air, drinking water, food, paint, and industrial releases (Adriano, 2001d). The Florida Department of Environmental protection (FDEP) has established a soil cleanup target level (SCTL) of 400 mg/kg for Pb in residential and commercial/industrial areas (FEDP, 2005). The interaction between Pb and soil components mainly includes specific adsorption, precipitation, and the formation of complexes or chelates with organic matter (Adriano, 2001d). Specific adsorption of Pb to Mn (hydr)oxide is proven to be greater than that to any other metal (hydr)oxides (Hettiarachchi et al., 2000). Due to the strong affinity of Pb for organic matter and its generally immobile nature, lead usually accumulates in the surface layers of soils (Adriano, 2001d). The mobility and bioavailability of Pb is highly site-specific (Turpeinen et al., 2000) and also depends on the forms and species of Pb (Adriano, 2001d).

To clean up Pb-contaminated soils, many remediation strategies have been proposed. Soil washing is one of the commonly used *ex-situ* technique, with a goal of transferring the contaminants from the solid to the aqueous phase (Grasso et al., 1997). Many extraction agents including acids, neutral salts, and chelating agents have been tested and showed successful applications under certain conditions (Cline and Reed, 1995; Isoyama and Wada, 2007; Kos and Leštan, 2003; Lin et al., 2001; Neilson et al., 2003; Reed et al., 1996; Van Benschoten et al., 1997). Electroremediation and phytoremediation are emerging techniques for removing metals from soils. Some laboratory and pilot-scale experiments have demonstrated the effectiveness of

electroremediation to extract Pb from soils (Altin and Degirmenci, 2005; Amrate et al., 2005; Chung and kang, 1999; Yang and Lin, 1998). To apply phytoremediation successfully, hyperaccumulators are required. By definition (Baker at al., 1994), a hyperaccumulator for Pb should reach more than 1000 mg-Pb /kg-shoot dry matter. Cui et al. (2004b) have shown that Indian mustard (*Brassica juncea* L.) and winter wheat (*Triticum aestivum* L.) can extract Pb from soils with the addition of elemental sulphur and EDTA; some other studies have shown that the use of vetiver grass (*Vetiveria zizanoides*), corn (*Zea mays* L.), or morning glory (*Ipomoea lacunose* L.) associated with the application of EDTA showed great potential as a remedial strategy (Hovsepyan and Greipsson, 2005; Kambhampati et al., 2003; Wilde et al., 2005).

In-situ Pb immobilization has been considered as a cost-effective alternative. Many amendments including coal fly ash, zeolite, natural organic matter, and gypsum- and lime-rich industrial by products have been utilized in chemical immobilization of Pb (Illera et al., 2004; Kumpiene at al., 2007; Shanableh and Kharabsheh, 1996), and numerous studies have indicated the successful immobilization of Pb by phosphorus-containing materials (Chen et al., 2003; Hettiarachchi et al., 2000; Hettiarachchi and Pierzynski, 2002; Melamed et al., 2003; Tang et al., 2004; Yang et al., 2001; Yoon et al., 2007).

In this study, we investigated the effectiveness of removing Pb from shooting range soils using magnetic separation. The toxicity of the soil extracts before and after magnetic treatment was determined by MetPLATE^{TM} assay and the 48-h *Ceriodaphnia dubia* acute toxicity test. Chemical analysis by ICP-AES was also performed to investigate Pb distribution in the soil matrix and extracts.

5.2 Material and Methods

5.2.1 Soils and Chemicals Used

Five soil samples were collected from different out-door shooting ranges in Florida. All these shooting facilities were operated for years. Soil 1 was collected from the mid-berm of a rifle shooting range in Ocala, Florida. Soil 2 and soil 3 were collected from the mid-berm of two different rifle areas at the same shooting range in Osceola, Florida. Soil 4 and soil 5 were collected respectively from a pistol range and a rifle range located in Gainesville, Florida, and both of them were sampled from the mid-berm. All soils samples were first air-dried, screened (sieve # 10; 2.0 mm particles) and homogenized. Table 5-1 shows the soil characteristics and the total Pb concentrations in the soil samples.

Iron filings (Fisher, 40 mesh) were placed in 1 M NaOH for 72 hours to increase the adsorption capacity and then washed thoroughly with distilled water before use (Yeager, 1998).

5.2.2 Toxicity of Soils under Study

Twenty milliliter of distilled water was added to 10 g of soil samples. The soil slurry was shaken for 4 hours at room temperature, and then centrifuged at 10,000 rpm for 15 minutes and the supernatant (soil extract) was removed with a pipet. The toxicity of the soil extracts were determined by measuring the EC_{50}s of the samples using the MetPLATE^{TM} toxicity assay (see Appendix A for detailed MetPLATE^{TM} protocol), and then the EC_{50}s were converted to toxicity units (TUs) according to Equation 3-1 (see Chapter 3). All soil samples as well as toxicity tests were run in triplicate.

5.2.3 Magnetic Separation of Pb from Shooting Range Soils

Twenty milliliter of distilled water was added to 10 g of soil samples. The soil slurry was shaken for 1 hour at room temperature. Then, 5% (0.5 g) of iron filings were added and the system was shaken for 3 hours followed by magnetic retrieval of the iron filings. The supernatant

(soil extract) was separated from the solid phase by centrifuging at 10,000 rpm for 15 minutes. The retrieved iron filings and the soil matrix following centrifugation were dried at 70°C overnight. Soil without iron treatment served as the control. Toxicity tests (i.e. MetPLATE^{TM} and the 48-h acute *Ceriodaphnia dubia* assay) were performed for all soil extracts before and after treatment. Chemical analysis was carried out for all factions of samples, including soil extracts, retrieved iron filings, and the soil matrix. Each soil sample was run in triplicate. Detailed procedures for MetPLATE^{TM} assay and the 48-h acute *Ceriodaphnia dubia* test were included in Appendix A.

5.2.4 Chemical Analysis

Chemical analysis was undertaken for all fractions, including the soil matrix, soil extracts and iron filings, before and after magnetic treatment. Soil extracts were digested using the U.S. EPA method 3010A (US EPA, 1992). Soils and iron filings were digested according to the U.S. EPA method 3050B (US EPA, 1996). All digested samples were analyzed for metals using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). See Appendix B for detailed digestion procedures.

5.3 Results and Discussion

5.3.1 Toxicity of Soils under Study

The total Pb concentration in the five soil samples varied from 1,538 mg/kg to 70,000 mg/kg, with an order of soil 1> soil 2> soil 5> soil 3> soil 4, which indicated Pb contamination of the shooting range soils. These findings support many recent studies that revealed very high Pb concentrations in the soils of shooting range (Cao et al., 2003; Chen and Daroub, 2002; Darling and Thomas, 2003; Hardison et al., 2004). Figure 5-1 shows the toxicity of the shooting range soil extracts determined by MetPLATE^{TM} assay. The toxicity units of these five soil extracts followed this order: soil 2 (36.6 TUs) > soil 3 (16.8 TUs) > soil 5 (14.2 TUs) > soil 1

(5.9 TUs)> soil 4 (4.5 TUs), which did not completely comply with the trend of the total Pb concentrations, possibly due to metal speciation and bioavailability. Therefore, it confirmed that like most trace metals, total Pb concentration in soils usually is not a good indicator of Pb bioavailability (Adriano, 2001d). Park et al. (2003) examined the bioavailability of metals at eight military shooting ranges in the Kyungkido and Kangwondo districts in Korea by measuring the exchangeable and soluble metal fractions as well as the metal content in the tissues of the plants growing at these sites. Although all of the sites were seriously contaminated with heavy metals, especially Pb and Cd, their results showed that only a small fraction of the total metal was soluble and as a result the metal bioavailability was very low.

In ecotoxicology, "bioavailability" can be broadly defined as the portion of a chemical in the environment which is available for biological action (e.g. uptake by an organism) (Adriano, 2001a). Generally speaking, bioavailability is a function of the solubility and mobility of metals (Adriano, 2001a), which mainly depends on the metal's chemical behavior, soil properties and the individual characteristics of the receptor (Siebielec et al., 2006). It is recognized that increasing soil pH leads to decrease of Pb content in plant roots. Besides, Pb has a strong affinity for organic matter and is generally immobile in soil (Adriano, 2001d). Pb can also be immobilized in soil by Fe and Mn oxides through specific adsorption and/or coprecipitation processes (Hettiarachchi and Pierzynski, 2004).

Various analytical methods have been used to assess metal availability, among which the chemical extraction technique is the most commonly used method. The soluble metal content in soil solution plus the exchangeable fraction provide a good measure of the plant-available amount (Adriano, 2001a). However, plant physiology and rhizosphere biochemistry can change the relationship between the extractant and plant tissue concentration (Basta et al., 2005). The

use of organisms (e.g. plant or soil microorganisms) to assess metal bioavailability could be more reliable but there are still concerns about the most suitable organisms and about the extrapolation of results from the laboratory to the field (Adriano, 2001a). Metal bioavailability and toxicity to soil microorganisms can be estimated by the impact of metals on the microbial community at either the population level (size of the population, community structure, species diversity, etc.) or at the functional level (e.g. respiration, element cycling, C and N mineralization, etc.) (Naidu et al., 2003b). Siebielec et al. (2006) evaluated the metal bioavailability and toxicity in 40 long-term contaminated soils sampled from the Tarnowskie Gory area (a metal mining area) of Poland. Metal availability was measured using chemical extraction, microbial activity and wheat (*Triticum aestivum*, L.) growth. Their study showed that despite high metal contents in most soils, the bioavailability of Zn, Pb, and Cd was relatively low and mostly independent on total metal contents. They also reported that soil contamination with metals did not reduce microbial activity, such as nitrification potential in soils, and the most contaminated soils had the highest microbial activity due to their relatively high organic matter and clay contents and neutral pH optimal for bacteria activity.

5.3.2 Evaluation of Magnetic Separation of Pb from Shooting Range Soils, Using MetPLATE™ and *Ceriodaphnia dubia* Acute Toxicity Tests

After a single magnetic treatment of the shooting range soils, as determined by MetPLATE™ (Table 5-2), all soil extracts became non-toxic with TU values less than 1, and the percent toxicity removal from the soils was generally higher than 77.8%. Pb toxicity removal from shooting range soils following magnetic treatment was also evaluated with the 48-hr *Ceriodaphnia dubia* acute toxicity test (Table 5-3). As discussed in Chapter 3, the 48-h *C. dubia* test showed higher TU values than the MetPLATE™ assay, due to the higher sensitivity of the daphnid test. The TU values of soil 1 and soil 2 were respectively 18.2 and 32.7 before

treatment, which then decreased to 1.5 and 1.3 after single magnetic treatment, and 91.6% and 95.9% of toxicity was removed from soil 1 and soil 2, respectively. The percent toxicity removal from Soil 3 and soil 4 was higher than 88.7%, which resulted in non-toxic soil extracts after magnetic treatment. As regards soil 5, the TU values of the soil extract decreased from 15.3 to 1.4 after treatment, which represents 91.0% toxicity removal.

5.3.3 Assessment of Pb Removal Efficiency Using Chemical Analysis

Following toxicity testing to assess Pb toxicity removal by the proposed magnetic treatment, we then used chemical analysis to study Pb distributions in the soil matrix and extracts. As shown in Table 5-4, for each soil, a large portion of Pb was retained in the soil matrix, and little amount of Pb was present in soil extracts, which also indicated the low bioavailability of these aged shooting range soils. Pb removal from soil extracts was very high, ranged from 78.0% to 98.3%, whereas much lower Pb removal (2.1% to 12.5%) was found from the soil matrix. Table 5-4 also shows that Pb was adsorbed to the iron filings and reached Pb concentrations ranging from 242.4 mg/kg for soil 4 and 3529.5 mg/kg for soil 1.

5.4 Conclusions

By comparing toxicity of the soil extracts and the total Pb in the soils, it is confirmed that total metal concentrations may not be used to predict the bioavailability and thus the toxicity of this metal in natural soils. As regards the effectiveness of the magnetic separation method on removing Pb from shooting range soils, the results have shown that a great reduction of toxicity generated by Pb was obtained after single magnetic treatment. The chemical analysis suggested that a large portion of Pb was retained in the soil matrix, and although Pb was removed from both of the soil matrix and soil extracts, the removal from soil matrix was always lower than that from the soil extracts. Moreover, the chemical analysis also demonstrated that Pb was indeed adsorbed and concentrated on the iron filings. Therefore, this magnetic separation method

showed great potential in decontaminating heavy metal-contaminated soils that have been aged for years under field condition.

Table 5-1. Soils characteristics

Characteristic	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
pH	6.2	5.1	5.6	6.4	6.8
% Organic carbon	0.1	0.4	0.4	0.4	0.5
% Organic matter	0.2	0.7	0.7	0.7	0.9
% Sand	86.6	78.3	89.4	93.8	88.0
% Silt	9.5	17.2	7.9	2.9	7.1
% Clay	3.9	4.5	2.7	3.3	4.9
CEC ($cmol_c/kg$)	11.1	11.0	5.8	16.5	24.9
Total Pb (mg/kg)	70,000	12,400	3,256	1,538	11,490

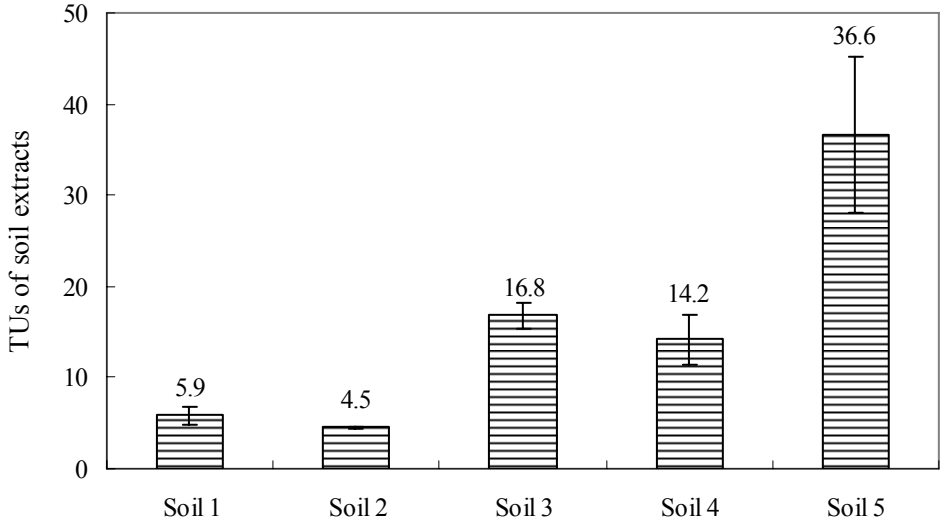


Figure 5-1. Toxicity of shooting range soil extracts, as determined by MetPLATE^{TM} assay (error bars represent standard deviation of three replicates)

Table 5-2. Lead toxicity removal from five shooting range soils by magnetic treatment, as determined by MetPLATE™

Soil ID	Pb^{2+} conc. in soil (mg/kg)	Treatment[a] times	EC_{50} of soil extract (% soil extract)		Toxicity units[c] of soil extract		Toxicity removal (%)
			No treatment	After Magnetic treatment	No treatment	After Magnetic treatment	
Soil 1	70,000	1	17.1 ± 2.9% [b]	>100%	5.9 ±1.0	<1[d]	>82.9%
Soil 2	12,400	1	2.8 ± 0.7%	>100%	36.6 ± 8.6	<1	>97.2%
Soil 3	3,256	1	6.0 ± 0.5%	>100%	16.8 ± 1.5	<1	>94.0%
Soil 4	1,538	1	22.2 ± 1.2%	>100%	4.5 ± 0.2	<1	>77.8%
Soil 5	11,490	1	7.2 ± 1.4%	>100%	14.2 ± 2.7	<1	>92.8%

[a] 5% (0.5 g) iron filings were added to the shooting range soils, and the contact time was 3 hours; [b] Mean of 3 replicates ± one standard deviation; [c] Toxicity units = $100/EC_{50}$; [d] Non-toxic.

Table 5-3. Lead toxicity removal from five shooting range soils by magnetic treatment, as determined by the 48-h acute *Ceriodaphnia dubia* test

Soil ID	Pb^{2+} conc. in soil (mg/kg)	Treatment[a] times	EC_{50} of soil extract (% soil extract)		Toxicity units[b] of soil extract		Toxicity removal (%)
			No treatment	After Magnetic treatment	No treatment	After Magnetic treatment	
Soil 1	70,000	1	5.5 ± 0.3%[c]	65.5 ± 0.8	18.2 ± 0.8	1.5 ± 0.02	91.6 ± 0.5%
Soil 2	12,400	1	3.1 ± 0.1%	75.4 ± 1.0%	32.7 ± 1.2	1.3 ± 0.02	95.9 ± 0.1%
Soil 3	3,256	1	3.0 ± 0.1%	>100%	33.1 ± 0.6	<1[d]	>97.0%
Soil 4	1,538	1	11.3 ± 0.2%	>100%	8.8 ± 0.1	<1	>88.7%
Soil 5	11,490	1	6.5 ± 0.1%	72.5 ± 5.3%	15.3± 0.3	1.4 ± 0.1	91.0 ±0.5%

[a] 5% (0.5 g) iron filings were added to the shooting range soils , and the contact time was 3 hours; [b] Toxicity units = $100/EC_{50}$; [c] mean of 3 replicates ± one standard deviation; [d] Non-toxic.

Table 5-4. Effect of magnetic treatment on the removal of Pb from shooting range soils, as determined by chemical analysis

Soil ID	Initial Pb conc. in soil (mg/kg)		Pb conc.[b] in soil matrix, extracts, and iron filings			Pb removal from soil fractions (%)	
			In soil matrix (mg/kg)	In soil extract (mg/L)	Adsorbed on iron filings(mg/kg)	From soil matrix	From soil extract
Soil 1	70,000	No treatment	67685±1152.6	10.7± 0.8	No iron filings added	2.1 ± 0.9%[d]	98.3 ± 0.01%
		Magnetic treatment[a]	66270±551.5	0.2± 0.01	3529.5 ± 274.1[c]		
Soil 2	12,400	No treatment	10932±166.9	31.4 ± 2.7	No iron filings added	9.0 ± 0.7%	93.2 ± 1.5%
		Magnetic treatment	9950.5±231.2	2.1 ± 0.3	916.1 ± 64.7[c]		
Soil 3	3,256	No treatment	2962.0 ±217.8	23.5 ± 5.0	No iron filings added	3.3 ± 2.0%	92.4 ± 0.6%
		Magnetic treatment	2862.0 ±149.9	1.8 ± 0.2	575.2 ± 92.8[c]		
Soil 4	1,538	No treatment	1328.5 ± 74.2	4.8 ±0.01	No iron filings added	12.5 ±0.5%	88.7 ± 1.3%
		Magnetic treatment	1163.1 ± 72.2	0.5 ± 0.1	242.4 ± 6.4[c]		
Soil 5	11,490	No treatment	9960 ± 149.9	18.0 ± 0.7	No iron filings added	7.1 ± 4.5%	78.0 ± 4.9%
		Magnetic treatment	9252.5±310.4	3.9 ± 0.7	737.3 ± 90.1[c]		

[a] 5% (0.5 g) iron filings were added to the contaminated soils , and the contact time was 3 hours; [b] Detected by ICP-AES; [c] Iron filings had a Pb background value of 56.8 mg/kg. This concentration was subtracted from the Pb concentration in filings after treatment; [d] Mean of 3 replicates ± one standard deviation.

CHAPTER 6
HEAVY METAL REMOVAL FROM SEDIMENTS USING MAGNETIC SEPARATION

6.1 Introduction

Sediments are considered a mixture of assorted materials that settle to the bottom of a water body (US EPA, 1993). During the sedimentation process, water will be trapped and entrained in the sediment, forming the pore water (Batley and Giles, 1979). Once toxicants enter into the aquatic system, they tend to be sorbed onto sediments which act as "sinks" that accumulate higher levels of chemicals than the overlying water column (Mendil and Uluözlü, 2007; Nowierski et al., 2006; Wang and Chen, 2000). Within the sediment matrix, chemical species tend to reach equilibrium between the pore water and solid phase; however, the concentrations of chemical species in the pore water, which are useful in determining sediment quality and contamination, are not necessarily the same as those in the overlying water (Bufflap and Allen, 1995).

Recently, issues related to heavy metal contaminated-sediments have been attracting increasing attention of regulatory agencies and researchers. Metals tend to accumulate in sediments and a variety of sediment constituents including clay minerals, iron oxides, manganese oxides, and organic matter are considered metal adsorbents (Jenne, 1995; Wang and Chen, 2000). The accumulated heavy metals in sediments may be remobilized by natural and man-made processes (Lin et al., 2003), and become available for biological uptake and then contaminate the food chain. Metals which are not directly available for biological uptake may become available later through changes in physicochemical conditions or erosion of sediment deposits (Tarras-Wahlberg and Lane, 2003). The cycling of trace metals in sediments is governed by precipitation and dissolution of minerals (Ouddane et al., 2004), whereas the mobility and toxicity of metals are generally affected by metal speciation, sediment composition,

occurrence of complexing agents, and fundamental physicochemical conditions, such as pH and Eh (Lin et al., 2003; Luther, 1995; Ouddane et al., 2004; Pardue et al., 1995; Tack et al., 1996). Like heavy metal fractionations in soil, sequential extraction methods can also be applied to sediments to divide the heavy metals into five fractions (exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic matter/sulfide-bound, and residual) (Lin et al., 2003; Pardo et al., 1990; Salomons and Förstner, 1980; Tessier et al., 1979). Metals in exchangeable, carbonate-bound, and Fe/Mn oxide-bound forms are considered to be more mobile and bioavailable than organic-bound and residual forms. Therefore, the determination of the amount of metal in each binding form is more useful than the measurement of total metal content in predicting the potential environmental effects caused by contaminated sediments (Lin et al., 2003).

Although numerous remediation methods have been tested and successfully applied to heavy metal contaminated-soils, much less is known about sediment treatment (Mulligan et al., 2001b). Particle size and organic matter content in sediments significantly affect the selection of treatment strategy. Fine textured sediments have a much higher affinity for all types of contaminants, which are more difficult to remediate (US EPA, 1993). The remediation of metal-contaminated sediments can be accomplished either *in situ* or *ex situ* by physical or chemical treatments (Mulligan et al., 2001b; US EPA, 1993). Pretreatment of dredged or excavated sediments such as dewatering is usually required to modify the physical and chemical characteristics of the sediments (US EPA, 2005). Currently, contaminated dredged sediments are mainly buried in landfills; however this cannot be considered as a long-term solution (Löser et al., 2005). Other main treatments for metal-contaminated sediments include solidification/stabilization, washing, and bioremediation. The purpose of solidification/stabilization is to reduce the mobility of contaminants by treating them with

reagents (lime, fly ash, cement, etc.); however, long-term monitoring is required since this process can be reversible (Mulligan et al., 2001b; US EPA, 1993). Sediment washing is primarily useful for sands and gravels, the washing solutions can be solely water or water in combination of organic solvents, chelating compounds, surfactants, acids or bases (Mulligan et al., 2001b; US EPA, 1993). Biological processes have recently been under development and gained increasing attention since they are environmentally friendly and economical (Chen and Lin, 2001). However, techniques for metal remediation are not as developed as those for organic contaminants (Mulligan et al., 2001b). Some studies have investigated the effectiveness and kinetics of bioleaching to decontaminate sediments (Chen and Lin, 2001; Löser et al., 2005, 2006, 2007). Metal removal by the bioleaching process is based on dissolving toxic metals in sulfuric acid which is produced by microorganisms through oxidation or reduction of sulfur compounds (Chen and Lin, 2001).

The objective of this research was to evaluate the effectiveness of removal of heavy metals (Cu^{2+}, Zn^{2+}, and Hg^{2+}) from four sediments using the proposed magnetic separation method which had been shown to be effective for treating metal-contaminated soils. The sediment extracts were tested by MetPLATE^{TM} assay and the 48-h *Ceriodaphnia dubia* acute toxicity test to assess the reduction of heavy metal toxicity in sediments. In addition, chemical analysis and mass balance studies were also performed to investigate heavy metal distribution in the sediment matrix and extracts.

6.2 Material and Methods

6.2.1 Sediments Used

Two types of sediments were collected at 4 different water bodies in Gainesville, Florida. The sandy sediments were sampled from the Little Hatchet Creek and Hogtown Creek, respectively. An organic rich sediment was collected from a pond near Home Depot in

Southwest Gainesville. The second organic rich sediment was obtained from Lake Alice on the University of Florida campus. All sediments were collected from the top few inches. Table 6-1 shows the main characteristics of the sediments under study. All sediments samples were first air-dried and stored in the refrigerator until use.

6.2.2 Chemicals Used

Three heavy metal solutions (Cu^{2+}, Zn^{2+}, and Hg^{2+}) were used. Copper solution was prepared from copper sulfate ($CuSO_4 \bullet H_2O$, Sigma®). Zinc solution was prepared from zinc chloride ($ZnCl_2$, Sigma®). Mercury solution was prepared from mercury chloride ($HgCl_2$) purchased from Mallinckrodt. Iron filings (Fisher, 40 mesh) were placed in 1M NaOH for 72 hours to increase the adsorption capacity and then washed thoroughly with distilled water before use (Yeager, 1998).

6.2.3 Sediment Heavy Metal Binding Capacity

The purpose of determining the heavy metal binding capacity (HMBC) of sediments is to get an idea of how much metal would be necessary for spiking sediments to produce metal toxicity. The spiked sediments would subsequently be used to assess the effectiveness of magnetic treatment.

Briefly, the methodology for testing sediment heavy metal binding capacity is similar to that used for assessing soil heavy metal binding capacity (SHMBC) (see Chapter 2). The sediments were first screened (sieve # 16; 1.19 mm particles) and homogenized. Then, serial dilutions of metal-spiked solutions were prepared in moderately hard water 60 mg/L Ca, 60 mg/L Mg, pH = 7.4-7.8) for sediment spiking. Three metal solutions containing Cu^{2+}, Zn^{2+}, or Hg^{2+} were prepared. Twenty milliliter of each solution were added to 5 g (dry weight) of sediment in 50-mL Erlenmeyer flasks. The flasks were covered with parafilm and placed on a shaker at 300 rpm for 4 hours. After shaking, the solid phase was separated from the pore water

by centrifugation at 10,000 rpm for 15 minutes. The metal toxicity of the sediment extracts was assayed with MetPLATE^{TM}, a microbial test which responds specifically to heavy metal toxicity (See Appendix A for detailed MetPLATE^{TM} procedure). Sediments spiked with moderately hard water served as negative controls. Regression analysis was then used to determine the EC_{50}s for both the sediments under study and the reference sediment. Two sets of reference material were used including Ottawa sand and one of the sediments which showed the lowest EC_{50} (i.e. bound the smallest amount of metals). The sediment HMBC was determined by dividing the EC_{50} for the sediment sample by the EC_{50} for the metal in the reference sediment as defined in Equation 6-1.

$$\text{Sediment HMBC} = \frac{EC_{50} \text{ of field sediment spiked with a given metal}}{EC_{50} \text{ of reference sediment spiked with the same metal}} \quad (6\text{-}1)$$

A detailed description of the HMBC procedure was discussed in Chapter 2 and the corresponding methodology was summarized in Figure 2-1. All HMBC tests and MetPLATE^{TM} toxicity tests were run in triplicate.

6.2.4 Magnetic Separation of Heavy Metals from Metal-Spiked Sediments

After the determination of the sediments heavy metal binding capacity, a sandy sediment and an organic rich sediment were selected to assess the effectiveness of removing Cu^{2+}, Zn^{2+}, and Hg^{2+} from spiked sediments by magnetic separation.

Fifty gram (dry weight) of sediment was spiked with 40 mL of Cu^{2+}, Zn^{2+} or Hg^{2+} solutions. Based on the HMBC values, two concentrations (125 mg/L and 250 mg/L) of heavy metal solutions were spiked into the Hogtown creek sandy sediment, resulting in 100 mg-metal/kg sediment and 200 mg-metal/kg sediment. For the organic rich sediment collected from the Home Depot pond, much higher concentrations, 500 mg/L and 1000 mg/L, were used, which generated metal concentrations of 400 mg/kg sediment, and 800 mg/kg sediment, respectively. The spiked sediment was shaken for 1 hour at room temperature, then 5% (2.5 g iron /50 g

sediment) of iron filings were added and the mixture was shaken for 3 hours at room temperature. Then, the iron filings were magnetically retrieved using a ferrimag rectangular magnet (Scientifics®, 152 ×102×25mm, 3.4 megagauss oersteds), and 60 mL of distilled water was added into the system to bring total solution volume to 100 mL. The sediment slurry was centrifuged at 10,000 rpm for 15 minutes. The supernatant (sediment extract) was removed with a pipet and was used after overnight settling in the refrigerator. After centrifugation the retrieved iron filings and the sediment matrix were dried at 70°C overnight. Toxicity testing was undertaken for all sediment extracts before and after magnetic treatment. Sediment without iron treatment served as the control. Chemical analysis by ICP-AES was performed for all factions, including sediment extracts, retrieved iron filings, and sediment matrix. Each sediment sample was run in triplicate.

6.2.5 Toxicity of Sediment Extracts

Two toxicity assays, MetPLATE™ and the 48-h *Ceriodaphnia dubia* acute bioassay were used to assess the toxicity of the sediment extracts before and after iron treatment. The EC_{50} of the sediment extract was determined by regression analysis, and was then converted to toxicity unit ($TU = 100/EC_{50}$). Higher TU values indicate higher toxicity. All toxicity tests were run in triplicate and the detailed test procedures are described in Appendix A.

6.2.6 Chemical Analysis

Chemical analysis was performed for all fractions, including the sediment matrix, sediment extracts and iron filings, before and after magnetic treatment. For Cu and Zn spiked sediment fractions, the sediment extracts were digested according to the U.S EPA method 3010A (US EPA, 1992), and sediments and iron filings were digested according to the U.S. EPA method 3050B (US EPA, 1996). Then, all digested samples were analyzed for total Cu and Zn using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). In the case of Hg-spiked

sediment, different digestion and analysis methods were employed. The U.S. EPA method 1631(US EPA, 2002b) was utilized for digesting sediment extracts, whereas the sediment matrix and iron filings were digested with a 7:3 (v: v) mixture of HNO_3/H_2SO_4 in 50 mL Teflon containers in a hot block overnight (adapted from Warner et al., 2003). All digested samples were then analyzed by SnCl2 (tin chloride) reduction technique (US EPA, 2002b) with a cold vapor atomic fluorescent spectroscopy (CV-AFS). Mass balance studies were also undertaken for the Cu^{2+}, Zn^{2+}, and Hg^{2+} spiked sediments. All samples were run in triplicate. Detailed digestion procedures are included in Appendix B.

6.3 Results and Discussion

6.3.1 Sediment Heavy Metal Binding Capacity

The methodology for assessing sediment heavy metal binding capacity (HMBC) is based on toxicity testing of extracts from metal-spiked sediments with MetPLATE^{TM}, a test specific for heavy metal toxicity (Bitton et al., 1994). Three metals (Cu, Zn, and Hg) were tested for their binding capacity to four sediments sampled in Gainesville, Florida. Table 6-2 shows the EC_{50}s (expressed as added metal in mg/kg sediment) of the three metals in the different sediments under study. As discussed in Chapter 2, the Ottawa sand displayed a very low binding capacity for metals, with EC_{50}s of 1.1 mg/kg for Cu, 0.9 mg/kg for Zn, and 1.5 mg/kg for Hg, which were lower than those shown by the sediments under study. Therefore, the Ottawa sand was still utilized as one of the reference sediments. Among the four sediments studied, the sandy sediment sampled from Little Hatchet Creek displayed the lowest EC_{50}s (highest toxicity to MetPLATE^{TM}) for the three metals tested, and its EC_{50}s, expressed as metal added to sediments in mg/kg, were 4.3 for Cu, 14.3 for Zn, and 18.4 for Hg (Table 6-2). Thus, the sediment from Little Hatchet Creek was employed as the second reference due to its lowest binding capacity for metals among the sediments under study. Another sandy sediment from Hogtown Creek showed

a little higher EC_{50}s (25.8 for Cu, 35.6 for Zn, and 31.2 for Hg) than Little Hatchet sediment, whereas, the EC_{50}s of the two organic rich sediments for the three metals, varied between 92.3 and 111.2 for Cu, between 122.6 and 139.7 for Zn, and between 162.7 and 644.1 for Hg, were much higher than those of the sandy sediments (Table 6-2).

The sediment heavy metal binding capacity for the three metals and four sediments tested is shown in Figure 6-1, using Ottawa sand as the reference. Figure 6-2 displays the sediments HMBC, using Little Hatchet sediment as the reference. In both figures, the organic rich sediments showed higher binding capacity towards the three metals than the sandy sediments due to their higher organic matter content (5.8%-10.5%). This finding consisted with the results shown in Chapter 2, that the organic rich soils had higher SHMBC than the sandy soils.

It is well known that organic matter content is one of the key factors controlling metal mobility and toxicity in soils and sediments (Adriano, 2001a, 2001b; Jenne, 1995; Ouddane et al., 2004). Through studying sediment cores collected from 20 lakes in the Muskoka region of Ontario, Canada, El Bilali et al. (2002) found that organic matter played a key role in the enrichment of Cu, Zn, Hg, Pb, and Cd in surface sediments. The HMBC concept was previously used to assay metal bioavailability in surface waters (Huang et al., 1999) and municipal landfill leachates (Ward et al., 2005). Chapter 2 showed the first application to date of the bioassay to assess the metal bioavailability in soils. Similarly, the sediment HMBC method could be used to predict the metal bioavailability to aquatic organisms. França et al. (2005) investigated the enrichment of heavy metals in benthic invertebrates and fish in three salt marsh areas in the Tagus estuary, Portugal. Their results revealed that although Hortas salt marsh (contained more sand and lower organic matter) contained lower Cu concentration than the other marshes, the enrichment of Cu in the invertebrates and fish in this area was the highest. Therefore, the total

metal concentrations in sediments may not give an accurate indication of metal bioavailability, since the sediment characteristics play an important role in determining metal solubility and toxicity. Our findings in this section, confirm that, at least for Cu, Zn and Hg, the Sediment HMBC for organic rich sediments is much higher than for sandy sediments. This research should be expanded to include more sediments and metals.

6.3.2 Use of MetPLATE™ to Evaluate the Effectiveness of Magnetic Separation of Cu, Zn and Hg from Spiked Sediments.

A sandy sediment and an organic rich sediment, collected respectively from Hogtown Creek and Home Depot Pond, were spiked with individual metal (Cu^{2+}, Zn^{2+}, or Hg^{2+}) solutions based on their HMBC values. For the three metals under study, the toxicity of the sediment extracts before and after magnetic treatment was determined by measuring the EC_{50}s. The EC_{50} was then converted to toxicity unit ($TU = 100/EC_{50}$). It is worth mentioning that higher TU values indicate higher toxicity. The percent toxicity removal was calculated according to Equation 3-2.

Table 6-3 shows that the toxicity removal efficiency from the spiked sandy sediment varied with the type of metal. Comparing the three heavy metals used, at equal concentrations, Zn resulted in the highest toxicity (or highest TU values) in the sediment extracts than Hg and Cu before treatment. Cu toxicity in the sandy sediment extracts before magnetic treatment varied from 10.4 TUs when the sample was spiked with 100 mg Cu^{2+}/kg sediment to 158.6 TUs when the sample was spiked with 200 mg Cu^{2+}/kg. As regards Hg^{2+}, toxicity was 25.1 TUs and 250.7 TUs at spiking levels of 100 mg and 200 mg Hg^{2+}/kg sediment, respectively. Zn toxicity was 63.7 TUs and 514.8 TUs at spiking levels of at 100 mg and 200 mg Zn^{2+}/kg sediment, respectively (Table 6-3). After a single magnetic treatment, the TU values of the sediment extracts were all less than 1 for all three heavy metals, which indicated significant reductions in

toxicity. The percent toxicity removal from Cu-, Zn-, and Hg-spiked sandy sediment was generally higher than 90.4% (Table 6-3).

With regard to metal-spiked organic rich sediment, as shown in Table 6-4, the toxicity (or TU values) generated by the three metals in sediment extracts before treatment followed this order: Zn> Cu > Hg. For Cu-spiked organic rich sediment, before treatment, the TU values of the sediment extracts were 12.3 and 70.1 at spiking levels of 400 mg and 800 mg Cu^{2+}/kg sediment, respectively. After a single magnetic treatment of Cu-spiked sediment, the TU values of the sediment extracts decreased to 1.7 and 1.9 at Cu^{2+} input concentrations of 400 mg and 800 mg/kg, respectively. This represents a toxicity reduction of 86.4% to 97.2%. In the case of Zn-spiked organic rich sediment, after a single magnetic treatment, the TUs of the sediment extracts decreased from 53.5 to 3.2 at 400 mg Zn^{2+}/kg sediment, and from 242.0 to 7.3 at 80 mg Zn^{2+}/kg sediment, generating a toxicity removal of 94.0% and 97.0%, respectively (Table 6-4). As regards Hg-spiked organic rich sediment, no toxicity (TU<1) was observed in the sediment extract after a single magnetic treatment at Hg spiking level of 400 mg/kg. At a spiking level of 800 mg Hg^{2+}/kg sediment, toxicity decreased from 49.9 TUs to 1.3 TUs, leading to a toxicity reduction of 97.3% (Table 6-4). Considering the effect of sediment characteristics, this magnetic treatment worked effectively in both sandy sediment and organic rich sediment. Moreover, the metal type did not exert significant effect on the metal removal efficiency.

6.3.3 Use of *Ceriodaphnia dubia* Acute Toxicity Test to Evaluate the Effectiveness of Magnetic Separation of Cu, Zn and Hg from Spiked Sediments.

Metal toxicity removal from sediments following magnetic treatment was also evaluated with the 48-hr *Ceriodaphnia dubia* acute toxicity test. The results are shown in Tables 6-5 and 6-6. Comparing with MetPLATE^{TM} assay, the 48-h *C. dubia* test showed higher TU values due to the higher sensitivity of the daphnid test, which had already been shown in Chapters3, 4, and 5.

However, as regards toxicity removal of Cu, Zn, and Hg from sediments following magnetic treatment, the two tests showed a similar trend. For Cu-, Zn-, and Hg-spiked sandy sediment, the toxicity removal from the sediments was generally higher than 90.4% when using MetPLATE^{TM}, and higher than 99.0% when using the *C. dubia* test. The sandy sediment spiked with 100 mg/kg Cu^{2+}, 200 mg/kg Cu^{2+}, or 100 mg/kg Zn^{2+} all became non-toxic after a single magnetic treatment. For Cu-, Zn-, and Hg-spiked organic rich sediment, after a single treatment, the toxicity removal from the metal-spiked sediments was higher than 83.0% using MetPLATE^{TM}, and ranged from 80.5% to 99.8% when using the *C. dubia* test.

6.3.4 Assessment of Metal Removal Efficiency Using Chemical Analysis

Besides toxicity tests, we also employed chemical analysis to assess the removal efficiency of Cu, Zn, and Hg from metal-spiked sediments, as well as the distributions of these three metals in the sediment matrix and extracts. All fractions, including the sediment matrix, sediment extracts and iron filings, before and after magnetic treatment from the sediments spiked with the highest Cu, Zn and Hg concentrations (200 mg-metal/kg for sandy sediment and 800 mg-metal /kg for organic rich sediment) were digested and chemically analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (for Cu and Zn) and cold vapor atomic fluorescent spectroscopy (CV-AFS) (for Hg).

For sandy sediments, as shown in Table 6-7, the metal removal from the sediment matrix was the highest for Hg (79.3%), followed by Cu (38.2%) and Zn (25.9%). However, the removal of Cu, Zn and Hg from sediment extracts was much higher, and varied from 95.8% to 99.5%. In addition, very high concentrations of Cu (1700.2 mg/kg), Zn (1886.5 mg/kg), and Hg (1923.4 mg/kg) were found in the retrieved iron filings, which demonstrated that the metals were indeed adsorbed and concentrated on the iron filings.

Table 6-8 shows the percent removal of Cu, Zn, and Hg re from organic rich sediment factions. Among the three metals studied, Hg showed the highest removal from both sediment matrix (58.5%) and extracts (93.3%). The removal of Cu and Zn from the sediment matrix were 18.5% and 27.9%, respectively. Cu removal from the sediment extracts was 69.3%, as compared to 35% removal for Zn. The concentrations of Cu, Zn and Hg adsorbed onto the iron filings followed this order: Hg (6,014.2mg/kg) > Cu (2,441.5mg/kg) > Zn (1,789.3mg/kg).

Oxides and oxyhydroxides of iron play a significant role in sequestering elements due to their large surface area and strong affinity for many elements (Parida et al., 1997). The sorptive properties of iron (hydr)oxides for various metals and metalloid including Cu, Cd, Zn, Pb, Co, Ni, and As have been extensively investigated (Bryce et al., 1994; Esmadi and Simm, 1995; Lee and Anderson, 2005; Nayak et al., 2006; Swallow et al., 1980; Wilkie and Hering, 1996; Yamaguchi and Okazaki, 2002). A study conducted by Namasivayam and Senthilkumar (1999) used Fe (III)/Cr (III) hydroxide as an absorbent to remove Cu^{2+} from aqueous solutions. Besides, Namasivayam and Ranganathan (1995) had investigated the adsorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} on Fe (III)/Cr (III) hydroxide. These studies showed that the adsorption of metal ions increased when the adsorbent concentration increased and the particle size decreased, and the adsorption obeyed the Freundlich isotherm model. However, much fewer studies on mercury adsorption onto iron (hydr)oxides have been carried out. Kim et al. (2004a) employed extended X-ray absorption fine structure (EXAFS) spectroscopy to study Hg^{2+} sorption to iron (hydr)oxides and their results indicated that Hg^{2+} formed inner-sphere sorption complexes to goethite (α-FeOOH) over a pH range of 4 to 8. Another study carried out by Kim et al. (2004b) revealed that chloride ligands resulted in reduced Hg^{2+} sorption to goethite, whereas sulfate could enhance the Hg^{2+} binding to goethite.

In all, the chemical analysis suggested that Cu, Zn, and Hg were removed from both the sediment extracts and the sediment matrix. However, for each heavy metal, in a given sediment, metal removal from the sediment extracts was always higher than that from the sediment matrix, which agreed with the results in chapter 3 on metal removal from soil fractions.

6.3.5 Mass Balance of Metals in Sediments

Tables 6-9 and 6-10 display the mass balance of Cu, Zn, and Hg in the spiked sandy sediment and organic rich sediment before and after magnetic treatment. As shown in these tables, a large portion of the metals was immobilized in the sediment matrix before treatment, but the metals associated with the sediment matrix and in the sediment extracts were both reduced following magnetic treatment, these findings are consistent with those derived from the mass balance study of metals in soils (see in chapter 3). The total recovery of metals was not significantly affected by the sediment type. In the sandy sediment, 87.4% of Cu was accounted for before treatment whereas 81.5% of Cu was accounted for after treatment. In the organic rich sediment, the mass balance accounted for 94.1% Cu before treatment and 91.2% after treatment. As regards Zn, the mass balance accounted for 77.4% and 83.5% Zn before magnetic treatment of the sandy and organic rich sediment, respectively. Following magnetic treatment, 81.2% and 70.8% of Zn was accounted for in the sandy and organic rich sediment, respectively. The total recovery of added Hg from sediments was somewhat lower than that of Cu and Zn. Before treatment, the recovery of Hg was 76.7% and 67.6% in the sandy sediment and the organic rich sediment, respectively. After magnetic treatment, only 60.7% and 65.1% of Hg was recovered from the sandy sediment and organic rich sediment, respectively. The lower recovery of Hg probably came from the digestion process of Hg-containing iron filings.

Comparing with *ex situ* treatment of contaminated sediments, fewer techniques can be used for *in situ* treatment due to a variety of limitations, such as saturated conditions, anaerobic

environments and ambient temperatures; however, *in situ* treatment is generally considered to be less expensive than *ex situ* treatment or disposal of contaminated sediments (US EPA, 1998b). Table1-6 (see Chapter 1) lists the commonly used *in situ* and *ex situ* techniques for sediment remediation by the U.S. EPA. In 1995, Environment Canada carried out a pilot-size (100 m ×100 m) demonstration project of capping metal and metalloid (Zn, Cu, Pb, Cr, Ni, Cd, As, and Hg) contaminated sediments in Hamilton Harbor, Lake Ontario. A layer of clean medium to coarse sand with the average thickness of 35 cm was placed at the site (Azcue et al., 1998). One year later, the mobility of trace elements through the cap material and the physical stability of the cap were assessed by Azcue et al. (1998). Significant reductions in the vertical fluxes (up to 80%) of all trace elements were observed after capping of the contaminated sediments; however, they also reported that a thin layer (1 to 3 cm thick) of fresh moderately contaminated sediments had started to develop on the top of the capping layer. Therefore, long-term monitoring of the cap is essential to ensure that its integrity has not been compromised by water body and other effects (US EPA, 1993).

Ex situ treatment of contaminated sediments usually involves dredging and/or pretreatment of sediments to remove debris and dewater the dredged sediments (Mulligan et al., 2001b). Dredging of sediments causes resuspension of sediment; however, the spread of resuspended sediment can be limited through the use of silt curtains (US EPA, 1993). US EPA (1993) divided the dredging methods into three categories including mechanical, hydraulic, and pneumatic dredge. The amount of dewatering depends on the type of dredging used and the technique to be used for treatment. Centrifugation, filtration or gravity thickening can be used for dewatering purposes (Mulligan et al., 2001b). Extraction of heavy metals from dredged sediments requires mobilization of metals since metals are often strongly retained in sediment under natural

conditions (Nystroem et al., 2006). Calmano et al. (1993) indicated that acidic and chelating agents were most effective in removing heavy metals from sediments. Extraction studies in laboratory scale have showed promising results. Yu et al. (1996) evaluated the remobilization abilities of EDTA (ethylenediamine tetraacetic acid), DTPA (diethylenetriamine pentaacetic acid), and EGTA (ethylene glycol tetraacetic acid) on zinc from Ell-Ren river sediment in Taiwan. Their results showed that DPTA had higher remobilization effect on Zn than EDTA and EGTA. McCready et al. (2003) compared the effectiveness of 1 M HCl and 0.05 M EDTA on extracting Zn, Pb, Cu, and Cd from sixty sediments with different textures collected from Sydney Harbor. They reported that 1M HCl extracted a large percentage (60-100%) of heavy metals in oxic sediments; whereas the extractability of metals with 0.05 M EDTA was generally lower by 20%. Sediment washing is a large scale application of extraction method; however, it cannot efficiently treat sediments with fine particles and high humic content or low permeability (US EPA, 1993).

Recently, electrodialytic remediation has also showed good results for remediation of heavy metal contaminated harbor sediments (Nystroem et al., 2005, 2006; Ottosen et al., 2007). Nystroem et al. (2006) tested the use of different desorbing agents (HCl, NaCl, citric acid, lactic acid, ammonium citrate and distilled water) in electrodialytic remediation of harbor sediment. Their results revealed that the use of desorbing agents did not generally enhance the heavy metal removal. The removal was 48% Cu, 80% Zn, 96% Pb, and 98% Cd when using distilled water. In another study, Ottosen et al. (2007) successfully removed Cu and Cd from a sediment sampled from Sisimiut Harbor, Greenland by electrodialytic remediation. The Cu concentration was reduced from 97 to 16 mg/kg and the Cd concentration was reduced from 0.55 to 0.03 mg/kg

after 28 days with an applied current density of 1.2 mA/cm^2, and the major removal of the two heavy metals was obtained during the first 3 days.

Ex situ stabilization/solidification is another technique suitable for sediments contaminated with inorganic and metals, however, fine particle sizes and the presence of soluble salts of manganese, tin, copper and lead can reduce the treatment effectiveness (US EPA, 1993). Besides, since immobilization leads to an increase in volume, larger areas of land are required for disposal. Cement- or silicate-based processes are useful for sediments and economical if the end product can be used for landfill closure or in other applications (Mulligan et al., 2001b). Müller and Pluquet (1998) investigated the effectiveness of five iron-bearing materials (red mud, sludge from drinking water treatment, bog iron ore, unused steel shot and steel shot waste) on immobilization of Cd and Zn in a contaminated sediment dredged from the port of Bremen (Germany). After treatment, the uptake of Cd and Zn by plants was reduced by 20-50%, and the NH_4NO_3 and DTPA (diethylenetriamine pentaacetic acid) extractable amounts of Cd and Zn were reduced by 50% and 20%, respectively. It was also demonstrated that red mud and Fe-bearing sludge were the most effective materials. Meng et al. (1991) treated a Cd-contaminated lake sediment with aluminum nitrate at pH 9.5. The NH_4OAc-extractable Cd in the sediment treated with 1.6 mmol aluminum per gram of sediment was 80% less than the extractable Cd in the untreated sediment. They also found that freeze-thaw treatment of the Al-treated sediment significantly reduced the volume of the settled solids and did not affect the tendency for the treatment to enhance Cd retention.

6.4 Conclusions

The sediment heavy metal binding capacity (HMBC) test showed that sediment HMBC varied with the type of sediment, with organic rich sediments displaying a much higher metal binding than sandy sediments. In addition, after demonstrating the use of magnetic separation in

treating heavy metal-contaminated soils in Chapter 3, we also examined the effectiveness of this treatment approach in removing Cu, Zn, and Hg from spiked sediments. The results of this study showed a significant reduction of toxicity generated by Cu, Zn or Hg in sediment extracts after a single magnetic treatment. As regards toxicity reduction in sediment extracts, the type of sediment and metal did not affect the treatment effectiveness. Moreover, chemical analysis suggested that all the three metals were removed from both the sediment matrix and the sediment extracts. However, metal removal from the sediment matrix was lower than that from the sediment extracts.

In conclusion, the results indicated that, in addition to decontaminating metal-contaminated soils, the magnetic treatment could also be used to treat metal contaminated sediments. Comparing with other sediment remediation techniques, this method is relatively easy to operate, and time-saving. Future studies on decontaminating natural/aged contaminated sediments would be necessary to assess the feasibility of applying this method under field conditions.

Table 6-1. Sediments characteristics

Characteristic	Little Hatchet Creek Sediment	Hogtown Creek Sediment	Home Depot Pond Sediment	Lake Alice Sediment
pH	6.0	5.7	5.8	5.9
Eh (mV)	313.4	288.6	381.1	347.8
% Organic carbon	0.0	0.4	6.4	6.1
% Organic matter	0.0	0.7	5.8	10.5
% Sand	62.0	99.4	87.0	95.7
% Silt	37.8	0.5	6.0	0.3
% Clay	0.2	0.1	7.0	4.0
CEC ($cmol_c/kg$)	3.6	8.3	192.9	218.5

Table 6-2. EC_{50}s (added metal in mg/kg sediment), as determined by MetPLATE™, of water extracts from four sediments and Ottawa sand

Sediment Type	Heavy Metal	EC_{50} (mg/kg sediment)
Ottawa Sand	Cu	1.1 ± 0.2*
	Zn	0.9 ± 0.1
	Hg	1.5 ± 0.3
Little Hatchet Creek sediment	Cu	4.3 ± 0.9
	Zn	14.3 ± 2.5
	Hg	18.4 ± 2.3
Hogtown Creek Sediment	Cu	25.8 ± 5.3
	Zn	35.6 ± 1.5
	Hg	31.2 ± 2.0
Lake Alice Sediment	Cu	111.2 ± 14.2
	Zn	139.7 ± 15.0
	Hg	644.1 ± 22.8
Home Depot Pond Sediment	Cu	92.3 ± 8.6
	Zn	122.6 ± 2.0
	Hg	162.7 ± 16.6

*mean of 3 replicates ± one standard deviation

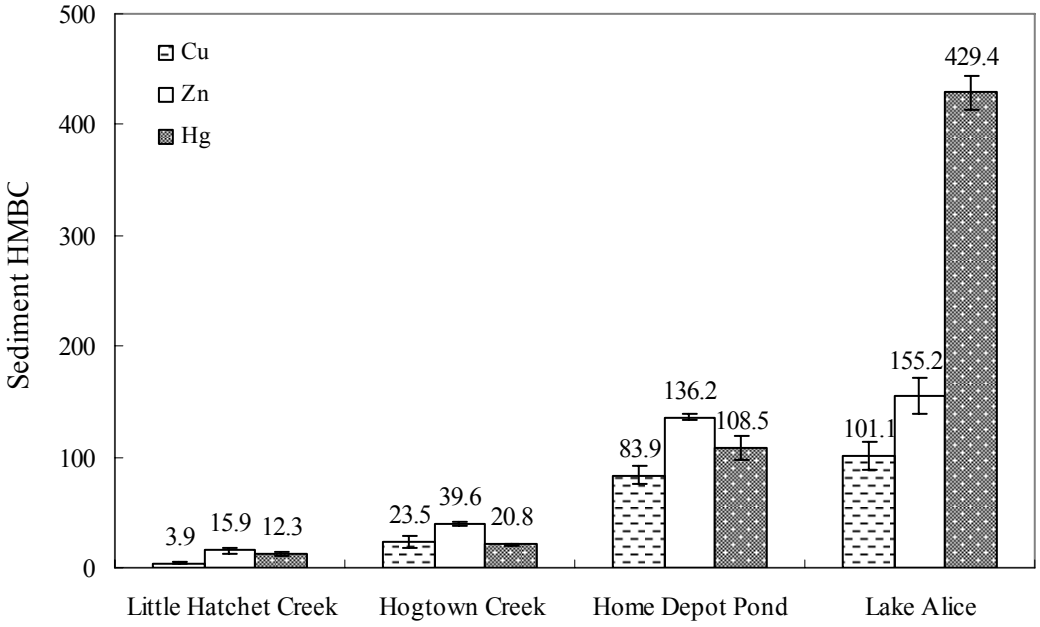


Figure 6-1. Sediment HMBC for three metals (Cu, Zn, Hg) and four sediments (Ottawa sand served as the reference).

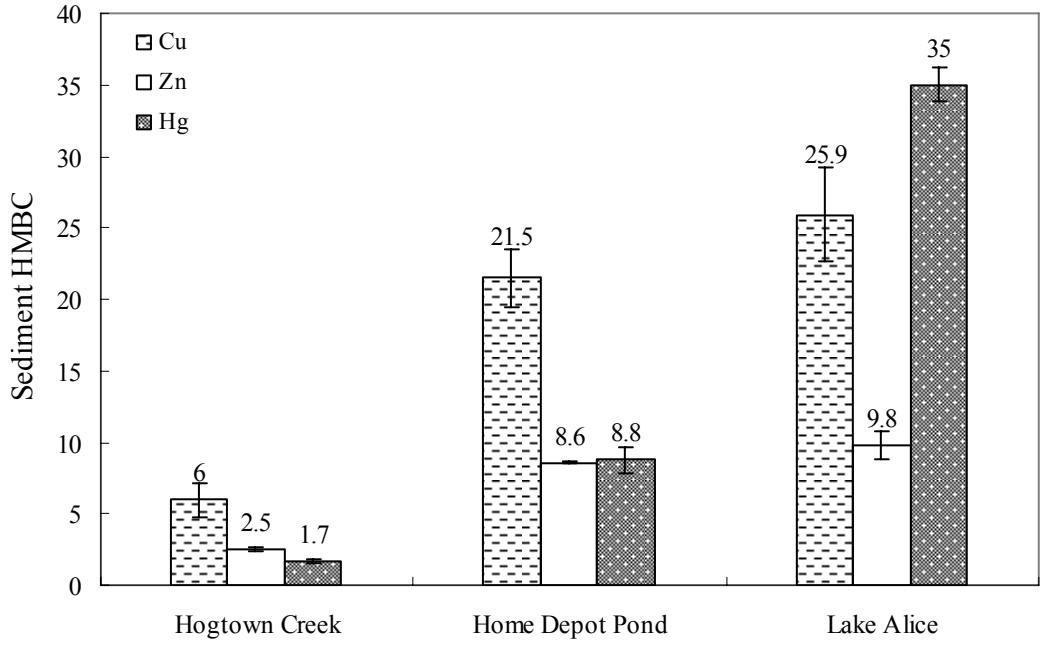


Figure 6-2. Sediment HMBC for three metals (Cu, Zn, Hg) and three sediments (Little Hatchet Creek sediment served as the reference).

Table 6-3. Copper, zinc and mercury toxicity removal from a spiked sandy sediment by magnetic treatment, as determined by MetPLATE™.

Heavy metal type	Heavy metal concentration in spiked sediment (mg/kg)	EC_{50} of sediment extract (% sediment extract)		Toxicity units[b] of sediment extract		Toxicity removal (%)
		No treatment	Magnetic treatment[a]	No treatment	Magnetic treatment	
Cu[c]	100	9.6 ± 1.1%[f]	>100%	10.4 ± 1.2	<1[g]	>90.4%
	200	0.6 ± 0.002%	>100%	158.6 ± 0.4	<1	>99.4%
Zn[d]	100	1.6 ± 0.02%	>100%	63.7 ± 0.6	<1	>98.4%
	200	0.2 ± 0.02%	>100%	514.8 ± 40.3	<1	>99.0%
Hg[e]	100	4.0 ± 0.08%	>100%	25.1 ± 0.5	<1	>96.0%
	200	0.4 ± 0.06%	>100%	250.7 ± 36.3	<1	>99.6%

[a] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [b] Toxicity units = $100/EC_{50}$; [c] 50 g of sediment was spiked with 40 mL of Cu solution containing 125 mg/L and 250 mg/L Cu^{2+}, respectively; [d] 50 g of sediment was spiked with 40 mL of Zn solution containing 125 mg/L and 250 mg/L Zn^{2+}, respectively; [e] 50 g of sediment was spiked with 40 mL of Hg solution containing 125 mg/L and 250 mg/L Hg^{2+}, respectively; [f] Mean of 3 replicates ± 1 standard deviation; [g] Non-toxic.

Table 6-4. Copper, zinc and mercury toxicity removal from a spiked organic rich sediment by magnetic treatment, as determined by MetPLATE™

Heavy metal type	Heavy metal concentration in spiked sediment (mg/kg)	EC_{50} of sediment extracts (% sediment extract)		Toxicity units[b] of sediment extracts		Toxicity removal (%)
		No Treatment	Magnetic treatment[a]	No treatment	Magnetic treatment	
Cu[c]	400	8.2 ± 0.8%[f]	60.1± 2.7%	12.3 ± 1.1	1.7 ± 0.1	86.4 ± 0.6%
	800	1.4 ± 0.11%	51.4 ± 0.2%	70.1 ± 5.4	1.9 ± 0.01	97.2 ± 0.2%
Zn[d]	400	1.9 ± 0.04%	30.9±1.1%	53.5 ± 1.2	3.2 ± 0.1	94.0 ± 0.1%
	800	0.4 ± 0.01%	13.9 ± 2.6	242.0 ± 6.6	7.3 ± 1.4	97.0 ± 0.5%
Hg[e]	400	17.0 ± 0.2%	>100%	5.9 ± 0.1	<1[g]	>83.0%
	800	2.0 ± 0.3%	78.5 ± 5.7%	49.9 ± 7.2	1.3 ± 0.1	97.3 ± 0.6%

[a] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [b] Toxicity units = $100/EC_{50}$; [c] 50 g of sediment was spiked with 40 mL of Cu solution containing 500 mg/L and 1000 mg/L Cu^{2+}, respectively; [d] 50 g of sediment was spiked with 40 mL of Zn solution containing 500 mg/L and 1000 mg/L Zn^{2+}, respectively; [e] 50 g of sediment was spiked with 40 mL of Hg solution containing 500 mg/L and 1000 mg/L Hg^{2+}, respectively; [f] Mean of 3 replicates ± 1 standard deviation; [g] Non-toxic.

Table 6-5. Copper, zinc and mercury toxicity removal from a spiked sandy sediment by magnetic treatment, as determined by the 48-h acute *Ceriodaphnia dubia* toxicity test

Heavy metal type	Heavy metal concentration in spiked sediment (mg/kg)	EC_{50} of sediment extracts (% sediment extract)		Toxicity units[b] of sediment extracts		Toxicity removal (%)
		No treatment	Magnetic treatment[a]	No treatment	Magnetic treatment	
Cu[c]	100	0.3 ± 0.02%[f]	>100%	342.2 ± 23.8	<1[g]	>99.7%
	200	0.03 ± 0.00%	>100%	3611.0 ± 27.2	<1	>99.98%
Zn[d]	100	0.61 ± 0.12%	>100%	168.5 ± 33.6	<1	>99.4%
	200	0.11 ± 0.01%	11.7 ± 0.1%	887.1 ± 51.6	8.5 ± 0.1	99.0 ± 0.0%
Hg[e]	100	0.27 ± 0.04%	76.1 ± 1.6%	373.0 ± 51.6	1.3 ± 0.02	99.6 ± 0.1%
	200	0.03 ± 0.01%	65.9 ± 4.0%	3532.2±723.8	1.5 ± 0.1	99.96 ± 0.01%

[a] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [b] Toxicity units = $100/EC_{50}$; [c] 50 g of sediment was spiked with 40 mL of Cu solution containing 125 mg/L and 250 mg/L Cu^{2+}, respectively; [d] 50 g of sediment was spiked with 40 mL of Zn solution containing 125 mg/L and 250 mg/L Zn^{2+}, respectively; [e] 50 g of sediment was spiked with 40 mL of Hg solution containing 125 mg/L and 250 mg/L Hg^{2+}, respectively; [f] Mean of 3 replicates ± 1 standard deviation; [g] Non-toxic.

Table 6-6. Copper, zinc and mercury toxicity removal from a spiked organic rich sediment by magnetic treatment, as determined by the 48-h acute *Ceriodaphnia dubia* toxicity test

Heavy metal type	Heavy metal concentration in spiked sediment (mg/kg)	EC_{50} of sediment extracts (% sediment extract)		Toxicity units[b] of sediment extracts		Toxicity removal (%)
		No treatment	Magnetic treatment[a]	No treatment	Magnetic treatment	
Cu[c]	400	2.4 ± 0.1%[f]	68.8 ± 5.3%	41.4 ± 1.9	1.5 ± 0.1	96.5 ± 0.4%
	800	0.16 ± 0.02	64.5 ± 0.8%	633.1 ± 87.2	1.6 ± 0.02	99.8 ± 0.04%
Zn[d]	400	1.2 ± 0.07%	6.5 ± 0.7%	85.3 ± 4.9	15.6 ± 1.6	81.7 ± 3.0%
	800	0.22 ± 0.03%	1.1 ± 0.03%	462.5 ± 68.9	89.1 ± 2.4	80.5 ± 3.4%
Hg[e]	400	1.5 ± 0.2%	34.5 ± 0.8%	69.1 ± 10.0	2.9 ± 0.06	95.8 ± 0.5%
	800	0.14 ± 0.01%	13.1 ± 0.2%	714.3 ± 28.3	7.5 ± 0.1	98.9 ± 0.1%

[a] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [b] Toxicity units = $100/EC_{50}$; [c] 50 g of sediment was spiked with 40 mL of Cu solution containing 500 mg/L and 1000 mg/L Cu^{2+}, respectively; [d] 50 g of sediment was spiked with 40 mL of Zn solution containing 500 mg/L and 1000 mg/L Zn^{2+}, respectively; [e] 50 g of sediment was spiked with 40 mL of Hg solution containing 500 mg/L and 1000 mg/L Hg^{2+}, respectively; [f] Mean of 3 replicates ± 1 standard deviation.

Table 6-7. Effect of magnetic treatment on the removal of Cu^{2+}, Zn^{2+} and Hg^{2+} from a spiked sandy sediment, as determined by chemical analysis.

Initial metal conc. in spiked sediment (mg/kg)[a]		Metal conc. in sediment matrix, extracts, and iron filings			Metal removal from sediment fractions	
		In sediment matrix (mg/kg)	In sediment extracts (mg/L)	Adsorbed on iron filings (mg/kg)	From sediment matrix (%)	From sediment extracts (%)
Cu^{2+} (200mg/kg)	No treatment	128.5 ± 3.6[c]	24.5 ± 2.9	No iron filings added	38.2 ± 3.1%	99.5 ± 0.1%
	Magnetic treatment[b]	80.4 ± 3.3	0.17 ± 0.06	1700.2 ± 152.6[d]		
Zn^{2+} (200mg/kg)	No treatment	98.5 ± 2.9	33.4 ± 0.4	No iron filings added	25.9 ± 6.3%	95.8 ± 1.0%
	Magnetic treatment	75.7 ± 5.4	1.4 ± 0.3	1886.5 ± 139.5[e]		
Hg^{2+} (200mg/kg)	No treatment	118.5 ± 3.1	17.5 ± 1.0	No iron filings added	79.3 ± 1.3%	98.0 ± 0.6%
	Magnetic treatment	24.5 ± 1.0	0.4 ± 0.1	1923.4 ± 22.7[f]		

[a] 50 g of sediment was spiked with 40 mL of Cu, Zn or Hg solution containing 250 mg/L metal; [b] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Iron filings had a Cu background value of 2503.3 mg/kg. This concentration was subtracted from the Cu concentration in filings after treatment; [e] Iron filings had a Zn background value of 13.1 mg/kg. This concentration was subtracted from the Zn concentration in filings after treatment; [f] Iron filings had an Hg background value of 0.02 mg/kg. This concentration was subtracted from the Hg concentration in filings after treatment.

Table 6-8. Effect of magnetic treatment on the removal of Cu^{2+}, Zn^{2+} and Hg^{2+} from a spiked organic rich sediment, as determined by chemical analysis.

Initial metal conc. in spiked sediment (mg/kg)[a]		Metal conc. in sediment matrix, extracts, and iron filings			Metal removal from sediment fractions	
		In sediment matrix (mg/kg)	In sediment extract (mg/L)	Adsorbed on iron filings (mg/kg)	From sediment matrix (%)	From sediment extract (%)
Cu^{2+} (800mg/kg)	No treatment	750 ± 50.7[c]	5.2 ± 0.2	No iron filings added	18.5 ± 2.0%	69.3 ± 3.4%
	Magnetic treatment[b]	611.7 ± 29.8	1.6 ± 0.1	2441.5 ± 365.9[e]		
Zn^{2+} (800mg/kg)	No treatment	627.7 ± 15.5	34.3 ± 1.3	No iron filings added	27.9 ± 2.0%	35.0 ± 3.8%
	Magnetic treatment	460.9 ± 23.2	22.3 ± 0.5	1789.3 ± 149.3[f]		
Hg^{2+} (800mg/kg)	No treatment	528.1 ± 22.8	6.4 ± 0.5	No iron filings added	58.5 ± 1.5%	93.3 ± 0.8%
	Magnetic treatment	219.0 ± 1.9	0.4 ± 0.02	6014.2 ± 769.1[g]		

[a] 50 g of sediment was spiked with 40 mL of Cu, Zn or Hg solution containing 1000 mg/L metal; [b] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] Iron filings had a Cu background value of 2503.3 mg/kg. This concentration was subtracted from the Cu concentration in filings after treatment; [e] Iron filings had a Zn background value of 13.1 mg/kg. This concentration was subtracted from the Zn concentration in filings after treatment; [f] Iron filings had an Hg background value of 0.02 mg/kg. This concentration was subtracted from the Hg concentration in filings after treatment.

Table 6-9. Mass balance of Cu, Zn and Hg in a spiked sandy sediment before and after magnetic treatment.

Initial metal mass (mg)[a]		Metal in sediment matrix (mg)	Metal in sediment extract (mg)	Metal adsorbed on iron filings (mg)	Total recovered metal mass (mg)	Total recovery of metal (%)
Cu (2 mg)	Before treatment	1.3± 0.04[c]	0.5 ± 0.06	No iron filings added	1.7 ± 0.1	87.4 ± 4.2%
	After treatment[b]	0.8 ± 0.03	0.003 ± 0.001	0.9 ± 0.08[d]	1.6 ± 0.1	81.5 ± 5.5%
Zn (2 mg)	Before treatment	0.9 ± 0.03	0.7 ± 0.01	No iron filings added	1.5 ± 0.03	77.4 ± 1.8%
	After treatment	0.7 ± 0.05	0.03 ± 0.01	0.9 ± 0.1[e]	1.6 ± 0.1	81.2 ± 4.6%
Hg (2 mg)	Before treatment	1.2 ± 0.03	0.35 ± 0.02	No iron filings added	1.6 ± 0.02	76.7 ± 2.4%
	After treatment	0.2 ± 0.01	0.01 ± 0.002	1.0 ± 0.01[f]	1.2 ± 0.01	60.7 ± 0.6%

[a] 50 g of sediment was spiked with 40 mL of Cu, Zn or Hg solution containing 250 mg/L metal ; [b] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] The amount of background Cu mass in iron filings was subtracted from the Cu mass in filings after treatment; [e] The amount of background Zn mass in iron filings was subtracted from the Zn mass in filings after treatment; [f] The amount of background Hg mass in iron filings was subtracted from the Hg mass in filings after treatment.

Table 6-10. Mass balance of Cu, Zn and Hg in a spiked organic rich sediment before and after magnetic treatment.

Initial metal mass (mg)[a]		Metal in sediment matrix (mg)	Metal in sediment extract (mg)	Metal adsorbed on iron filings (mg)	Total recovered metal mass (mg)	Total recovery of metal (%)
Cu (8 mg)	Before treatment	7.4 ± 0.5[c]	0.1 ± 0.004	No iron filings added	7.5 ± 0.5[d]	94.1 ± 6.4%
	After treatment[b]	6.0 ± 0.3	0.03±0.003	1.2 ± 0.2[d]	7.3 ± 0.4	91.2 ± 5.3%
Zn (8 mg)	Before treatment	6.0 ± 0.2	0.7 ± 0.03	No iron filings added	6.7 ± 0.2	83.5 ± 2.2%
	After treatment	4.3 ± 0.2	0.4 ± 0.01	0.9 ± 0.1[e]	5.7 ± 0.2	70.8± 1.9%
Hg (8 mg)	Before treatment	5.3 ± 0.2	0.1 ± 0.01	No iron filings added	5.4 ± 0.2	67.6 ± 2.9%
	After treatment	2.2 ± 0.02	0.01 ± 0.0005	3.0 ± 0.4	5.2 ± 0.4	65.1 ± 4.6%

[a] 50 g of sediment was spiked with 40 mL of Cu, Zn or Hg solution containing 1000 mg/L metal; [b] 5% (2.5 g) of iron filings were added to the spiked sediment, and the contact time was 3 hours; [c] Mean of 3 replicates ± one standard deviation; [d] The amount of background Cu mass in iron filings was subtracted from the Cu mass in filings after treatment; [e] The amount of background Zn mass in iron filings was subtracted from the Zn mass in filings after treatment; [f] The amount of background Hg mass in iron filings was subtracted from the Hg mass in filings after treatment.

CHAPTER 7
PLANT GROWTH STUDY TO DEMONSTRATE METAL REMEDIATION BY MAGNETIC SEPARATION

7.1 Introduction

The Environment may be polluted by heavy metals as a result of industrial activities, such as mining, smelting, electroplating, energy production, military operations, or sewage sludge disposal (Lim et al., 2004; Nedelkoska and Doran, 2000; Quartacci et al., 2005). Land application of sewage sludge is more economical than incineration or disposal into landfills (Blais et al., 2004). However, a large portion of sewage sludge from industrial sources often contains toxic metals, such as lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), copper (Cu) and zinc (Zn), that can persist in the top cultivated layer of soils (Wani et al., 2007). If these metals are phytoavailable, they may accumulate in plants and pose potential threat to humans and grazing animals (Boularbah et al., 2006). Also, soil phytotoxicity may cause disappearance of natural vegetation cover which could exert risk for the surrounding areas (Boisson et al., 1999). Some metals like Cd and Zn are very mobile in soils and thus readily available to plants (Madejón et al., 2004).

Metal phytoavailability is mainly determined by the metal speciation (Notten et al., 2005), soil characteristics, such as pH, texture, organic matter and clay content, cation exchange capacity and redox potential (Planquart et al., 1999), and duration of contact with the surface binding these metals (Naidu et al., 2003a). Other factors including the distribution of metals across the soil profile and rooting depth are also able to affect the uptake of metals by plants (Notten et al., 2005). Naidu et al. (2003a) have shown that at any given total Cd concentration, the phytoavailable metal fraction is higher in Oxisols than in Vertisols unless the pH of Oxisols is higher than 6 to enhance their binding capacity of Cd. Vegetation can be used to indicate or monitor environment contamination by heavy metals (Mertens et al., 2005; Pugh et al., 2002).

For example, mosses have been used as bioindicators in many countries in the Northern Hemisphere to estimate the atmospheric deposition of metal particles (Denayer et al., 1999). Madejón et al. (2004) have surveyed the content of eight trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb, Zn) in leaves and stems of white poplar (*Populus alba*) trees, and found a significant and positive correlation between the concentration of trace elements in poplar leaves and the soil available Cd and Zn, which indicated that poplar leaves could be used as biomonitors for soil pollution of these two metals.

To quantitatively predict the transfer of metals from soils to plants, both mechanistic and empirical models have been proposed. Since mechanistic models are based on plant and soil parameters which are difficult to determine, many more studies employed empirical models, and linear functions are preferred in most cases (Krauss et al., 2002). However, in reality, plant uptake of metals is a non-linear process. Krauss et al. (2002) evaluated the use a curvilinear Freundlich-type function ($C_{Plant} = b \times C_{Soil}^{a}$, where b and a are the empirical Freundlich coefficients) to predict Cd, Cu, Pb, and Zn concentrations in wheat (*Triticum aestivum* L.) grain and leaf (C_{Plant}) from soil concentrations (C_{Soil}). They showed that this Freundlich-type function was suitable to predict Cd and Zn concentrations in wheat grain and leaf from the EDTA-extractable metal concentrations, whereas the prediction of Cu and Pb concentrations was poorer.

Recently, studies have been carried out to investigate the metal tolerance mechanisms in different plant species (Clemens, 2001; Horiguchi, 1987; Meharg, 2005; Sasaki et al., 1995). The mechanisms involve exclusion, active removal, biosorption, precipitation or bioaccumulation in external and intracellular spaces which can influence the metal solubility and the bioavailability to plants (Carlot et al., 2002). The bioaccumulation of metals in plants depends on both the plant species and the type of metal (Naidu et al., 2003a). Hyperaccumulation is a sub-class of metal

resistance of plant species, which is less common than exclusion strategies (Meharg, 2005). The first hyperaccumulators characterized were members of Brassicaceae and Fabaceae families (Salt et al., 1998). Presently, more than 400 plant species have been identified as metal hyperaccumulators (Tang et al., 2003). The potential use of hyperaccumulators to remediate heavy metal-contaminated soils has attracted researchers' interests. For instance, *Alyssum bertolonii*, *A. tenium*, and *A. troodii* are recognized hyperaccumulators of Ni, and *Thlaspi caerulescens* is a recognized hyperaccumulator of Cd and Zn (Nedelkoska and Doran, 2000). Sahi et al. (2002) reported that *Sesbania drummondii* possessed the ability of hyperaccumulating Pb. In addition, a hyperaccumulator of As, *Pteris vittata* L. (brake fern) was discovered by Ma et al. (2001). These studies on metal hyperaccumulating plants have revealed the feasibility of utilizing phytoremediation as a mean to clean up metal-contaminated soils. However, although phytoremediation is being regarded as a promising, economical, and environmentally friendly remediation alternative, some limitations have been addressed by researchers. The major problem affects plant remediation efficiency is that some of the metals are immobile in soils and thus their bioavailability is limited to the root surfaces (Wu et al., 2004). Moreover, the majority of the hyperaccumulators have very low biomass production (Tang et al., 2003), and the disposal of contaminated crop material is another concern (Sas-Nowosielska, et al., 2004).

In this chapter, we investigated the ability of MetPLATE^{TM}, a bacterial toxicity test, in predicting heavy metal phytoavailability in different types of soils, as well as the use of plants to assess the effectiveness of magnetic separation for removing heavy metals from soils.

7.2 Material and Methods

7.2.1 Assessment of Cu Phytotoxicity Using MetPLATE™

7.2.1.1 Soils used

Three soil types were used to study the suitability of the MetPLATE toxicity test to predict heavy metal phytoavailability. A sandy soil was sampled from the top 4 feet at the McCarty Woods on the University of Florida campus, which is representative of the soils prevailing in North Central Florida. An organic rich soil was collected from the first few top inches along Hogtown Creek in Gainesville, FL. A mixed soil contained 50% (w/w) of the red sandy soil (sampled from Perdido landfill in Cantonment, FL) and 50% (w/w) of the organic rich soil. Table 7-1 shows the main characteristics of the soils under study. All soils samples were first air-dried, screened (sieve # 10; 2.0 mm particles) and homogenized.

7.2.1.2 Soil spiking with Cu

According to the soil Cu binding capacity obtained in Chapter 2, different ranges of Cu solution (prepared from $CuSO_4 \cdot H_2O$, Sigma®) were spiked into the three types of soils. The sandy soil was spiked with 25, 50, and 100 mg/kg of Cu; the organic rich soil was spiked with 50, 500, and 700 mg/kg of Cu; while the mixed soil was spiked with 100, 200, and 500 mg/kg of Cu. The spiked soils were air-dried for 10 days to reach equilibrium and promote the adsorption of the added metal, and then screened (sieve # 10; 2.0 mm particles) and homogenized again prior to toxicity tests and pot experiments.

7.2.1.3 Pot experiment

The pot experiment was performed in a greenhouse under controlled conditions (temperature 25 ± 3 °C, day-night cycle 16/8 h), and two plant species, lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*), were used. Five hundred gram of spiked soil was placed in each plant pot (5-inch diameter). NH_4NO_3 and KH_2PO_4 were applied as fertilizers at the rates

of 0.43 and 0.33 g/kg, respectively (Wu et al., 2004). Nine seeds of each plant species were sown in each pot and thinned to four seedlings for lettuce and three seedlings for Indian mustard after 14 days. All pots were watered daily with distilled water by adjusting the water content to 70% of the soil water holding capacity, and fertilized once per week to maintain vigorous plant growth (water holding capacity of each type of soil was determined in advance). For each type of soil spiked with one Cu concentration, three replicates were set up for each plant species. Non-spiked soils served as controls.

After eight weeks, all plants were harvested and the shoots (aboveground parts) were separated from the roots. The length of the shoots were measured and recorded. Then, the shoots and roots were washed with distilled water, and oven dried at 70°C for 24 h to measure their dry biomass. The dried plant tissue was then ground through a 20 mesh sieve (0.85 mm opening), and the Cu concentration in plant roots and shoots were measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) after wet acid digestion of the plant tissue using concentrated nitric acid and 30% hydrogen peroxide (Mills and Jones, 1996). The detailed plant digestion procedure was included in Appendix B.

7.2.1.4 Toxicity of soils, as determined by MetPLATE™

The toxicity of the spiked soils that were used for growing plants was tested by the MetPLATE™ assay. Twenty milliliter of distilled water were added to 10 g of spiked soil, and the soil mixture was shaken for 4 hours. Then the soil slurry was centrifuged at 10,000 rpm for 15 minutes. The supernatant (soil extract) was removed with a pipet. The MetPLATE™ microbial test was carried out according to Bitton et al. (1994), and the detailed procedure is described in Appendix A. To determine the EC_{50} for the soil extracts, 4 to 5 dilutions of the soil extracts were prepared, and a regression analysis was used to calculate the EC_{50}. Moderately

hard water (60 mg/L Ca, 60 mg/L Mg, pH = 7.4 -7.8) was used as the negative control. All experiments were run in triplicate.

7.2.2 Use of Plants to Evaluate the Effectiveness of Magnetic Separation on Cu-Spiked Soils

7.2.2.1 Soils preparation

The same sandy soil sampled from the McCarty Woods on the University of Florida campus was spiked with Cu^{2+} solution (prepared from $CuSO_4 \bullet H_2O$, Sigma®) to reach a concentration of 100 mg Cu^{2+}/kg soil. Another organic rich soil (organic soil 2), purchased from a local landscaping store, was spiked to reach a concentration of 500 mg Cu^{2+}/kg soil. The spiked soils were air-dried for 10 days, and then screened (sieve # 10; 2.0 mm particles) and homogenized prior to iron treatment followed by magnetic separation. The iron filings (Fisher, 40 mesh) were placed in 1M NaOH for 72 hours to increase the adsorption capacity and then washed thoroughly with distilled water before use (Yeager, 1998).

7.2.2.2 Treatment of spiked soils

Treatment design. To investigate the effectiveness of magnetic separation of Cu from spiked soils, as well as the potential effect of iron on plants, the following five treatments were designed for plant study: (1) control (non-spiked soil); (2) control with 5% (5g iron/ 100 g soil) of iron filings; (3) spiked soil with iron treatment, and the iron filings were then magnetically retrieved; (4) spiked soil with iron treatment, but the iron filings were not magnetically retrieved (to investigate the effect of Cu immobilization in soil); (5) spiked soil without iron treatment. For the sandy soil, two concentrations of iron filings (2.5% and 5%) were used and magnetically retrieved, whereas only 5% of iron filings were used to treat Cu-spiked organic rich soil 2. Due to the large amount of soil required for growing plants, each treatment was performed in several batches.

Magnetic separation of Cu from soils. For Cu-spiked sandy soil and organic rich soil 2, 80 ml of distilled water were added to 100 g of spiked soil, and the soil slurry was shaken for 1 hour. Then, iron filings (2.5% and 5% for sandy soil, 5% for organic rich soil 2) were added to the soil slurry, followed by shaking for another 3 hours. Then, the iron filings were retrieved by a Ferrimag rectangular magnet (Scientifics®, 152 ×102×25mm, 3.4 megagauss oersteds). Each soil sample treated by the same amount of iron filings from different batches were combined and mixed thoroughly, air-dried, and homogenized for growing plants.

Iron immobilization of Cu in soils. The procedure of this treatment was the same as that described above except that the added iron filings (5%) were not removed from the soil. Therefore, after treatment, the soil and the iron filings were air-dried and homogenized for growing plants.

7.2.2.3 Pot experiment

Soils with the various treatments mentioned above were used to grow plants in clay pots under controlled conditions in a greenhouse. The pot experiment in this section was the same as that described in Section 7.2.1.3. The same two plant species, lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*), were planted. For each type of treatment including controls, three replicates were set up for each plant species.

After eight weeks, all plants were harvested, and the length and biomass of the shoots and roots were recorded. The Cu concentration in digested plant tissue was also measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

7.2.2.4 Toxicity of soils used for growing plants

The effect of different treatments on the toxicity of the sandy soil and the organic rich soil 2 used for growing plants was also tested by MetPLATE™ assay. One hundred milliliter of distilled water was added to 50 g of soil, and the soil mixture was shaken for 4 hours followed by

centrifugation at 10,000 rpm for 15 minutes. The supernatant (soil extract) was removed with a pipet and was tested for toxicity after overnight settling in the refrigerator. All experiments including toxicity tests were run in triplicate. Moderately hard water (60 mg/L Ca, 60 mg/L Mg, pH = 7.4 -7.8) served as the negative control. The detailed procedure for MetPLATE^{TM} test was included in Appendix A.

7.3 Results and Discussion

7.3.1 Assessment of Cu Phytotoxicity Using MetPLATE^{TM}

7.3.1.1 Cu phytotoxicity

The growth of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) in Cu-spiked soils was investigated. Three types of soils (sandy soil, organic rich soil, and mixed soil) were spiked with different Cu concentrations based on their Cu binding capacity (see Chapter 2). Compared with the control (i.e. non-spiked) soil, no Cu phytotoxicity was observed in lettuce and mustard during germination and growth in 25 mg/kg and 50 mg/kg Cu-spiked sandy soil. However, in 100 mg/kg Cu-spiked sandy soil, after germination, purple spots started to appear on the leaves of lettuce and mustard, and the seedlings of the plants stopped growing and eventually died, which indicated Cu phytotoxicity. Figures 7-1 and 7-2 show the dry biomass of lettuce and mustard grown in spiked sandy soil, respectively. The dry biomass varied between 2.5 g/pot to 2.7 g/pot for shoots, and 0.7 g/pot to 0.8 g/pot for roots (Figure 7-1). No significant difference (5% level according to the Tukey's Studentized Range (HSD) Test) was observed regarding the dry biomass of lettuce shoots and roots grown in the control, 25 mg/kg, and 50 mg/kg Cu-spiked sandy soils. As regards Indian mustard, when the Cu input concentration was 25 mg/kg, the dry biomass of shoots (3.5 g/pot) and roots (1.2 g/pot) did not differ significantly from those grown in the control soil. However, when the Cu concentration was increased to 50 mg/kg, the dry biomass of mustard decreased to 2.9 g/pot for shoots and 0.9 g/pot for roots, and

the reduction of biomass was significant at the 5% level by performing Tukey's Studentized Range (HSD) Test (Figure 7-2). The effect of Cu concentrations in sandy soil on shoots length of the two plant species was displayed in Figure 7-3. As regards lettuce, shoot length did not significantly differ in 0 mg/kg (control), 25 mg/kg, and 50 mg/kg Cu-spiked sandy soil, and ranged from 20.9 cm to 24.5 cm. For Indian mustard, shoot length was 24.3 cm and 22.3 cm in 0 mg/kg and 25 mg/kg Cu-spiked soil, respectively. However, the shoot length was 18.6 cm at 50 mg/kg Cu concentration, which was significantly different from the control soil. Therefore, both plants did not grow at 100 mg/kg Cu while soil spiking at 50 mg/kg Cu caused little inhibition only to Indian mustard, and 25 mg/kg Cu did not inhibit the growth of any of the two plant species.

With regard to Cu phytotoxicity in organic rich soil, Figures 7-4, 7-5, and 7-6 show the dry weight and height of lettuce and Indian mustard grown in 0 mg/kg (control), 50 mg/kg, 500 mg/kg, and 700 mg/kg Cu-spiked organic rich soil. In 50 mg/kg Cu-spiked organic rich soil, the dry biomass of both plant species was slightly enhanced (6.7 g/pot and 1.7 g/pot for lettuce shoots and roots, respectively; 6.0 g/pot and 1.5 g/pot for mustard shoots and roots, respectively), as compared to the control plants (5.5 g/pot and 1.2 g/pot for lettuce shoots and roots, respectively; 5.4 g/pot and 1.2 g/pot for mustard shoots and roots, respectively). Besides, the shoots length of lettuce (20.8 cm) and Indian mustard (28.0 cm) in 50 mg/kg Cu-spiked organic rich soil were very close to those grown in control soil (22.6 cm for lettuce and 30.5 cm for mustard). When Cu concentration was increased to 500 mg/kg soil, the growth of lettuce was moderately inhibited. Although the shoots length of lettuce (18.2 cm) did not significantly differ from that of the control plant (22.6 cm), the dry biomass of shoots (3.6 g/pot) and roots (0.8 g/pot) significantly decreased. As regards Indian mustard, the growth of mustard in 500 mg/kg

Cu-spiked organic rich soil was severely inhibited, with total dry biomass (shoots plus roots) of 0.04 g/pot and shoots length of only 2.1 cm. In 700 mg/kg Cu-spiked organic rich soil, the growth of both lettuce and Indian mustard were severely inhibited. As shown in Figure 7-4, the dry biomass of lettuce shoots and roots were only 0.2 g/pot and 0.04 g/pot, respectively. The dry biomass of mustard grown in 700 mg/kg Cu-spiked organic rich soil was 0.02 g/pot for shoots and 0.01 g/pot for roots (Figure 7-5). In addition, the shoots length of lettuce and mustard decreased to 6.2 cm and 2.1 cm (see Figure 7-6).

In the case of Cu-spiked mixed soil, the growth of lettuce was not affected in 100 mg/kg and 200 mg/kg Cu-spiked mixed soil. Figure 7-7 shows that, in 0 mg/kg (control), 100 mg/kg and 200 mg/kg Cu-spiked mixed soil, the dry biomass of lettuce varied from 2.2 g/pot to 2.4 g/pot for shoots and 0.17 g/pot to 0.21 g/pot for roots. The shoots length of lettuce in 100 mg/kg (26.4 cm) and 200 mg/kg (25.5 cm) Cu-spiked mixed soil was very close to that grown in the control soil (24.9 cm) (Figure 7-8). When the Cu concentration increased to 500 mg/kg soil, the growth of lettuce was moderately inhibited. The shoots length of lettuce decreased to 14.5 cm (Figure 7-7), and the plant yield decreased to 0.6 g/pot for shoots and 0.03 g/pot for roots (Figure 7-8). The data for Indian mustard were unfortunately lost due to heavy pest infestation.

It is known that at sufficiently high concentration, heavy metals can cause severe damage to physiological and biochemical activities of plants (Nicholls and Mal, 2003), such as the interruption of essential enzymes' activities, photosynthetic processes, and nutrient uptake (Sayed, 1999). Many researchers have investigated the phytoxicity of heavy metals to different plant species (Davis and Beckett, 1978; Fjallborg and Dave, 2004; Luo and Rimmer, 1995; Mukherji and Gupta, 1972; Nicholls and Mal, 2003; Sonmez et al., 2006). Mukherji and Gupta (1972) studied the effect of Cu^{2+} on the growth of lettuce (*Lectuca sativa*) seedlings in cupric

sulfate solution, and they found that their root growth was completely inhibited at a Cu^{2+} concentration of 0.05 M, and the seed germination stopped at a Cu^{2+} concentration of 0.1 M. They also reported that the inhibition of root growth was relatively stronger than that of hypocotyl growth. Davis and Beckett (1978) investigated the toxic effect of copper, zinc and nickel in young barley (*Hordeurn vulgare* L.), wheat (*Triticum aestivum* L.), rape (*Brassica napus* L.), lettuce (*Lactuca sativa* L.) and ryegrass (*Lolium perenne*). Their results indicated that the dry biomass of these young plants was independent on the concentration of Cu, Ni, or Zn in their photosynthesizing tissues until reach to a critical concentration (upper critical level). Another study conducted by Nicholls and Mal (2003) assessed the effects of Cu and Pb on the growth of an invasive weed, *Lythrum salicaria*, in a sandy soil which was spiked with single metal solution and a mixture of Cu and Pb to reach 1000 mg/kg and 2000 mg/kg metal concentrations. 55 days after spiking, the aboveground parts of the plants were completely withered and died.

7.3.1.2 Toxicity of soils, as determined by MetPLATE™

It is known that the total metal content in soils is a poor indicator of its mobility, toxicity and (phyto)availability. Metal speciation is an important factor controlling the metal uptake by plants. Generally, water-soluble and exchangeable forms of metals are most available to plants (Tokahoglu and Kartal, 2004). A study conducted by Zhang et al. (1998) showed that the Zn and Mn contents in plant were positively correlated with their Fe/Mn oxides-bound fraction in the soil; however, Ca uptake was negatively correlated with Ca bound to carbonates in the soil. Sequential extraction techniques have been used extensively to identify phytoavailable forms of metals in soils (Cajuste et al., 2000; Szakova et al., 2005; Tokahoglu and Kartal, 2004; Zhang et al., 1998). However, the multi-step extraction methods are usually very time-consuming;

therefore, we investigated the feasibility of using a rapid heavy metal-specific microbial test, MetPLATE^{TM}, to predict heavy metal phytotoxicity in different types of soil.

The toxicity of the three types of soil (sandy, organic rich, and mixed soil) used for growing plants was tested, and the results are shown in Table 7-2. For each spiked soil, the EC_{50} (expressed as % soil extract) of the soil extracts generally decreased as the Cu concentration increased, showing a raise in soil toxicity. In 25 mg/kg and 50 mg Cu^{2+}/kg sandy soil, 50 mg Cu^{2+}/kg organic rich soil, and 100 mg Cu^{2+}/kg mixed soil, the EC_{50}s of the soil extracts were all greater than 100%, showing very little toxicity. Similarly, no Cu phytotoxicity was found in the plants grown in the above Cu-spiked soils. In 100 mg Cu^{2+}/kg sandy soil, the EC_{50} of the soil extract decreased to 15.3% while in Cu-spiked organic rich soil, the EC_{50}s of the soil extracts were 3.7% and 2.4% at Cu concentrations of 500 mg/kg and 700 mg/kg, respectively. With regard to 200 mg/kg and 500 mg/kg Cu-spiked mixed soil, the EC_{50}s of the soil extracts were 47.7% and 11.5%, respectively.

To find a potential relationship between MetPLATE^{TM} toxicity and phytotoxicity, the percent inhibition of undiluted soil extract was also tested by MetPLATE^{TM}. Among all spiked soils at different Cu concentrations, the following did not cause phytotoxicity: 25 mg/kg and 50 mg Cu^{2+} /kg sandy soil, 50 mg Cu^{2+}/kg organic rich soil, and 100 mg/kg and 200 mg Cu^{2+}/kg mixed soil. The percent inhibition of theses undiluted soil extracts varied from non toxic to 67.8%, as determined by MetPLATE^{TM}. However, for the spiked soils that caused moderate to high phytotoxicity, the percent inhibition of their soil extracts ranged from 84.8% to 90.1%. Therefore, based on the results, we could preliminarily conclude that if a soil extract generates around 90% inhibition by MetPLATE^{TM} assay, this soil could probably cause phytotoxicity in lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*).

7.3.1.3 Cu uptake by lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*)

Table 7-3 presents the Cu concentrations in plant shoots and roots grown in different types of soils. Generally speaking, in Cu-spiked soils, the plant uptake of Cu increased with the increase of the input Cu concentration, indicating an increase in phytoavailable Cu. Comparing the two plant species, in the same type of soil, the Cu concentration in Indian mustard shoots was generally higher than that in lettuce shoots; however, the roots uptake of Cu in these two plant species was very close. Besides, for both lettuce and mustard, their roots always accumulated much more Cu than the shoots. A study carried out by Jordao et al. (2006) found similar results. They reported that in Cu-enriched vermicompost-amended soil, the Cu concentrations in the roots of lettuce (*Lactuca sativa*) (76.3 to 244.6mg/kg) were much higher than those in lettuce leaves (5.9 to 13.9mg/kg). Fargasova (2001) also showed that the accumulation of Cd, Cu, Zn, Pb, and Fe in mustard (*Sinapis alba*) seedlings was higher in the roots than in the shoots, no matter whether the metals were added individually or in pairs. By studying Cu uptake from solutions by lettuce (*Lactuca sativa*), Cheng and Allen (2001) reported that Cu uptake by plant roots was affected by free Cu ion activity, pH, and the concentration of other competing ions such as Ca^{2+}. Moreover, Cu concentration in lettuce shoots was much lower than that in lettuce roots.

As shown in Table 7-3, chemical analysis data for plants grown in 100 mg/kg Cu-spiked sandy soil are not available, due to plant death. However, as the spiked Cu concentration increased from 0 mg/kg (control) to 50 mg/kg, the uptake of Cu by lettuce increased from 4.4 mg/kg to 16.6 mg/kg in shoots, and from 5.7 mg/kg to 344.4 mg/kg in roots. Likewise, the Cu concentrations in Indian mustard increased from 5.2 mg/kg to 65.6 mg/kg in shoots, and from 7.4 mg/kg to 359.7 mg/kg in roots. Plant growth was not inhibited at these Cu levels as shown by the pot experiments. Davis and Beckett (1978) investigated the minimum concentrations of Cu in

plant shoots necessary to cause toxic reactions. They reported that the critical Cu^{2+} concentrations in plant shoots to cause phytotoxicity were 19 mg/kg for spring barley (*Hordeurn vulgare* L.), 21 mg/kg for ryegrass (*Lolium perenne*), 21 mg/kg for lettuce (*Lactuca sativa* L.), 16mg/kg for rape (*Brassica napus* L.), and 18 mg/kg for wheat (*Triticum aestivum* L.) Their findings confirm our pot experiments results that no phytotoxicity was observed in 50 mg/kg Cu-spiked sandy soil since the lettuce shoots concentration was only 16.6 mg/kg, less than 21 mg/kg.

In Cu-spiked organic rich soil, as the increase of the Cu input concentration from 0 mg/kg (control) to 50 mg/kg soil, the Cu concentrations in the plant shoots slightly increased, from 3.5 mg/kg to 6.7 mg/kg in lettuce shoots, and from 4.7 mg/kg to 8.4 mg/kg in mustard shoots; whereas the Cu concentrations in the plant roots increased largely from 8.4 mg/kg to 79.9 mg/kg for lettuce and 7.4 mg/kg to 71.4 mg/kg for mustard. With further increase of the Cu input concentration to 500 mg/kg soil, Cu uptake by lettuce increased to 50.2 mg/kg in shoots and 670.0 mg/kg in roots; however, the dry biomass of Indian mustard was too low to perform chemical analysis on the Cu content. At 700mg Cu^{2+}/kg organic rich soil, only lettuce shoots generated enough biomass for chemical analysis, with a Cu concentration of 106.4 mg/kg. In addition, one common Cu input concentration (50 mg/kg soil) was shared by the sandy soil and organic rich soil. Comparing the Cu uptake by plants grown in these two soils, we found that for the same plant, the uptake of Cu from the sandy soil was higher than that from the organic rich soil, which confirmed the higher metal binding capacity of the organic soil as discussed in Chapter 2.

Table 7-3 also shows the Cu concentrations in lettuce harvested from the mixed soil. For 0 mg/kg (control), 100 mg/kg, 200 mg/kg, and 500 mg/kg Cu-spiked mixed soil, the Cu

concentrations in lettuce shoots increased from 7.8 mg/kg to 318.5 mg/kg, whereas the uptake of Cu by lettuce roots was much higher, ranged from 51.7 mg/kg to 1199.0 mg/kg.

7.3.2 Evaluation of Iron Treatment on Cu-Spiked Soils Using Plant Study

The effectiveness of different iron treatments followed by magnetic separation on 100 mg/kg Cu-spiked sandy soil and 500 mg/kg Cu-spiked organic rich soil 2 were assessed, using plant growth experiment in a greenhouse under controlled conditions. Besides, the effect of added iron filings on plant growth was also investigated. Iron is an essential nutrient for plants; however, it can cause phytotoxicity when hyperaccumulated or maldistributed within plant cells (Pich et al., 2001).

As mentioned in Section 7.2.2.2, the experiment consisted of the following five treatments: (1) control (non-spiked soil); (2) control with 5% iron filings; (3) spiked soil with iron treatment, and the iron filings were then magnetically retrieved; (4) spiked soil with iron treatment, but the iron filings were NOT magnetically retrieved (iron immobilization); (5) spiked soil without iron treatment.

7.3.2.1 Effect of treatments on plant growth in a sandy soil

Figures 7-9 and 7-10 present the effect of these treatments on the dry biomass of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in a sandy soil. Figure 7-11 shows the shoots length of these two plant species. As shown in Figures 7-9 and 7-11, the growth of lettuce, as shown by determination of dry biomass and shoots length, did not differ significantly in control soil (no Cu^{2+} added) with 5% iron (2.1 g/pot for shoots dry biomass, 0.43 g/pot for roots dry biomass, and 22.9 cm for shoots length) and in control soil without iron (2.2 g/pot for shoots dry biomass, 0.67 g/pot for roots dry biomass, and 25.0 cm for shoots length), indicating that the added iron filings did not affect the growth of lettuce at 5% concentration. In non-treated sandy soil, lettuce could not survive due to high phytoxicity. However, after 2.5% or 5%

iron treatment, the growth of lettuce was substantially enhanced. The dry weight (0.56 g/pot for roots; 3.1 g/pot for shoots) and height (24.8 cm) of lettuce harvested from 2.5% iron treated soil was also close to the plants grown in control soils. Moreover, the growth of lettuce in 5% iron treated sandy soils was also completely restored. The plant weight and height did not significantly differ between pots where iron was retrieved (0.56 g/pot for roots, 3.0 g/pot for shoots, and 27.3 cm for shoots length) and pots where the iron was not retrieved from the soil (0.52 g/pot for roots, 3.2 g/pot for shoots, and 27.5 cm for shoots length) (Figures 7-9 and 7-11).

Similar results were obtained as regards the growth of Indian mustard in sandy soil (Figures 7-10 and 7-11). No significant effect of iron filings was observed on the growth of mustard. The dry weight and height of Indian mustard grown in control soil (no Cu^{2+} added) with 5% iron (4.6 g/pot for shoots, 1.6 g/pot for roots, and 23.4 cm for shoots length) were quite close to that grown in control soil without iron filings (4.2 g/pot for shoots, 1.9 g/pot for roots, and 22.3 cm for shoots length). Indian mustard could not survive in non-treated sandy soil; however, its growth was significantly improved in iron-treated soil. After the 5% iron treatment, the growth of Indian mustard was also completely restored. Moreover, the plant weight and height did not significantly differ between soils where iron was retrieved (1.8 g/pot for roots, 4.2g/pot for shoots, and 21.2cm for shoots length) and soils where the iron was not retrieved (1.6 g/pot for roots, 4.4 g/pot for roots, and 24.8 cm for shoots length). In the 2.5% iron-treated sandy soil, as compared with plants in the control soil, the dry plant biomass (2.4 g/pot for shoots and 0.5 g/pot for roots) was somewhat lower, although the shoots length of Indian mustard (20.0 cm) was not significantly different. This indicated that treatment with 2.5% iron filings was not as effective as 5% iron filings for reducing Cu phytotoxicity towards Indian mustard.

7.3.2.2 Effect of treatments on plant growth in organic soil 2

As shown in Figures 7-12 and 7-13, and 7-14, the growth of lettuce and Indian mustard in control soil with or without iron filings showed no significant difference. However, in non-treated 500 mg/kg Cu-spiked organic rich soil 2, the growth of lettuce and Indian mustard was significantly inhibited, with reduced dry biomass of 1.2 g/pot (shoots) and 0.2 g/pot (roots) for lettuce, and 0.21 g/pot (shoots) and 0.03 g/pot (roots) for mustard. Moreover, the height of lettuce and Indian mustard grown in non-treated organic rich soil 2 also decreased to 15.5 cm and 4.6 cm, respectively. After 5% iron treatment followed by magnetic separation, the growth of lettuce and Indian mustard was improved to the similar level of plants grown in non-contaminated soil. In 5% iron treated organic rich soil 2 where the iron filings were magnetically retrieved, the dry biomass and shoots length of lettuce were 5.8 g/pot (shoots), 0.7 g/pot (roots), and 38.9 cm, and the dry biomass and height of Indian mustard were 8.1g/pot for shoots, 2.9g/pot for roots, and 29.0cm. When the iron filings were not removed, the dry weight and height for plants were 4.3 g/pot (shoots), 0.6 g/pot (roots) and 32.3 cm for lettuce. For Indian mustard, the dry weight and height for plants were 7.5 g/pot (shoots), 2.9 g/pot (roots) and 27.3 cm for mustard (see Figures 7-12, 7-13, and 7-14).

Many other researchers have also used plant growth to study the effectiveness of different soil amendments in reducing heavy metal mobility and bioavailability in contaminated soils (Boisson et al., 1999; Castaldi et al., 2005; Chen et al., 2000; Smeulders et al., 1983a, b; Smeulders and Vandegeijn, 1983). Boisson et al. (1999) applied hydroxyapatite (HA) to treat a metal (Zn, Pb, Cu, Cd) and As-contaminated soil, and the treatment effectiveness was examined by growing maize (*Zea mays* L.) and bean (*Phaseolus vulgaris*) in treated and non-treated soil. Their data indicated that plant growth was partly restored at 0.5% and 1% hydroxyapatite treated soils. However, at 5% hydroxyapatite application rate, plant growth was inhibited again which

may due to the simultaneous immobilization of essential nutrients. In another study reported by Castaldi et al. (2005), the effects of three amendments (zeolite, compost, and calcium hydroxide) on the immobilization of Pb, Cd and Zn in a contaminated soil were determined and their influence on white lupin (*Lupinus albus*) growth was also investigated. Results showed that the growth of white lupin was enhanced in amended soils. With respect to unamended soil, the plant shoots biomass increased with a factor of 1.8 (soil amended with zeolite), 3.6 (soil amended with compost), and 3.1 (soil amended with calcium hydroxide), and the roots biomass increases with a factor of 1.4 (soil amended with zeolite), 5.6 (soil amended with compost), and 4.8 (soil amended with calcium hydroxide).

7.3.3 Toxicity of Iron Treated soils, as Determined by MetPLATE™

The above plant study has shown the effectiveness of iron treatment on Cu-spiked soils. It was found that following iron treatment, the phytoxicity caused by Cu was substantially decreased. We parallelly used the MetPLATE™ assay to test the toxicity of these iron-treated soils used for growing plants. The results are shown in Table 7-4. With regard to sandy soil, no toxicity was found in the control soil with 5% iron filings. After 2.5% and 5% iron treatment, whether the iron filings were magnetically retrieved or not, the treated soil extracts hardly showed any toxicity (7.5% to 8.5% inhibition), with EC_{50}s all greater than 100%, which agreed with the plant study showing no phytotoxicity in these treated soils. However, in non-treated 100mg/kg Cu-spiked sandy soil, the EC_{50} of the soil extract was 15.4%, and the non diluted soil extract generated an inhibition of 86.8% by MetPLATE™, which caused very high phytotoxicity resulting in plant death.

The toxicity of the organic rich soil 2 with or without iron treatments was also displayed in Table 7-4. The plant study showed that the growth of lettuce and Indian mustard was significantly inhibited in the non-treated 500 mg/kg Cu-spiked organic rich soil 2, whereas in 5%

iron-treated soils, no phytotoxicity was observed. After performing MetPLATE^{TM} toxicity test to the same soils, we found that the non-treated soil extract produced an inhibition of 78.2%; however, after 5% iron treatment, the EC_{50}s of the soil extracts were all greater than 100%, and much lower percent inhibitions (16.6% in iron retrieved soil extract and 14.9% in iron immobilized soil extract) were generated. The MetPLATE^{TM} assay showed consistent data with the plant study. In a study carried out by Wundram et al. (1996), a new phytotoxicity test, based on the inhibition of photosynthesis of the green algae *Chlamydomonas reinhardtii*, was investigated and compared with other phytotoxicity tests using *Lemna* (growth) and *Lepidium* (root elongation). The experiment was conducted in solutions with distinct heavy metals and solutions with complex mixture of heavy metals. Their data indicated that *Chlamydomonas* was most sensitive to Hg and Cd, whereas *Lepidium* and *Lemna* had highest sensitivity to Cu and Cd. In the solution containing a mixture of various heavy metals including Cd, Cu, Zn, and Pb, *Chlamydomonas* showed the highest sensitivity. Trapp et al. (2000) developed a short-term acute toxicity assay based on the change of transpiration of willow tree cutting grown in contaminated solution. The sensitivity of the test was evaluated with 3,5-dichlorophenol and the EC_{50}s were found between 5.8 and 9.6 mg/L after 48-h and 72-h exposure, which were similar to the results from algal (*Scenedesmus subspicatus* and *Raphidocelis subcapita*) growth tests.

7.3.4 Copper Uptake by Plants in Treated and Non-treated Soils

Chemical analysis by ICP-AES was also performed for lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) harvested from treated and non-treated sandy soil and organic rich soil 2. For each plant species, in the same soil, the uptake of Cu accumulated mainly in the roots, and this is agreed with results referred by other authors (Castaldi et al., 2005; Cheng and Allen, 2001; Fargasova, 2001; Jordao et al., 2006). Moreover, the shoots Cu concentration in Indian

mustard was generally higher than that in lettuce; however, the roots uptake of Cu in these two plant species was similar. These finding agreed with the results shown in Section 7.3.1.3.

7.3.4.1 Copper uptake by plants grown in sandy soil

As shown in Table 7-5, Cu uptake by lettuce shoots in sandy soil followed this order: 2.5% iron treated soil - iron retrieved (18.9 mg/kg) > 5% iron treated soil - iron immobilized (13.1 mg/kg) ≈ 5% iron treated soil - iron retrieved (12.0 mg/kg) > control soil with 5% iron (7.2mg/kg) ≈ control soil without iron (6.4 mg/kg). However, the uptake of Cu by lettuce roots in control with 5% iron (109.0 mg/kg) was significantly higher than that in control without iron filings (9.2 mg/kg), which was probably due to the Cu background in iron filings (2503.3 mg/kg). Besides, 125.4 mg/kg and 182.3 mg/kg of Cu were found respectively in lettuce roots at 5% and 2.5% iron treated sandy soil where the iron filings were magnetically retrieved. When the 5% iron filings were not removed from the treated sandy soil, a little higher Cu concentration (204.3 mg/kg) was found in lettuce roots, which may also result from the Cu background in the iron filings.

Table 7-5 also shows the Cu uptake by Indian mustard in treated and non-treated sandy soil, which followed the same trend as the Cu uptake by lettuce. The shoots Cu concentrations of mustard grown in the control with and with iron filings were 7.4 mg/kg and 5.5 mg/kg, respectively. The shoots Cu uptake by mustard from 5% and 2.5% iron-treated sandy soil were somewhat enhanced, varied from 30.9 mg/kg to 51.3 mg/kg. As regards roots Cu uptake by Indian mustard, 115.4 mg/kg Cu was found in the roots grown in 5% iron treated control, as compared to 8.3 mg/kg in roots from non-treated control (i.e. no iron filings added). Besides, the roots Cu concentrations in iron treated soil varied from 135.2 mg/kg to 215.9 mg/kg, with the highest value found in the soil where the 5% iron filings were not magnetically retrieved.

Chemical analysis for plants grown in 100 mg/kg Cu-spiked sandy soil was not available due to plant death.

7.3.4.2 Copper uptake by plants grown in organic rich soil 2

The Cu contents in lettuce and Indian mustard harvested from the organic rich soil 2 are displayed in Table 7-6. The shoots Cu contents in control soil with 5% iron (7.4mg/kg for lettuce and 5.4 mg/kg for mustard) were very close to that in control soil without iron (6.6 mg/kg for lettuce and 5.1 mg/kg for Indian mustard). Without iron treatment, in 500mg/kg Cu-spiked organic soil 2, the shoots Cu uptake was 75.3mg/kg for lettuce and 128.9 mg/kg for mustard. However, after iron treatment, substantial decrease of Cu content in plant shoots was obtained, indicating a drop of phytoavailable Cu. The shoots Cu uptake in 5% iron-treated organic rich soil 2, where iron filings were magnetically retrieved, was 18.4 mg/kg for lettuce and 25.4 mg/kg for mustard. When the iron flings were not removed, a little higher Cu concentrations, 30.0 mg/kg and 31.5 mg/kg, were respectively found in the shoots of lettuce and Indian mustard.

With regard to Cu uptake in plant roots, as shown in Table 7-6, 10.4 mg/kg and 9.4 mg/kg Cu were found in the roots of lettuce and Indian mustard, respectively, grown in control soil without the addition of iron filings. In the presence of 5% iron, the Cu content in roots grown in non-spiked organic rich soil 2 (control) increased to 61.7 mg/kg for lettuce and 55.0 mg/kg for mustard. However, these values were lower than those found in the sandy soil (109.0 mg/kg for lettuce and 115.4 mg/kg for mustard) due to the higher Cu binding capacity of organic rich soil as shown in Chapter 2. Without iron treatment, in 500 mg/kg Cu-spiked organic rich soil 2, 1131.2 mg/kg and 1025.2 mg/kg Cu was accumulated in the roots of lettuce and Indian mustard, respectively. However, after treatment with 5% iron filings, the roots Cu content was significantly reduced to 398.6 mg/kg for lettuce and 352.9 mg/kg for Indian mustard when the iron filings were magnetically retrieved. However, when the iron filings were not removed from

the treated soil, 448.9 mg/kg and 460.7 mg/kg Cu was found in the roots of lettuce and Indian mustard, respectively.

Lombi et al. (2002a) utilized a Fe-oxide rich material (red mud) to immobilize heavy metals (Cd, Zn, Cu, Ni, and Pb) in contaminated soils, at an application rate of 2% (w/w). After performing sequential extraction experiment, they found that this soil amendment shifted metals from the exchangeable forms to the Fe-bound fractions. In a companion paper (Lombi et al., 2002b), Lombi and his colleagues also examined the effects of the above soil amendment by using biological indicators, such as plants growth, metal uptake, and soil microbial activity. The results showed that the application of red mud reduced phytotoxicity of heavy metals, enhanced plant yields and decreased the metal concentrations in plants, which supports quite well the findings in our study.

7.4 Conclusions

The feasibility of using PletPLATE^{TM} toxicity test to predict Cu phytotoxicity was first investigated in this Chapter. The results of the plant study showed that lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) could not survive in 100 mg/kg Cu-spiked sandy soil, whereas in 25 mg/kg and 50 mg/kg Cu-spiked sandy soil, no phytotoxicity was observed. In Cu-spiked organic rich soil, 50 mg/kg Cu concentration did not cause any plant inhibition; however, at 500 mg/kg and 700 mg/kg Cu concentrations, the growth of the plants was significantly inhibited, indicating the phytotoxicity of Cu. In 100 mg/kg, 200 mg/kg, and 500 mg/kg Cu-spiked mixed soil, only the highest Cu concentration, 500 mg/kg soil, inhibited the growth of lettuce to some extent. The data for Indian mustard grown in mixed soil was unfortunately lost due to heavy pest infestation. The toxicity of the above soils was also tested, using MetPLATE^{TM}. We concluded that if a soil extract showed approximately 90% inhibition by MetPLATE^{TM} assay, this soil could probably cause phytotoxicity in lettuce (*Lactuca sativa*) and

Indian mustard (*Brassica juncea*). Moreover, chemical analysis on Cu content in plant tissue suggested that the plant uptake of Cu increased with the increase of the input Cu concentration, indicating the increase of phytoavailable Cu. Besides, for each plant in the same type of soil, roots could always accumulate much more Cu than shoots. Comparing the two plant species, the shoots Cu concentration in Indian mustard was generally higher than that in lettuce; however, the roots uptake of Cu in these two plant species was similar.

In addition, we employed pot experiments to demonstrate the effectiveness of iron treatment followed by magnetic separation for reducing phytoavailable Cu. The results indicated that iron filings at 5% concentration did not exert any adverse effect on plant growth. In Cu-spiked sandy soil and organic rich soil 2, the plant growth in non-treated soil was substantial inhibited. However, after iron treatment, whether the iron filings were magnetically retrieved or not, the growth of lettuce and Indian mustard was significantly enhanced, and the plant height and weight were similar to those observed in control soils that were not spiked with Cu. Moreover, chemical analysis showed a great reduction of the Cu content in plant shoots and roots after iron treatment of the Cu-spiked organic rich soil 2, which also indicated the decrease of phytoavailable Cu.

In all, we conclude that MetPLATE^{TM} toxicity test showed great potential in predicting Cu phytotoxicity. The effectiveness of the iron treatment for reducing phytoavailable Cu in soils was also confirmed by the plant study.

Table 7-1 Soils Characteristics

Characteristic	Sandy soil	Organic soil	Mixed soil	Organic soil 2
pH	5.7	5.7	5.5	5.3
Eh (mV)	422	403	374.7	337
% Organic carbon	0.5	6.4	3.3	4.1
% Organic matter	1.6	18.8	9.6	12.2
% Sand	96.9	93.2	92.0	92.6
% Silt	0.02	2.0	2.6	0.8
% Clay	3.06	4.8	5.4	6.6
CEC ($cmol_c/kg$)	14.1	230.1	122.3	107.8

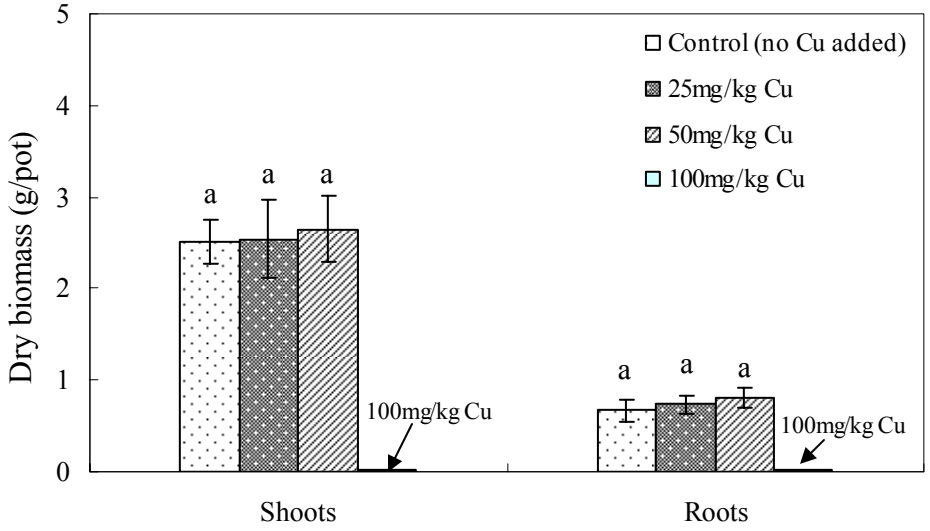


Figure 7-1. Effect of Cu concentrations on dry biomass of shoots and roots of lettuce (*Lactuca sativa*) grown in spiked sandy soil (No growth was observed in lettuce grown 100 mg/kg Cu-spiked sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

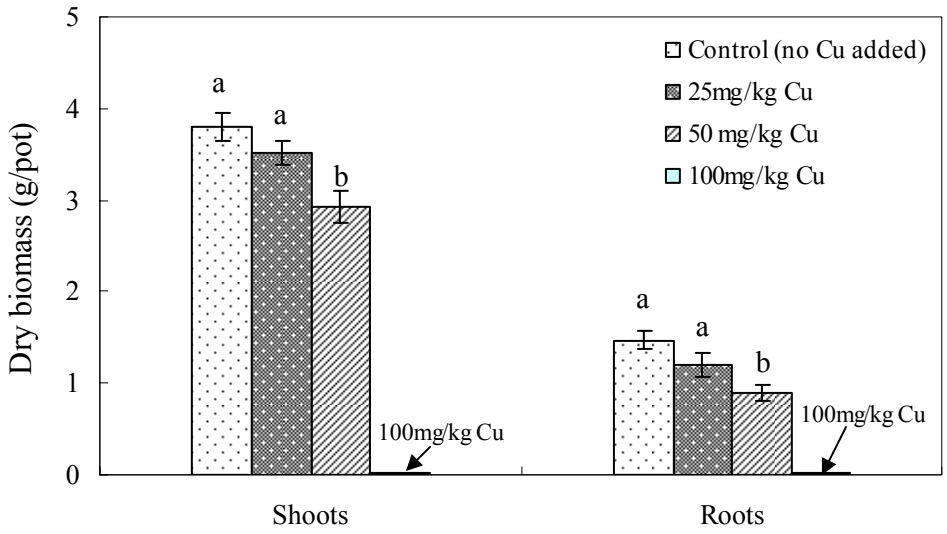


Figure 7-2. Effect of Cu concentrations on dry biomass of shoots and roots of Indian mustard (*Brassica juncea*) grown in spiked sandy soil (No growth was observed in 100 mg/kg Cu-spiked sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

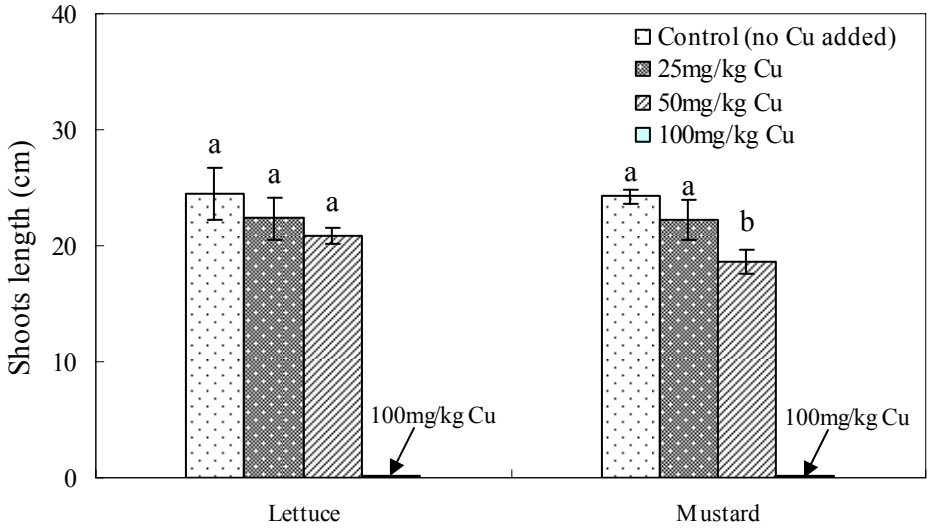


Figure 7-3. Effect of Cu concentrations on shoots length of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in spiked sandy soil (No growth was observed in lettuce and mustard grown in 100 mg/kg Cu-spiked sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

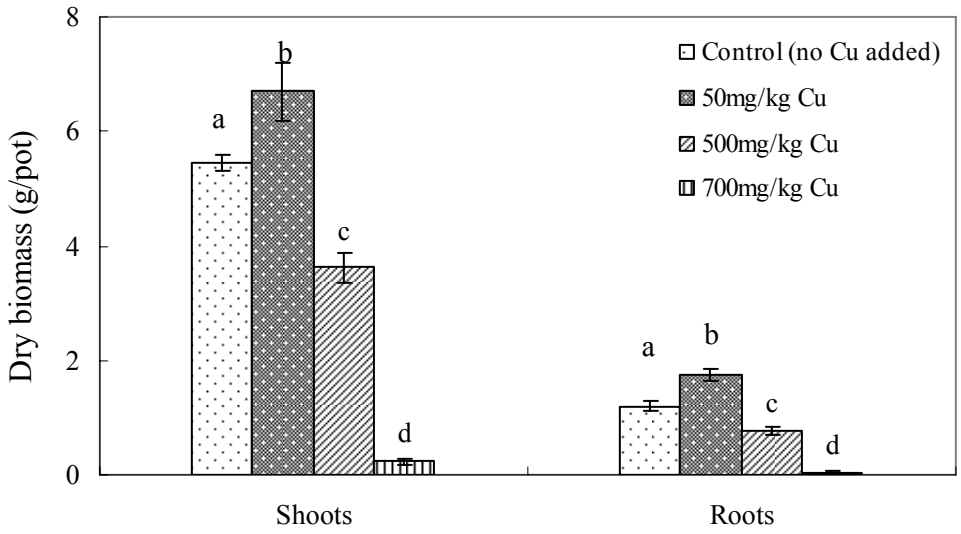


Figure 7-4. Effect of Cu concentrations on dry biomass of shoots and roots of lettuce (*Lactuca sativa*) grown in spiked organic rich soil (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

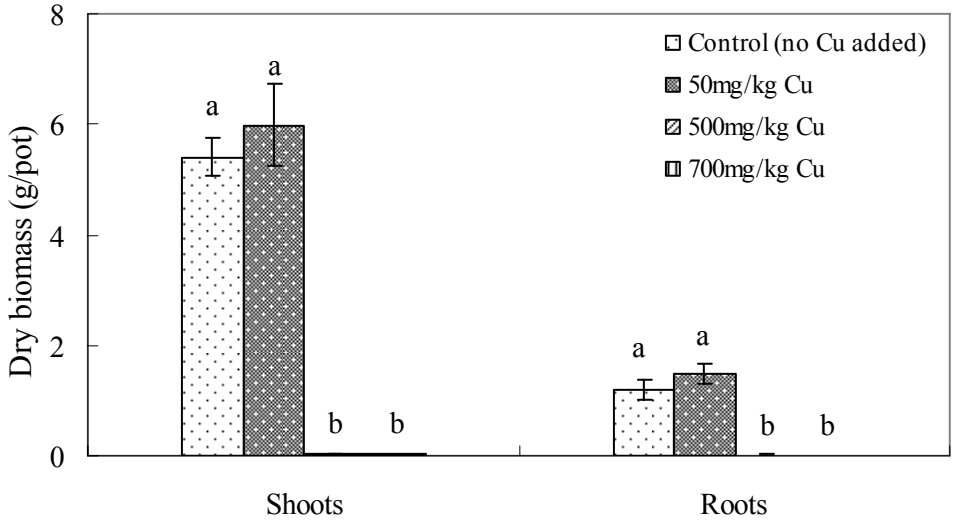


Figure 7-5. Effect of Cu concentrations on dry biomass of shoots and roots of Indian mustard (*Brassica juncea*) grown in spiked organic rich soil (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

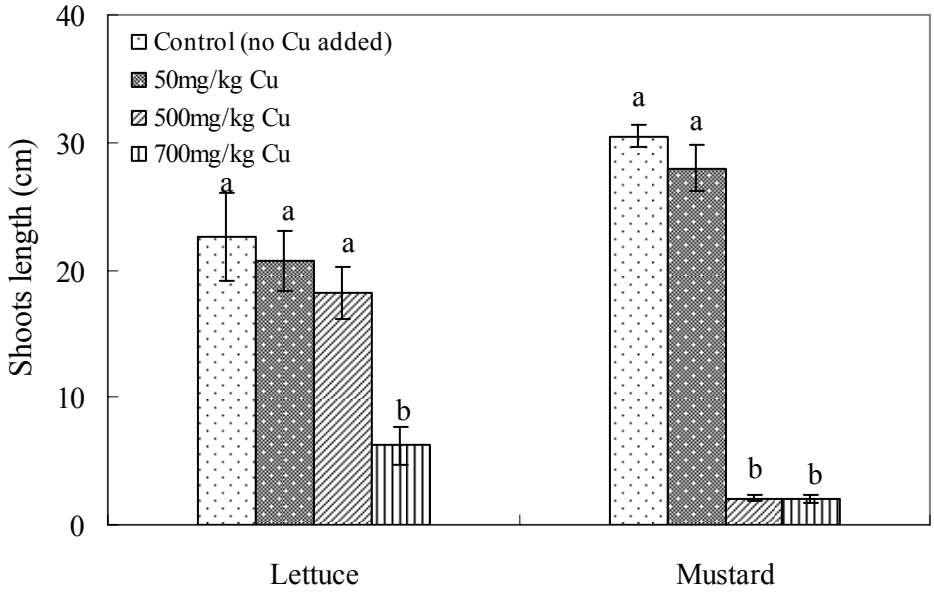


Figure 7-6. Effect of Cu concentrations on shoots length of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in spiked organic rich soil (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

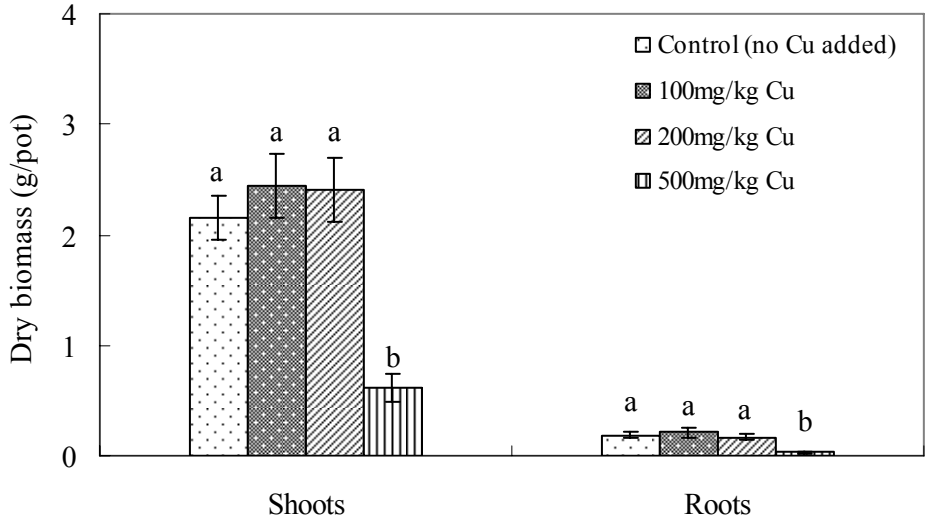


Figure 7-7. Effect of Cu concentrations on dry biomass of shoots and roots of lettuce (*Lactuca sativa*) grown in spiked mixed soil (Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

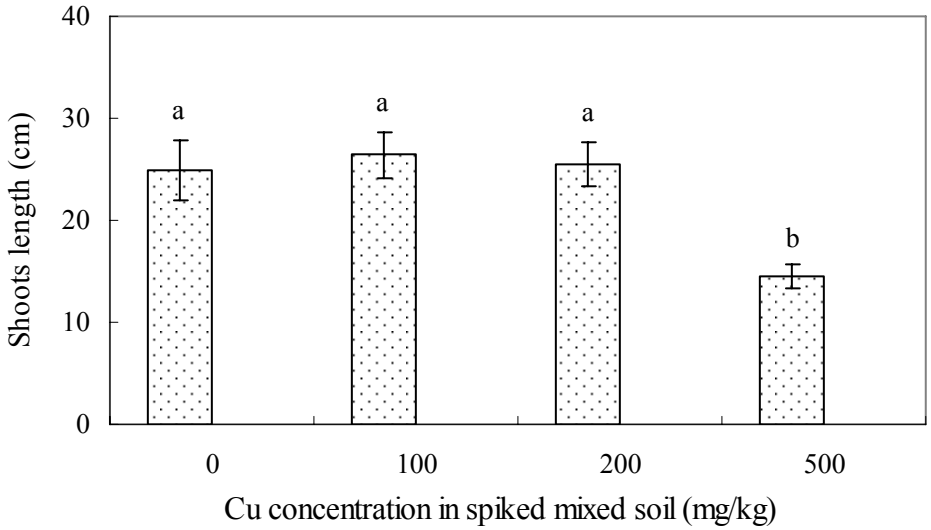


Figure 7-8. Effect of Cu concentrations on shoots length of lettuce (*Lactuca sativa*) grown in spiked mixed soil (Error bars represent standard deviation of three replicates. Values followed by the same letter do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

Table 7-2. Copper toxicity in spiked sandy soil, organic rich soil, and mixed soil used for growing plants, as determined by MetPLATE™.

Soil Type	Cu Conc. in spiked soil (mg/kg)	EC_{50} of soil extract (% soil extract)	% Inhibition of undiluted soil extract
Sandy	25	>100%	Not Toxic
	50	>100%	31.4 ± 2.1%
	100	15.3 ± 2.0%[a]	89.9 ± 0.4%
Organic rich	50	>100%	23.6 ± 3.0%
	500	3.7 ± 0.1%	89.9 ± 0.9%
	700	2.4 ± 0.06%	90.1 ± 0.8%
Mixed	100	>100%	31.6 ± 1.1%
	200	47.7 ± 1.3%	67.8 ± 4.2%
	500	11.5 ± 0.6%	84.8 ± 2.3%

[a] Mean of 3 replicates ± 1 standard deviation

Table 7-3. Copper uptake by lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in spiked sandy soil and organic soil, as determined by chemical analysis.

Soil Type	Initial Cu conc. in spiked soil (mg/kg)	Cu conc. in shoots (mg/kg)		Cu conc. in roots (mg/kg)	
		Lettuce	Mustard	Lettuce	Mustard
Sandy	0	4.4 ± 0.6[a]	5.2 ± 0.9	5.7 ± 1.4	7.4 ± 0.8
	25	7.6 ± 1.5	27.0 ± 3.2	123.8 ± 12.3	132.1 ± 15.3
	50	16.6 ± 2.1	65.6 ± 8.5	344.4 ± 0.6	359.7 ± 24.6
	100	N/A[b]	N/A[b]	N/A[b]	N/A[b]
Organic rich	0	3.5 ± 1.1	4.7 ± 1.3	7.4 ± 1.1	8.4 ± 0.9
	50	6.7 ± 1.2	8.4 ± 0.8	71.4 ± 3.4	79.9 ± 3.8
	500	50.2 ±13.5	N/A[c]	670.0 ± 6.2	N/A[c]
	700	106.4 ± 19.4	N/A[c]	N/A[c]	N/A[c]
Mixed	0	7.8 ± 0.9	N/A[d]	51.7 ± 2.5	N/A[d]
	100	36.9 ± 6.1	N/A[d]	243.9 ± 26.4	N/A[d]
	200	66.2 ± 11.9	N/A[d]	706.3 ± 43.4	N/A[d]
	500	318.5 ± 46.0	N/A[d]	1199.0 ± 113.2	N/A[d]

[a] Mean of 3 replicates ± 1 standard deviation; [b] Not available due to death of the plants; [c] Not available due to low biomass; [d] Data were lost due to heavy pest infestation.

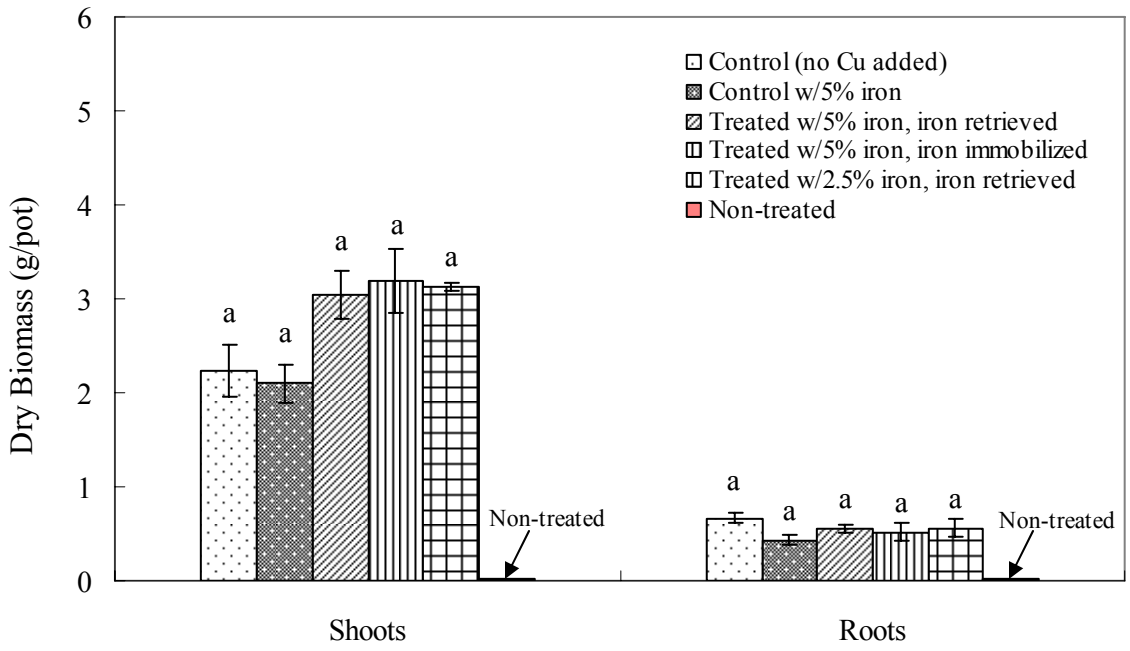


Figure 7-9. Effect of different treatments on dry biomass of shoots and roots of lettuce (*Lactuca sativa*) grown in a sandy soil (Cu concentration in spiked sandy soil was 100 mg/kg before treatment. No growth was observed for lettuce grown in non-treated sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

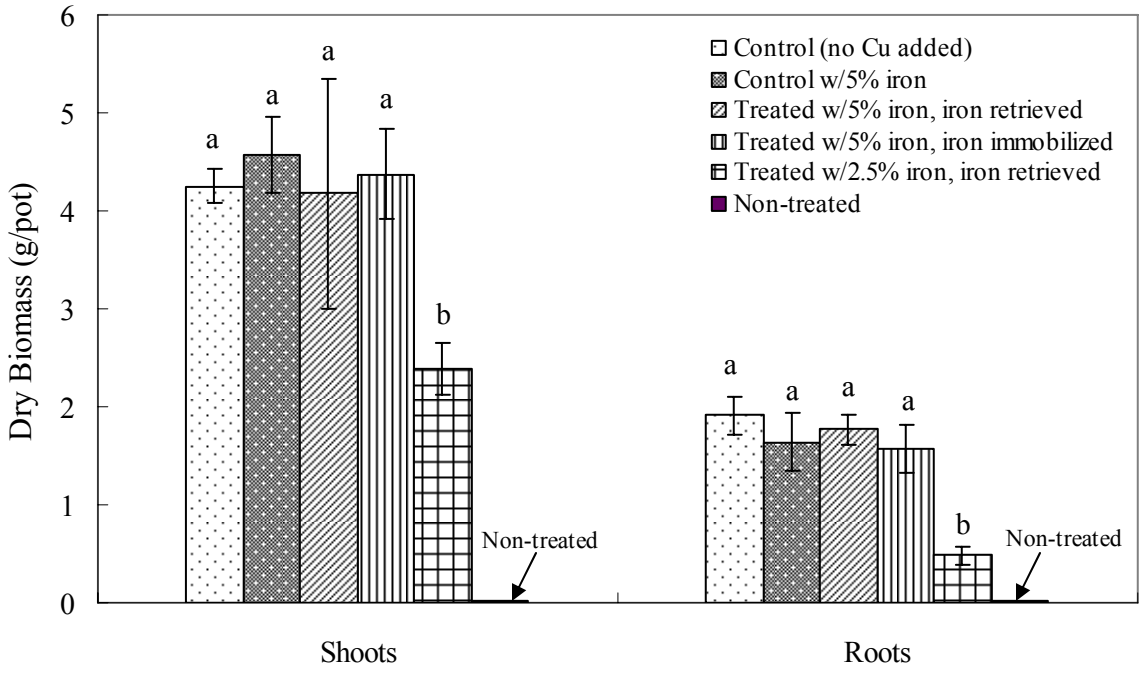


Figure 7-10. Effect of different treatments on dry biomass of shoots and roots of Indian mustard (*Brassica juncea*) grown in a sandy soil (Cu concentration in spiked sandy soil was 100 mg/kg before treatment. No growth was observed for Indian mustard grown in non-treated sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

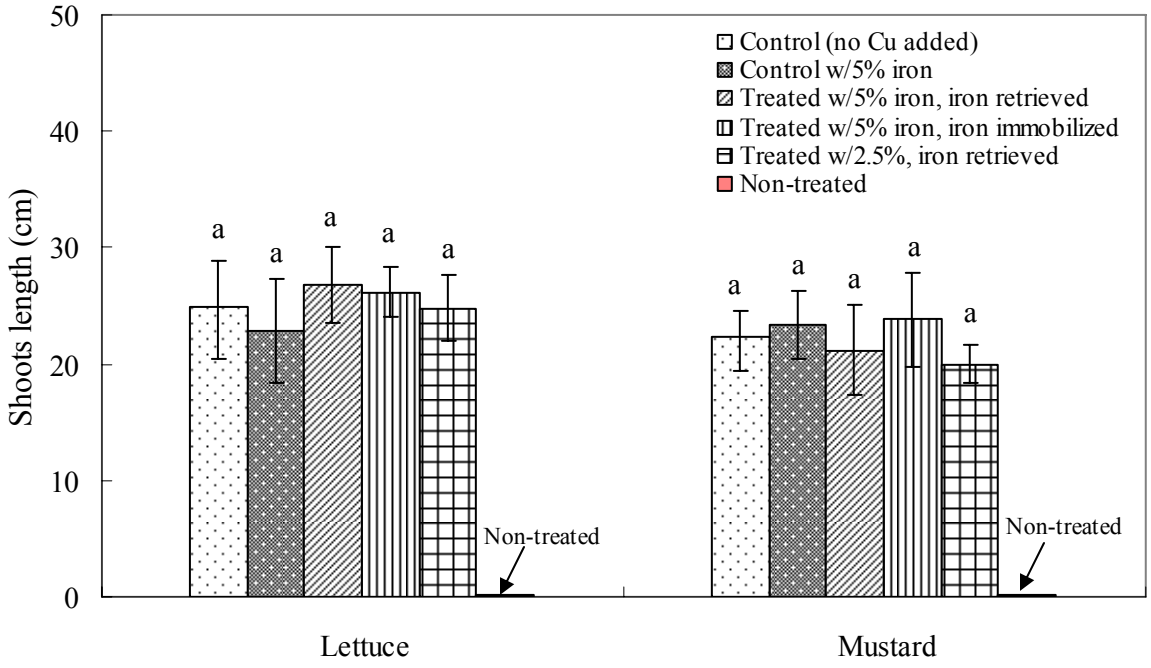


Figure 7-11. Effect of different treatments on shoots length of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in sandy soil (Cu concentration in spiked sandy soil was 100 mg/kg before treatment. No growth was observed in both Lettuce and Indian mustard grown in non-treated sandy soil. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

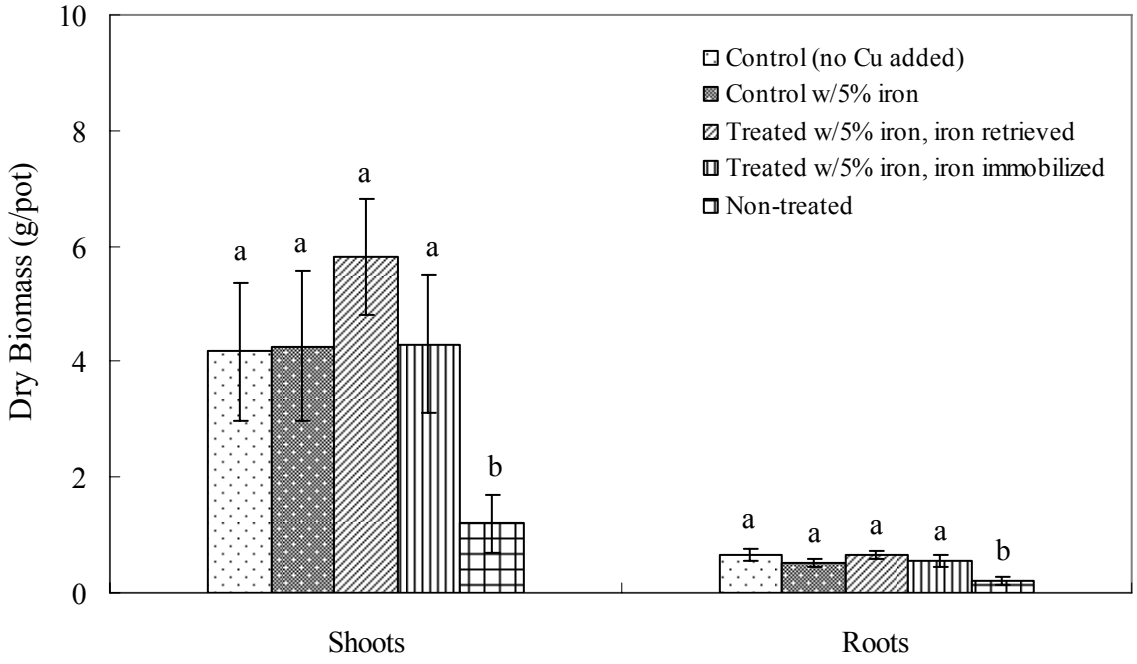


Figure 7-12. Effect of different treatments on dry biomass of shoots and roots of lettuce (*Lactuca sativa*) grown in organic rich soil 2 (Cu concentration in spiked organic soil 2 was 500 mg/kg before treatment with iron filings. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

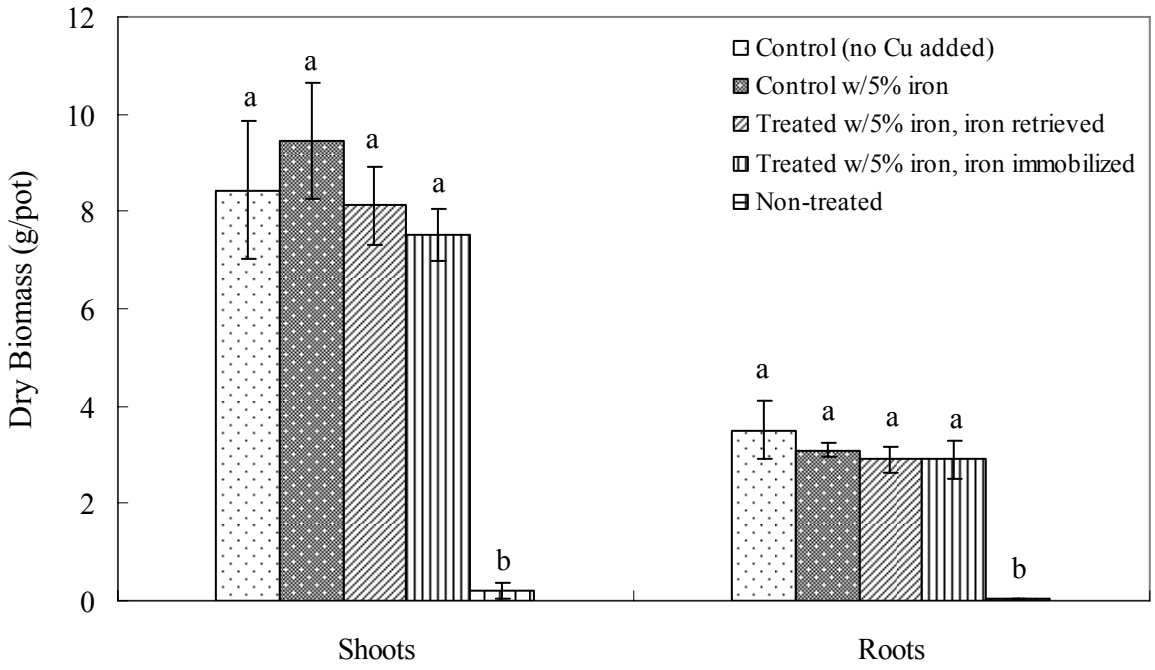


Figure 7-13. Effect of different treatments on dry biomass of shoots and roots of Indian mustard (*Brassica juncea*) grown in organic rich soil 2 (Cu concentration in spiked organic soil 2 was 500 mg/kg before treatment. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

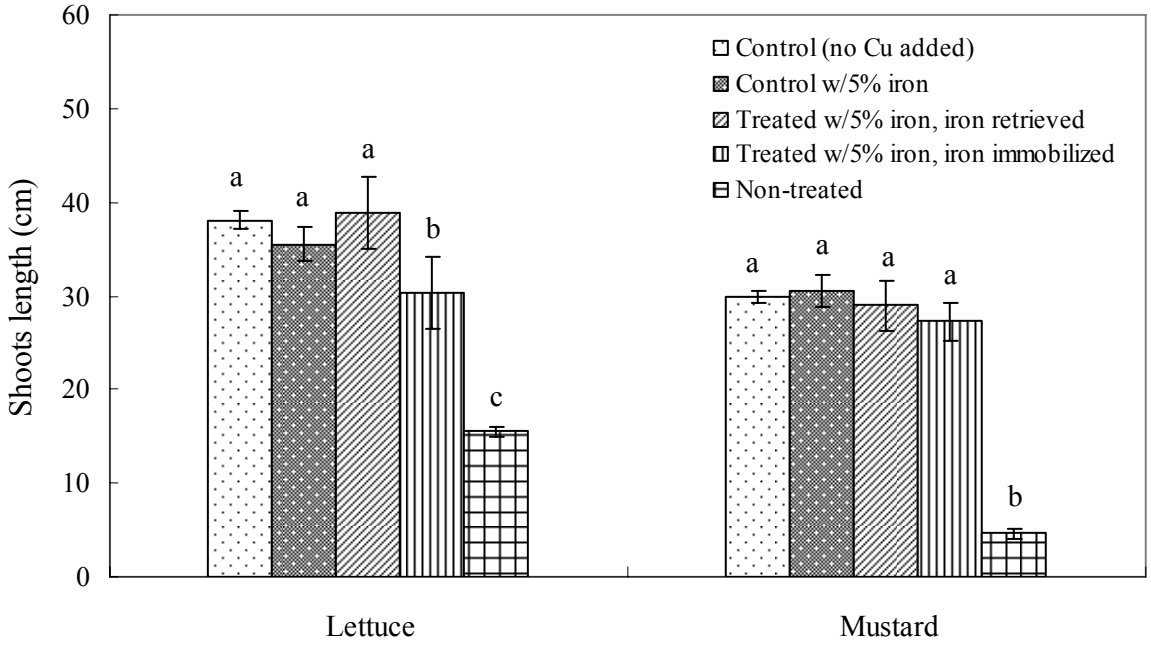


Figure 7-14. Effect of different treatments on shoots length of lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in organic rich soil 2 (Cu concentration in spiked organic soil 2 was 500 mg/kg before treatment. Error bars represent standard deviation of three replicates. Values followed by the same letter within the same group do not differ significantly at the 5% level according to the Tukey's Studentized Range (HSD) Test).

Table 7-4. Effect of different treatments on copper toxicity in sandy soil and organic rich soil 2 used for growing plants, as determined by MetPLATE™

Soil Type	Initial Cu conc. in spiked soil (mg/kg)	Treatment	EC_{50} of soil extract (% soil extract)	% Inhibition of undiluted soil extract
Sandy	0	Control w/ 5% of iron	>100%	-0.9 ± 0.2%[a]
	100	Treated w/ 5% of iron, iron retrieved	>100%	8.2 ± 0.2%[b]
	100	Treated w/ 5% of iron, iron immobilized	>100%	7.5 ± 0.9%
	100	Treated w/ 2.5% of iron, iron retrieved	>100%	8.4 ± 1.5%
	100	Non-treated	15.4 ± 3.4%	86.8 ± 0.1%
Organic rich	0	Control w/ 5% of iron	>100%	11.9 ± 1.8%
	500	Treated w/ 5% of iron, iron retrieved	>100%	16.6 ± 1.8%
	500	Treated w/ 5% of iron, iron immobilized	>100%	14.9 ± 2.7%
	500	Non-treated	15.9 ± 0.4%	78.2 ± 1.6%

[a] Non-toxic; [b] Mean of 3 replicates ± 1 standard deviation.

Table 7-5. Copper uptake by lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in treated and non-treated sandy soil, as determined by chemical analysis

Plant Type	Initial Cu conc. in spiked soil (mg/kg)	Treatment	Cu conc.[a] in shoots (mg/kg)	Cu conc. in roots (mg/kg)
Lettuce	0	Control	6.4 ± 0.5	9.2 ± 0.7
	0	Control w/ 5% of iron	7.2 ± 2.0	109.0 ± 7.2
	100	Treated w/ 5% of iron, iron retrieved	12.0 ± 2.0	125.4 ± 11.7
	100	Treated w/ 5% of iron, iron immobilized	13.1 ± 1.9	204.3 ± 10.9
	100	Treated w/ 2.5% of iron, iron retrieved	18.9 ± 2.7	182.3 ± 8.9
	100	Non-treated	N/A[b]	N/A[b]
Mustard	0	Control	5.5 ± 1.9	8.3 ± 2.5
	0	Control w/ 5% of iron	7.4 ± 0.3	115.4 ± 19.0
	100	Treated w/ 5% of iron, iron retrieved	35.7 ± 0.3	135.2 ± 0.9
	100	Treated w/ 5% of iron, iron immobilized	30.9 ± 0.5	215.9 ± 11.7
	100	Treated w/ 2.5% of iron, iron retrieved	51.3 ± 5.6	198.8 ± 6.6
	100	Non-treated	N/A[b]	N/A[b]

[a] Mean of 3 replicates ± 1 standard deviation; [b] Not available due to plant death.

Table 7-6. Copper uptake by lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) grown in treated and non-treated organic rich soil 2, as determined by chemical analysis

Plant Type	Initial Cu conc. in spiked soil (mg/kg)	Treatment	Cu conc. in shoots (mg/kg)	Cu conc. in roots (mg/kg)
Lettuce	0	Control	6.6 ± 1.7[a]	10.4 ± 1.9
	0	Control w/ 5% of iron	7.4 ± 1.3	61.7 ± 8.0
	500	Treated w/ 5% of iron, iron retrieved	18.4 ± 2.6	398.6 ± 26.8
	500	Treated w/ 5% of iron, iron immobilized	30.0 ± 8.5	448.9 ± 18.4
	500	Non-treated	75.3 ± 3.8	1131.2 ± 79.6
Mustard	0	Control	5.1 ± 0.1	9.4 ± 2.1
	0	Control w/ 5% of iron	5.4 ± 1.8	55.0 ± 5.9
	500	Treated w/ 5% of iron, iron retrieved	25.4 ± 2.5	352.9 ± 47.9
	500	Treated w/ 5% of iron, iron immobilized	31.5 ± 4.9	460.7 ± 43.5
	500	Non-treated	128.9 ± 11.1	1025.2 ± 37.2

[a] Mean of 3 replicates ± 1 standard deviation

CHAPTER 8
SUMMARY AND CONCLUSIONS

8.1 Summary

Heavy metal contamination of soils and sediments is a worldwide issue. The main purpose of this research was to investigate the effectiveness of a magnetic treatment method for removing heavy metals from contaminated soils and sediments. The treatment approach was based on adsorbing the metal contaminants onto iron filings seed and removing the metal-laden filings by magnetic separation.

This dissertation covered six experimental studies. The first study dealt with a toxicological approach to assess the heavy metal binding capacity of different types of soils. The toxicity test used was MetPLATE^{TM}, a rapid bacterial assay that is specific for heavy metal toxicity. The binding capacity of soils towards Cu^{2+}, Zn^{2+}, and Hg^{2+} was assessed. In the second study we determined the conditions for adsorption of the metals to iron filings followed by magnetic treatment. The parameters studied include the concentrations of added iron filings, level of soil saturation, and the contact time between the iron filings and the soil matrix. The effectiveness of magnetic treatment on heavy metal (Cu^{2+}, Cd^{2+}, and Zn^{2+}) removal from artificially contaminated soils was also evaluated. Toxicity tests, chemical analysis, mass balance study, and sequential extraction of metals were all performed for treated and non-treated soils. In the following study, we investigated the effects of aging on the magnetic separation process, as well as the change of Cu and Zn toxicity in aged soils. In the fourth study we investigated the magnetic separation of Pb from shooting range soils, and the fifth study focused on removing Cu^{2+}, Zn^{2+}, and Hg^{2+} from artificially contaminated sediments by the same magnetic separation method. In the final study, we discussed the feasibility of using MetPLATE^{TM} assay to predict

heavy metal phytotoxicity, as well as the use of plants to assess the effectiveness of magnetic separation for removing Cu from soils.

8.2 Conclusions

Based on the above studies, the following conclusions were drawn:

- A novel toxicological approach is proposed to determine the heavy metal binding capacity of soils. Cu^{2+}, Zn^{2+}, and Hg^{2+} binding capacity for different types of soils followed this trend: Georgia clay rich soil > organic rich soils > sandy soils.

- The recovery of iron filings from a sandy soil was very high under all three soil saturation conditions (dry soil, soil at field capacity, and water-saturated soil). For red sandy soil, organic rich soil, and Georgia clay rich soil, the recovery of iron filings from saturated soil was lower than that from soils under dry and field capacity conditions.

- The conditions for magnetic separation of metals from soils were: An iron filings concentration of 5% (w/w) and a contact time between iron filings and the soil matrix of 3 hours.

- Cd-spiked soils showed higher toxicity than Cu- and Zn- spiked soils. Besides, at the same concentration, the toxicity of each metal in different soils was shown to follow this trend: sandy soils> Georgia clay rich soil > organic rich soil.

- The magnetic treatment method proposed worked best on Cu-spiked soils, followed by the Zn-spiked soils and the Cd-spiked soils. In addition, the effectiveness of this magnetic treatment was not significantly affected by the type of soil in Cu-spiked soils. However, as regards Zn- and Cd-spiked soils, this method worked better in sandy soils than in organic rich soil and Georgia clay rich soil.

- A large portion of the added metals was immobilized in the soil matrix. After magnetic treatment, Cu, Zn, and Cd were removed from both the soil extracts and the soil matrix. However, for each heavy metal, in the same soil, metal removal from the soil extract was always higher than that from the soil matrix.

- After magnetic treatment, the removal of Cu, Zn, and Cd was the greatest from the exchangeable fraction, followed by the carbonate fraction in sandy soil. In organic rich soil, the removal of these metals was found in all fractions, in which the highest removal was from the organic-bound phase, and the second highest removal was from the exchangeable phase for Cu, and the Fe-Mn oxide phase for Zn and Cd.

- Energy dispersive X-ray spectroscopy (EDS) confirmed the adsorption of Cu, Zn, and Cd on magnetically retrieved iron filings.

- The retrieved iron filings could be regenerated in 1 N HNO_3 for 1 hr and then retreated by 1 M NaOH for 72 hrs prior to reuse. The regenerated iron filings worked as well as fresh iron filings even after two regeneration cycles.

- To simulate field conditions, we studied the effect of soil aging on Cu toxicity in soils. As in the sandy soil, Cu toxicity did not show significant change during the initial 4-month dry aging as determined by both MetPLATE^{TM} and the 48-h *Ceriodaphnia dubia* test. However, after 20 wet-dry cycles, Cu toxicity decreased gradually with time. Zn toxicity in aged sandy soil gradually decreased after 2-month aging as shown by both toxicity tests. In organic rich soil, Cu toxicity did not change significantly when using *C.dubia* test, however, MetPLATE^{TM} showed slightly increase in Cu toxicity. Zn toxicity in aged organic rich soil only showed some decrease after the 12th wet-dry cycle by both toxicity tests. No significant reduction in magnetic separation efficiency was observed as regards Cu and Zn removal from aged soils.

- The magnetic separation method also showed great potential in treating Pb contaminated shooting range soils. Our proposed treatment achieved a great reduction of toxicity generated by Pb in soils. Moreover, Pb was removed from both the soil matrix and the soil extracts, although the removal from the soil matrix was always lower than that from the soil extracts.

- We also used the toxicological approach to assess the heavy metal binding capacity of aquatic sediments. Organic rich sediments showed higher metal (Cu^{2+}, Zn^{2+}, and Hg^{2+}) binding capacity than sandy sediments.

- Cu^{2+}, Zn^{2+}, and Hg^{2+} could also be removed effectively from sediments by magnetic separation. The type of sediment and metal did not affect the treatment effectiveness. The metals were removed from both the sediment matrix and the sediment extracts. However, as observed in soils, metal removal from the sediment matrix was lower than that from the sediment extracts.

- The sensitivity of the toxicity tests were shown in this order: the 96-h *Selenastrum capricornutum* test > the 48-h *Ceriodaphnia dubia* test > MetPLATE^{TM} assay. However, the three tests showed similar trends as regards toxicity removal of metals from soils and sediments.

- To investigate Cu phytotoxicity, we conducted a plant growth study and the following results were obtained. Lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) could not survive in 100 mg/kg Cu-spiked sandy soil, whereas in 25 mg/kg and 50 mg/kg Cu-spiked sandy soil, no phytotoxicity was observed. In Cu-spiked organic rich soil, 50 mg/kg Cu concentration did not cause any growth inhibition of both Lettuce and Indian mustard; however, at 500 mg/kg and 700 mg/kg soil Cu concentrations, plant growth was significantly inhibited. In 100 mg/kg, 200 mg/kg, and 500 mg/kg Cu-spiked mixed soil, only the highest Cu concentration, 500 mg/kg soil, inhibited the growth of lettuce (*Lactuca sativa*) to some extent. The data for Indian mustard was lost due to heavy pest infestation.

- Cu uptake by Lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) increased as the increase of the Cu input concentrations in soils. Plant roots always accumulated more Cu than the aerial shoots. Furthermore, Cu concentration in the shoots of Indian mustard was generally higher than that in the shoots of lettuce. However, Cu uptake by the roots was similar in both plants.

- MetPLATE^{TM} toxicity test showed great potential in predicting metal phytotoxicity. If a soil extract showed approximately 90% inhibition by MetPLATE^{TM} assay, this soil could probably cause phytotoxicity in lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*).

- The plant growth study also demonstrated the effectiveness of the proposed magnetic treatment on reducing Cu phytoavailability. After treatment, whether the iron filings were magnetically retrieved from the soil or not, the growth of Lettuce (*Lactuca sativa*) and Indian mustard (*Brassica juncea*) was significantly enhanced, and the plant height and weight were very similar to plants grown in control soils (i.e. no Cu added). The Cu content in plant shoots and roots also significantly decreased after treatment.

- The plant growth study confirmed the results obtained through the use of short-term toxicity tests such as MetPLATE^{TM}. This suggests that these short-term toxicity tests could be used to assess the effectiveness of present and future treatment methods for soil and sediment decontamination.

APPENDIX A
DETAILED PROCEDURE FOR TOXICITY TESTS

A.1 MetPLATE™ Procedure

MetPLATE™ is a rapid, quantitative microbial assay developed by Bitton et al. (1994). The particularity of this test is that it is specific for heavy metal toxicity and is not affected by relatively high concentrations of organic toxicants. MetPLATE™ assay is based on inhibition of the activity of β-galactosidase in a mutant strain of *Escherichia coli*. The MetPLATE™ kit includes freeze-dried *E. coli* (bacterial reagent), moderately hard water (diluent), buffered chromogenic enzyme substrate (chlorophenol-red β-galactopyranoside (CPRG)), a positive control, and a 96-well microplate. In the presence of the active enzyme, CPRG will change from yellow to red-purple. Otherwise, absence or reduced color change indicates the inhibition caused by heavy metals. The degree of color change can be quantified by reading the optical density with a microplate spectrophotometer at 570nm. The percent inhibition was calculated according to Equation A-1.

$$\%\text{Inhibition} = \frac{(\text{Negative control absorbance} - \text{Sample absorbance})}{\text{Negative control absorbance}} \times 100\% \qquad \text{(A-1)}$$

To determine the EC_{50} (the concentration that causes 50% inhibition of the test organisms in a given sample), we usually first undertook a range-finding toxicity test to determine the range of concentrations that could cover the EC_{50} point, then 4 to 5 dilutions of the sample within this range were prepared prior to the definitive test. Then, a regression analysis was performed by plotting the percent inhibitions produced by these sample dilutions versus their concentrations, and the EC_{50} was calculated from the Equation A-2.

$$EC_{50} = \frac{50 - \text{Yintercept}}{\text{Slope}} \qquad \text{(A-2)}$$

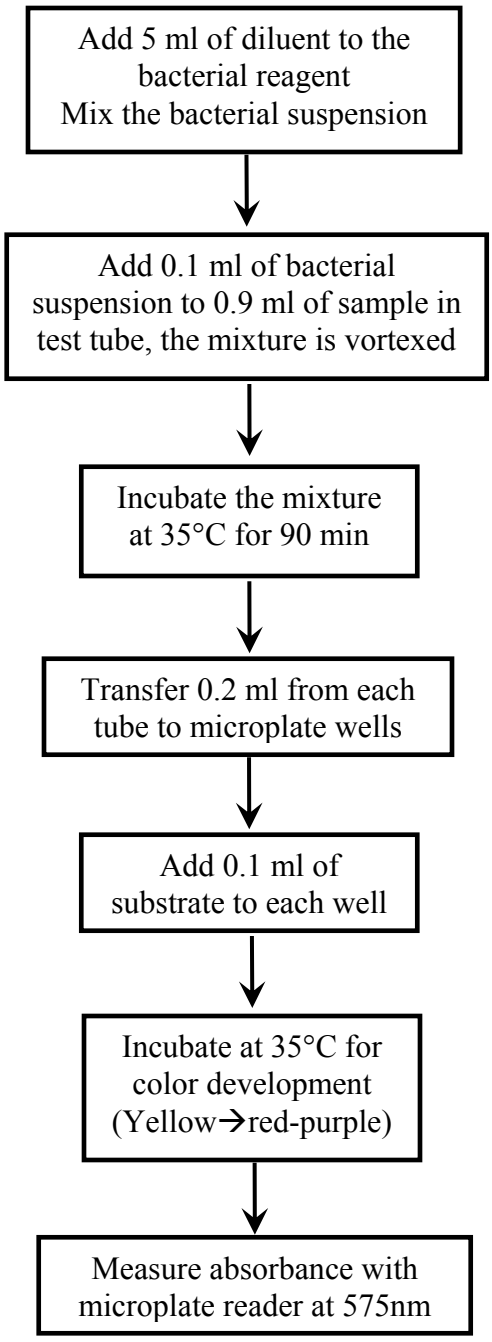


Figure A-1. MetPLATE™ protocol

A.2 48-h *Ceriodaphnia dubia* Acute Toxicity Test

A.2.1 Preparation of culture medium and food

Moderately hard water (MHW) was used as the growth medium to culture the daphnids. The characteristics of MHW are summarized in Table A-1. The food of *Daphnia* consisted of concentrated *Selenastrum capricornutum* suspension (3.5×10^7 cells/mL) and YCT (a mixture of yeast, cereal leaves and trout chow). The preparation of YCT started with a 7-day digestion of trout chow. Five gram of trout chow was added into 1 L of nanopure water, and the mixture was blended in a blender at low speed for 5 minutes. The trout chow solution was then transferred to a digestion apparatus where continuous aeration was maintained by using a glass pipette attached to an aquaculture pump. After 7 days, the digestion was complete and the digestate was transferred to a 1 L graduated cylinder and covered with Parafilm. Then, the cylinder was placed in the refrigerator to settle for at least 1 hour. Meanwhile, on day 7 of the trout chow digestion, 5 g of cereal leaves was added into 1 L of nanopure water in a blender, and the mixture was blended at low speed for 5 minutes. Then, the cereal leaves suspension was transferred to a 1 L graduated cylinder, covered with Parafilm and placed in the refrigerator to settle for at least 1 hour. Simultaneously, on day 7 of the trout chow digestion, 5 g of dry yeast were also added to 1 L of nanopure water, and the mixture was blended at low speed for 5 minutes. However, the yeast suspension was not allowed to settle and was combined immediately with trout chow and cereal leaves in the next step. Six hundred milliliter of each of YCT components (yeast suspension, trout chow and cereal leaves supernatant) were combined in a 2 L pitcher by sieving through a fine mesh (55 μm). The mixture was mixed thoroughly and transferred to a half gallon bottle labeled as "Pre-YCT". Ten milliliter of the Pre-YCT was placed in an aluminum weight dish and dried overnight at 70°C, and the total suspended solids (TSS) content of the pre-YCT

was then adjusted to 1800 mg/L by the addition of nanopure water as needed. The YCT food was apportioned into 400 mL plastic bottles, labeled, and stored in the freezer (-40°C) until use.

Table A-1. Chemical parameters of moderately hard water (MHW)

Parameters	Amount
$NaHCO_3$	96 mg/L
$CaSO_4 \cdot 2H_2O$	60 mg/L
$MgSO_4$	60 mg/L
KCl	4 mg/L
Hardness	80-100 (mg/L as $CaCO_3$)
Alkalinity	60-70
pH	7.4-7.8

A.2.2 Maintenance of *Ceriodaphnia dubia* cultures

A starter culture of *Ceriodaphnia dubia* was donated by Hydrosphere Research (Gainesville, FL). The Daphnids were cultured in 1 L glassware containing 500 mL of MHW and kept in a Pervical^{TM} environmental chamber (model # E-30 BX) at 25°C with a day-night cycle of 16/8 h. The daphnids were fed every other day with equal volume of YCT (6.67 ml) and *Selenastrum capricornutum* suspension (6.67 ml) per liter of culture. Neonates (less than 24-hour old) were separated from adults daily and used for toxicity test or for starting new cultures.

A.2.3 Test procedure

The 48-h *Ceriodaphnia dubia* bioassay was carried out according to the U.S. EPA's standard method (US EPA, 2002). To determine the EC_{50} for a given sample, five or more sample dilutions were prepared. MHW served as the diluent as well as the negative control. All sample dilutions and negative control were prepared in triplicate. Neonates (less than 24-hour old) were separated from adults and fed 2 hours prior to starting the test. Five neonates were exposed in plastic cups containing 20 ml of sample dilution or negative control. The test containers were placed in the same environmental chamber (model # E-30 BX) at 25°C with a

day-night cycle of 16/8 h. After 48 hours, the test containers were placed on a light table and the number of motile and dead daphnids was recorded. The percent inhibition was calculated as followed:

$$\%\text{Inhibition} = \frac{(\#\text{ of motile in negative control} - \#\text{ of motile in sample})}{\#\text{ of motile in negative control}} \times 100\% \qquad \text{(A-3)}$$

A.3 96-h *Selenastrum capricornutum* Chronic Toxicity Test

A.3.1 Preparation of algal medium

The preliminary algal assay procedure (PAAP) medium was prepared from eight nutrient stock solutions, including magnesium chloride ($MgCl_2 \cdot 6H_2O$), calcium chloride ($CaCl_2 \cdot 2H_2O$), sodium nitrate ($NaNO_3$), magnesium sulfate ($MgSO_4 \cdot 7H_2O$), potassium bicarbonate (K_2HPO_4), sodium bicarbonate ($NaHCO_3$), disodium ethylenediaminetetracetate (EDTA), and the "trace salts" solution (FDEP, 1997). Table A-2 lists the concentration of each salt component. The PAAP medium was prepared by combining 1ml of each major salt solution with 1mL of the trace salt solution in a 1 L volumetric flask filled with nanopure water. The pH of the PAAP medium was adjusted to 7.5 ± 0.1 with either 0.1 N NaOH or 0.1 N HCl. The medium was then filtered through a 0.45 μm pre-sterilized filter and stored in the refrigerator before use. PAAP medium without EDTA was used for toxicity testing, while PAAP with EDTA was used for growing algae cultures.

A.3.2 Maintenance of *Selenastrum capricornutum* cultures

A pure culture of *S. capricornutum* was obtained from Hydrosphere Research (Gainesville, FL). The algae culture was grown in PAAP medium (with EDTA) under controlled conditions (25°C, 24 hours light source (400 ± 40ft-c)). To maximize the light reflection and minimize the temperature change, black plastic sheeting was used to surround the light unit, and the interior of the sheeting was covered with aluminum foil. Besides, continuous aeration was also provided by

putting a 1 mL glass pipette in the culture which was connected to an air pump. The culture flask was covered with Parafilm and shaken at least once per day. After 3-5 days, the algae cells were used for toxicity test; after 7 days, a small portion of the mature algae culture was transferred to 2 L fresh medium to start a new culture. The surplus 7-day old algae culture was concentrated by centrifugation and then resuspended in a small volume of distilled water. The density of this recovered algae solution was maintained at of 3.5×10^7 cells/mL and served as *Ceriodaphnia dubia* food.

Table A-2. Components of preliminary algal assay procedure (PAAP) medium

Major salts	Concentration (g/L)
$MgSO_4\cdot7H_2O$	14.700
$MgCl_2\cdot6H_2O$	12.164
$CaCl_2\cdot2H_2O$	4.410
$NaHCO_3$	7.500
$NaNO_3$	25.500
K_2HPO_4	0.818
EDTA	0.300
Trace salts	**Concentration (mg/L)**
H_3BO_3	185.520
$MnCl_2$	415.380
$FeCl_3\cdot6H_2O$	159.760
$ZnCl_2$	3.270
$CoCl_2\cdot6H_2O$	1.428
$Na_2MoO_4\cdot2H_2O$	7.260
$CuCl_2\cdot2H_2O$	0.012

Source: FDEP, 1997.

A.3.3 Algal assay procedure

The 96-h chronic *Selenastrum capricornutum* test was carried out according to the U.S. EPA's standard method (US EPA, 2002).

To determine the EC_{50} of a given sample, 5 or more sample dilutions were prepared. PAAP medium (without EDTA) was used as sample diluent as well as negative control. All sample dilutions and negative control were prepared in triplicate. Fifty milliliter of each sample dilution was added to a 125 ml autoclaved Erlenmeyer flask with styrofoam stopper, and the sample

solution was then inoculated with 1 mL of algae inoculum to reach a cell density of 5×10^5 cells/mL. To prepare the inoculum, 10 ml of 3-5 days old algae suspension was centrifuged at 4,000 rpm for 15 minutes. The supernatant was discarded and the algae seed was resuspended in 20 ml of PAAP medium (without EDTA). Then the density of the algae seed was determined with a hemacytometer under a microscope. According to the density, the inoculum was prepared by diluting the seed with PAAP solution (without EDTA) to a final density of 5×10^5 cells/mL. All inoculated flasks were then placed under the fluorescent lights and were shaken and rearranged at least once per day. After 96 hours exposure, the cell density in each flask was measured with a hemacytometer under a microscope. The percent inhibition was calculated by the following Equation:

$$\%\text{Inhibition} = \frac{(\text{Negative control cell density} - \text{sample cell density})}{\text{Negative control cell density}} \times 100\% \qquad \text{(A-4)}$$

APPENDIX B
DETAILED PROCEDURE FOR TOTAL METAL ANALYSIS

B.1 U.S. EPA Method 3010A

The U.S. EPA method 3010A is an acid digestion procedure for aqueous samples and extracts. After digestion, the total metals in the sample can be analyzed by flame atomic absorption spectroscopy (FLAA) or inductively coupled argon plasma spectroscopy (ICP). The following elements can be digested by this method: Al, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mn, Mg, Mo, Ni, K, Se, Na, TI, V, and Zn (US EPA, 1992).

Fifty milliliter aliquot of the well-mixed sample was transferred to a 150 mL Griffin beaker and 3 mL of concentrated nitric acid (HNO_3) was added, the beaker was covered with a ribbed watch glass and placed on hotplate under the hood. The liquid was evaporated to a low volume (5 mL) without boiling. Then, the beaker was allowed to cool, and another 3 mL of concentrated HNO_3 was added. The beaker was covered with a watch glass and refluxed on the hotplate. Additional concentrated HNO_3 was added and the system was refluxed continuously until the digestate was light in color or did not change in appearance with continued refluxing. The system was evaporated to 3 mL without boiling. The beaker was allowed to cool, and then 10 ml of 1:1(v/v, diluted in distilled water) hydrochloric acid (HCl) was added. The beaker was covered with a watch glass and refluxed for an additional 15 minutes. The beaker was allowed to cool. The digestate was slowly filtered through a filter (Whatman 42 ashless) into a 50 mL volumetric flask. The beaker, watch glass, filter, and funnel were rinsed with distilled water, and the solution was brought to 50 ml with distilled water. The sample was now ready for analysis by inductively coupled plasma spectrometry.

B.2 U.S. EPA Method 3050B

The U.S. EPA method 3050B is an acid digestion procedure for solid samples including sediments, sludges, and soils. The following elements can be analyzed by flame atomic absorption spectroscopy (FLAA) or inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion: Al, Sb, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, V, Mg, Mn, Mo, Ni, K, Ag, Na, TI, V, and Zn (US EPA, 1996).

One to two gram of sample was weighed out in a 250 mL Erlenmeyer flask. Ten milliliter of 1:1(v/v, diluted in distilled water) nitric acid (HNO_3) was added, and the flask was covered with a ribbed watch glass and placed on the hotplate under the hood. The system was refluxed for 10-15 minutes without boiling. The flask was removed from the hotplate and 5 mL of concentrated HNO_3 was added. The system was refluxed again without boiling for 30 minutes. If brown fumes were generated, additional 5 mL of concentrated HNO_3 was added until no more fumes were formed. The system was evaporated to 5 mL without boiling for a maximum of 2 hours. The flask was allowed to cool and 2 mL of distilled water and 3 mL of 30% Hydrogen Peroxide (H_2O_2) were added. The system was refluxed and additional 1 mL of 30% H_2O_2 was added to a maximum of 10 mL until effervescence subsided. The system was refluxed until the sample volume was 5 mL. Then 10 mL of concentrated hydrochloric acid (HCl) was added, and the system was refluxed again for 15 minutes. The flask was allowed to cool. The digestate was slowly filtered through a filter (Whatman 42 ashless) into a 100 mL volumetric flask. The Erlenmeyer flask, watch glass, filter, and funnel were rinsed with distilled water, and the solution was brought to 100 mL with distilled water. The sample was now ready for analysis by inductively coupled plasma spectrometry.

B.3 Total Mercury Determination

Aqueous sample. The U.S. EPA method 1631 was used for determination of mercury (Hg) in filtered and non-filtered water by cold vapor atomic fluorescence spectroscopy (CV-AFS). The method detection limit (MDL) for Hg has been determined to be 0.2 ng/L when no interferences are present (US EPA, 2002). Fifty milliliter of sample containing 0.5-1% hydrochloric acid was oxidized in a 125 mL Teflon bottle by adding 0.5 mL of bromine monochloride (BrCl). The sample was then allowed to digest overnight. After oxidation, 0.2 mL of 30% hydroxylamine hydrochloride (300 g of $NH_2OH{\cdot}HCl$ was dissolved in 1000 mL of nanopure water) was added. The mixture was swirled and allowed to react for at least 5 minutes. The sample was now ready for analysis by CV-AFS.

Solid sample. The method to digest solid sample was adapted from Warner et al. (2003). Point five to one gram of sample was weighed in 50 mL Teflon hot block digestion tube. Twenty milliliter of a mixture of HNO_3/H_2SO_4 (7:3, v: v) was added to the Teflon tube. The tube was capped loosely and the sample was allowed to digest in a hot block at 100°C overnight. Then, the sample was allowed to cool, and another 30 mL of distilled water was added. The sample was now ready for analysis by CV-AFS.

After digestion, all digestates were analyzed for total Hg by stannous chloride ($SnCl_2$) reduction technique using a CV-AFS.

B.4 Plant Digestion for Total Metal Analysis

A wet acid digestion procedure using nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) was utilized for total metal analysis in plant tissue (Mills and Jones, 1996). Point five gram of dried (70°C) and ground (20 mesh) plant tissue was weighed in a 150 mL Griffin beaker, and 8 mL of concentrated HNO_3 was added. The sample was then allowed to digest overnight. On the next day, the beaker was covered with a watch glass and heated for one hour

on a hot plate at 120°C. Then, the beaker was removed from the hot plate and was allowed to cool. Four milliliter of 30% H_2O_2 was added, and the addition of 30% H_2O_2 was repeated until the digest was colorless. After the digestion was complete, the watch glass was removed and the residue was taken to dryness at 80˚C. Once all the acid had been evaporated, the beaker was immediately taken off the hot plate and allowed to cool. The residue was then dissolved in 10 mL of 1:10 HNO_3 (v/v). The sample was now ready for analysis by inductively coupled plasma spectrometry.

APPENDIX C
ADDITIONAL MATERIALS FOR PLANT STUDY

Figure C-1. Phytotoxicity of 100 mg/kg Cu to lettuce (*Lactuca sativa*) in sandy soil after 4 weeks exposure

Figure C-2. Phytotoxicity of 100 mg/kg Cu to Indian mustard (*Brassica juncea*) in sandy soil after 4 weeks exposure

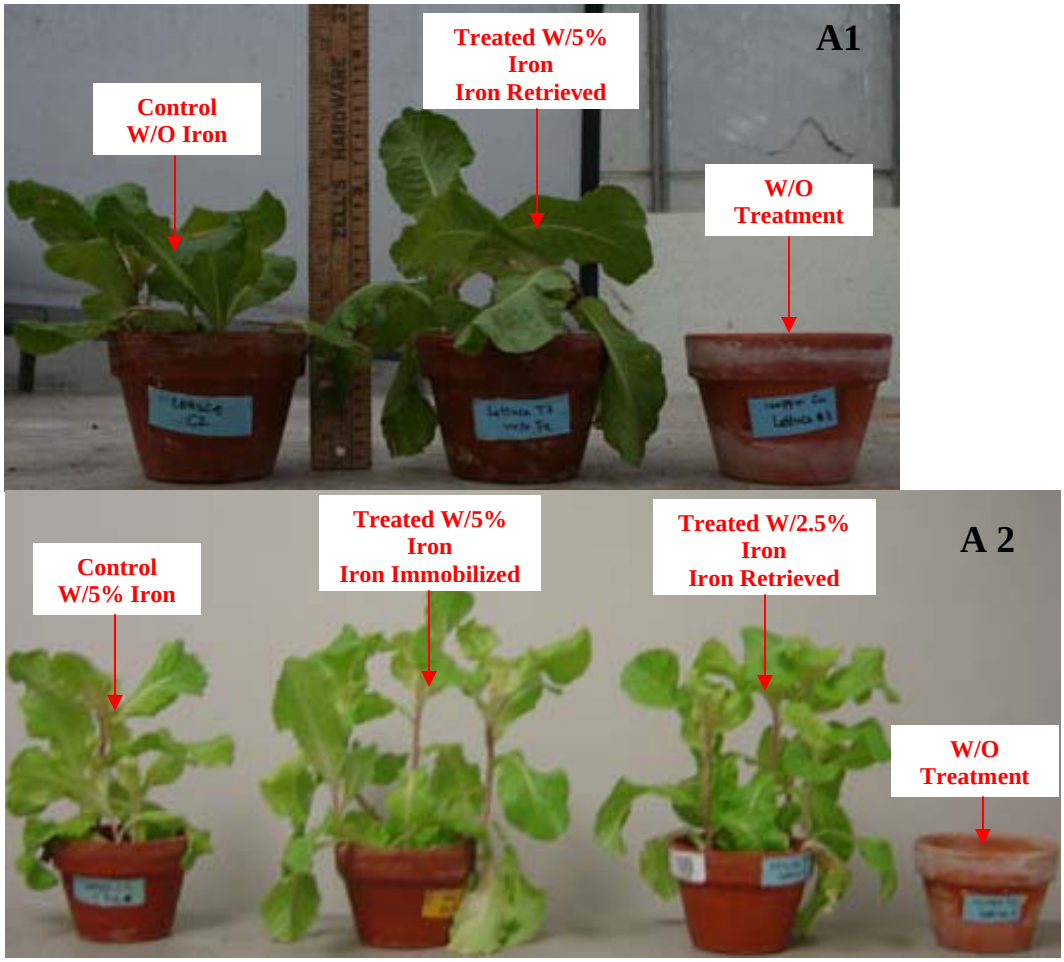


Figure C-3. Effect of iron treatment on the growth of Lettuce (*Lactuca sativa*) in sandy soil. A1 and A2) front view. B) Top view. (The sandy soil was spiked with 100 mg/kg Cu before iron treatment. Lettuce could not survive in non-treated soil).

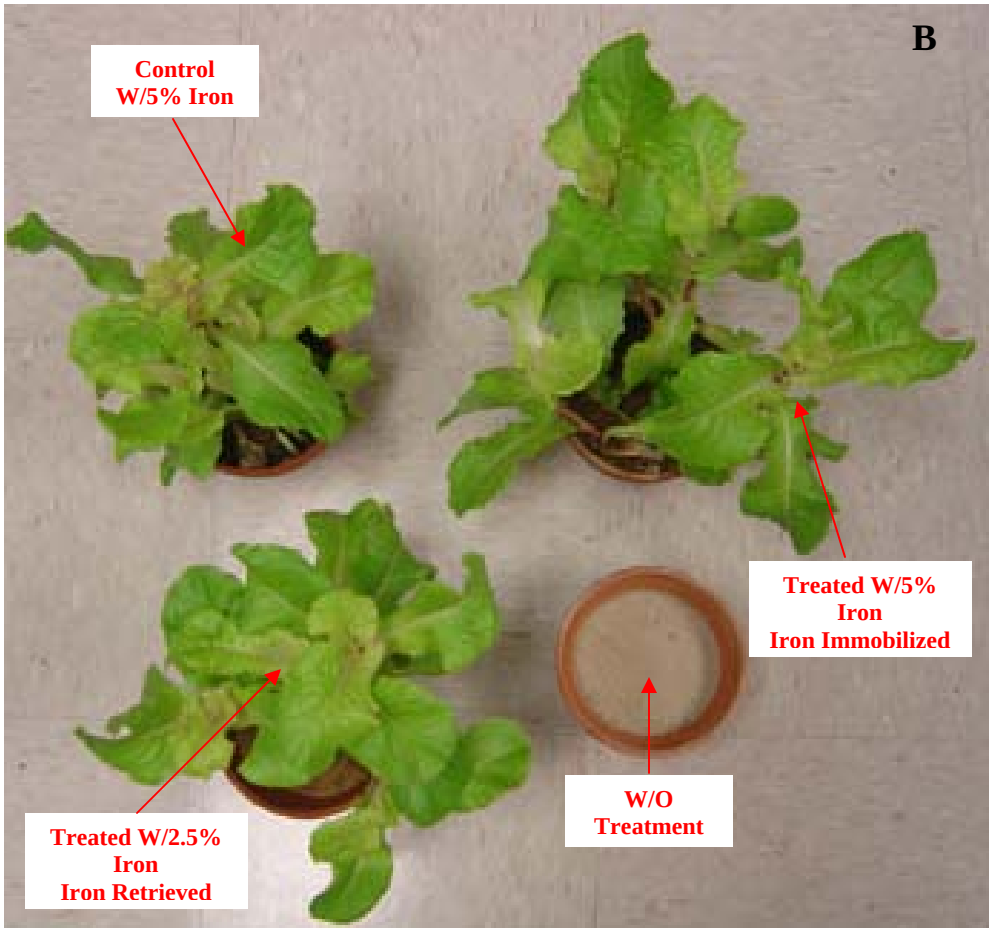


Figure C-3. Continued.

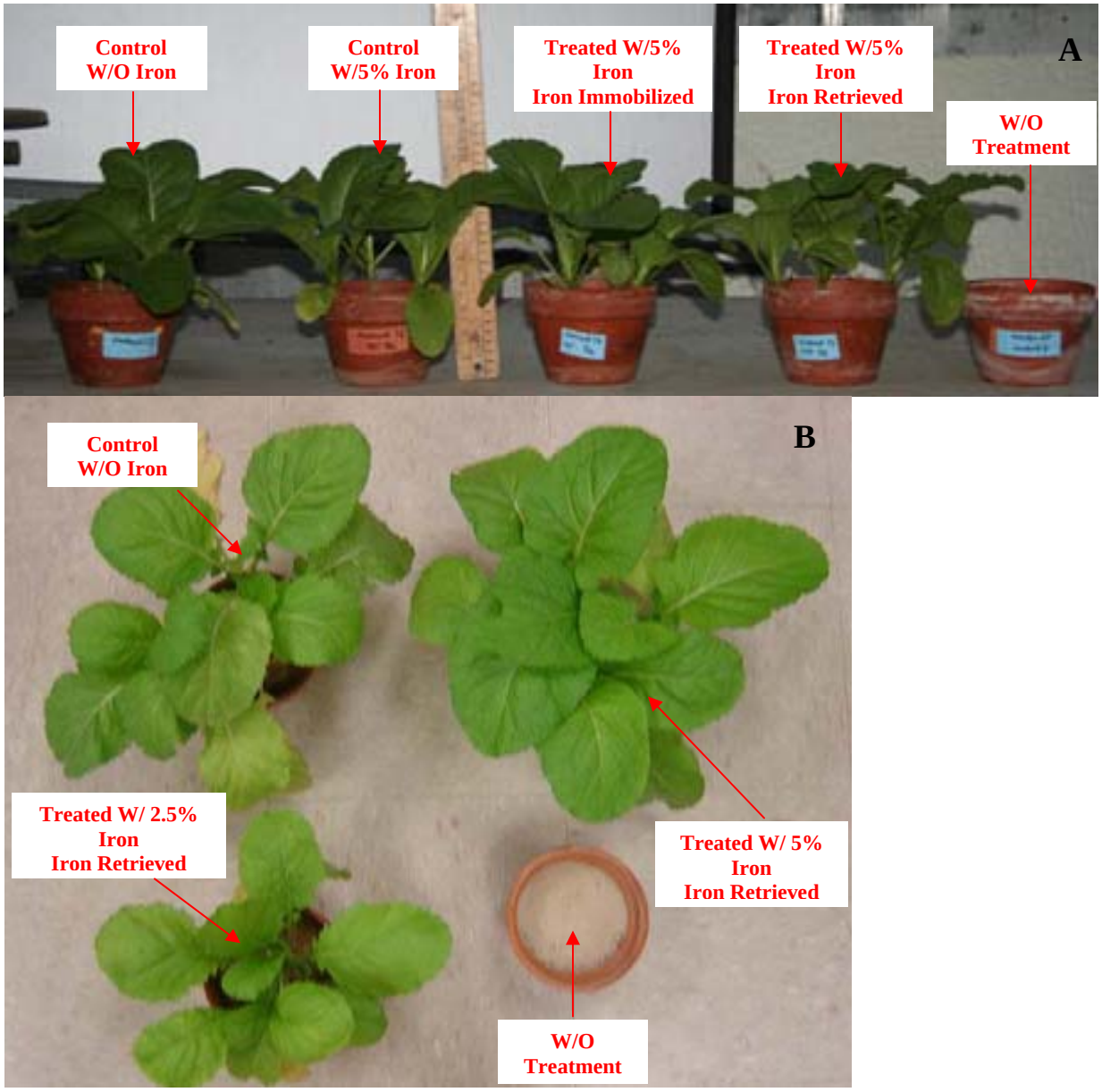


Figure C-4. Effect of iron treatment on the growth of Indian mustard (*Brassica juncea*) in sandy soil. A) Front view. B) Top view. (The sandy soil was spiked with 100 mg/kg Cu before iron treatment, plants grown in non iron-treated soil did not grow).

Figure C-5. Effect of iron treatment on the growth of lettuce (*Lactuca sativa*) in organic rich soil 2. A) Front view. B) Top view. (The organic rich soil 2 was spiked with 500 mg/kg Cu before iron treatment).

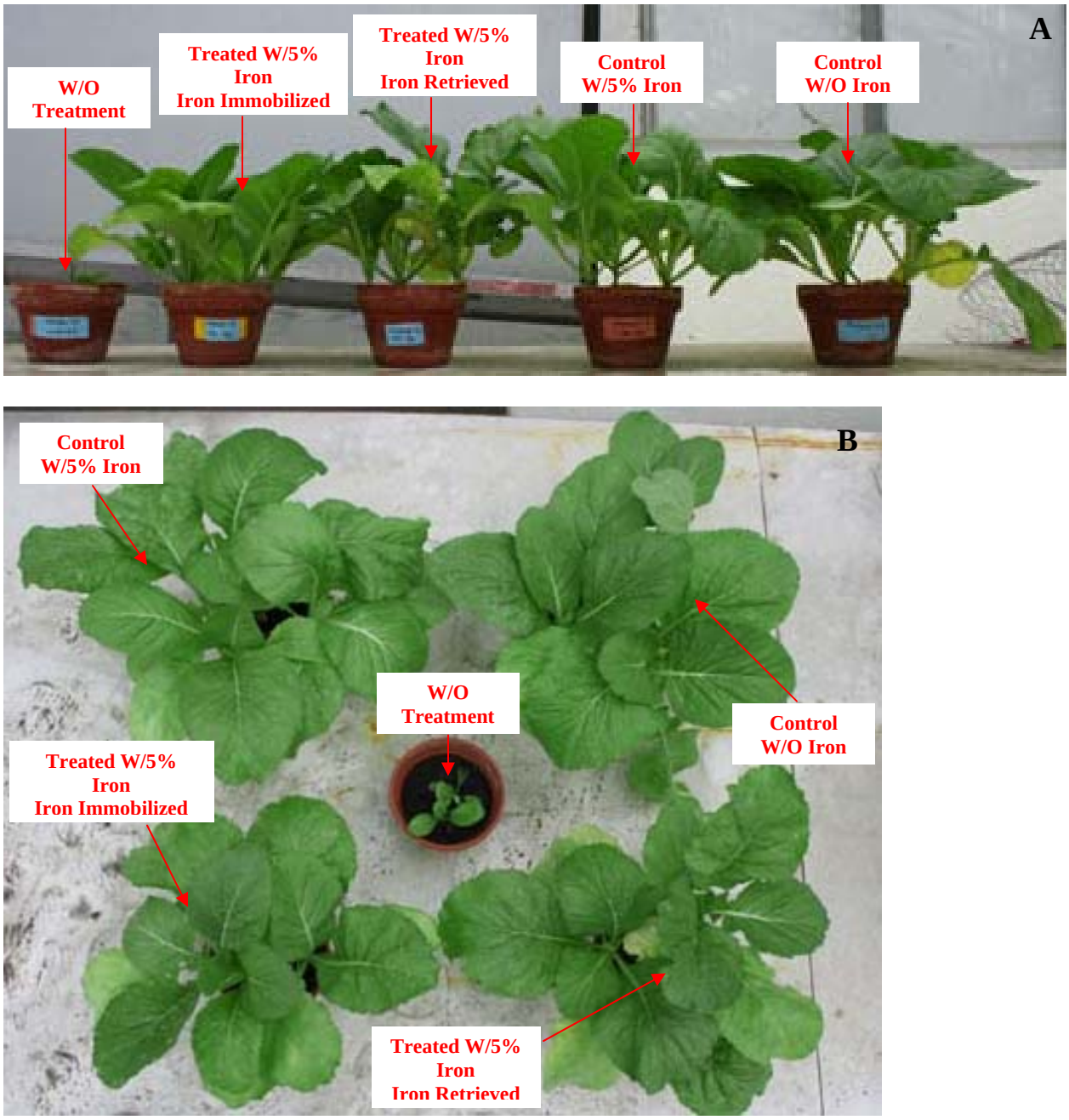


Figure C-6. Effect of iron treatment on the growth of Indian mustard (*Brassica juncea*) in organic rich soil 2. A) Front view. B) Top view. (The organic rich soil 2 was spiked with 500 mg/kg Cu before iron treatment).

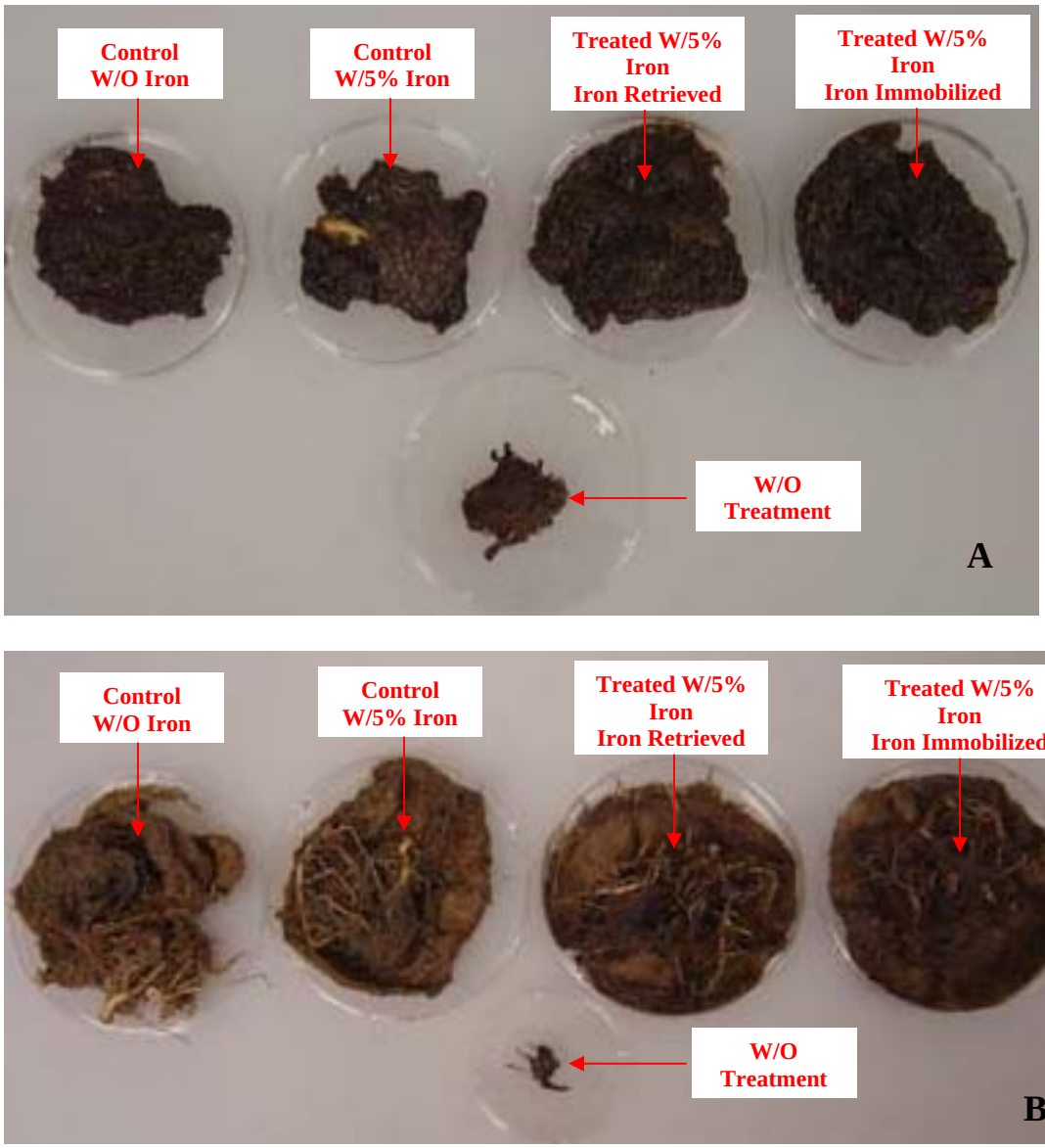


Figure C-7. Effect of iron treatment on plant roots in organic rich soil 2. A) Lettuce (*Lactuca sativa*) roots. B) Indian Mustard (*Brassica juncea*) roots. (The organic rich soil 2 was spiked with 500 mg/kg Cu before iron treatment).

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BIOGRAPHICAL SKETCH

Nan Feng, born in Liaoning province, China, is the only child of Jianguo Feng and Shuying Zhang. Nan Feng received her bachelor's degree in environmental science from Nankai University in 2003. Upon completion of her undergraduate study, she was admitted as a graduate student with an Alumni Fellowship award in the Department of Environmental Engineering and Sciences at the University of Florida, where she started her doctoral research in soil remediation under the direction of Dr. Gabriel Bitton.