FATE, TRANSPORT, AND ENVIRONMENTAL AVAILABILITY OF CU(II) APPLIED IN CATFISH AQUACULTURE PONDS AND ENHANCED IMMOBILIZATION OF SOIL-BOUND LEAD USING A NEW CLASS OF STABILIZED IRON PHOSPHATE NANOPARTICLES

Except where reference is made to the work of others, the work described in this dissertation is my own or was done in collaboration with my advisory committee. This dissertation does not include proprietary or classified information.

Ruiqiang Liu

Certificate of Approval:

Mark O. Barnett
Associate Professor
Civil Engineering

Dongye Zhao, Chairman
Associate Professor
Civil Engineering

Claude E. Boyd
Professor,
Fisheries and Allied
Aquacultures

Joey N. Shaw
Associate Professor
Agronomy and Soils

Yucheng Feng
Associate Professor
Agronomy and Soils

Joe F. Pittman
Interim Dean
Graduate School

FATE, TRANSPORT, AND ENVIRONMENTAL AVAILABILITY OF CU(II) APPLIED IN CATFISH AQUACULTURE PONDS AND ENHANCED IMMOBILIZATION OF SOIL-BOUND LEAD USING A NEW CLASS OF STABILIZED IRON PHOSPHATE NANOPARTICLES

Ruiqiang Liu

A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama
May 10, 2007

FATE, TRANSPORT, AND ENVIRONMENTAL AVAILABILITY OF CU(II) APPLIED IN CATFISH AQUACULTURE PONDS AND ENHANCED IMMOBILIZATION OF SOIL-BOUND LEAD USING A NEW CLASS OF STABILIZED IRON PHOSPHATE NANOPARTICLES

Ruiqiang Liu

Signature of Author

Date of Graduation

VITA

Ruiqiang Liu, son of Wuwang Liu and Xuede Zhang, was born in Taiyuan, Shanxi, China on February 3, 1971. He earned his bachelors degree in Environmental Engineering from Taiyuan University of Technology, China in 1994. He earned his M.S. in environmental engineering from the same university in 1997. Since August 2002, he has been a Ph.D. student in the Department of Civil Engineering. During his stay at Auburn, he has produced one U.S. patent (pending), four journal papers (two published, one in review, another being submitted) and two technical reports, and delivered five presentations at various national meetings.

DISSERTATION ABSTRACT

FATE, TRANSPORT, AND ENVIRONMENTAL AVAILABILITY OF CU(II) APPLIED IN CATFISH AQUACULTURE PONDS AND ENHANCED IMMOBILIZATION OF SOIL-BOUND LEAD USING A NEW CLASS OF STABILIZED IRON PHOSPHATE NANOPARTICLES

Ruiqiang Liu

Doctorate of Philosophy, May 10, 2007
(M.S., Taiyuan University of Technology, 1997)
(B.S., Taiyuan University of Technology, 1994)

226 Typed Pages

Directed by Dongye Zhao

Copper sulfate has been the most commonly used algaecide for about a century in the U.S. to control the off-flavor problem caused by blue-green algae in channel catfish (*Ictalurus punctatus)* ponds. In 2001, the ~80,000 hectares of channel catfish ponds in the U.S. received a total dose of 4,000,000 kg of $CuSO_4 \cdot 5H_2O$ or 1,000,000 kg of Cu^{2+}. However, no detailed studies have been available pertaining to the potential adverse impacts of the copper applied in catfish ponds on human and environmental health.

A pilot-scale study and various field measurements at commercial ponds were conducted to investigate the environmental fate of copper applied as an algaecide in catfish ponds. In the pilot study, a total of 774 g Cu(II) were applied to an experimental catfish pond over a period of 16 summer weeks. Copper mass balance indicated that

virtually all Cu(II) applied was retained in the sediment. Approximately 0.01% of the total Cu applied was taken up by fish and 0.1% remained in pond water. Data from three commercial fishponds of different ages (1-25 years) and with different sediment types (acidic, neutral and calcareous) supported the pilot-scale observation. Field monitoring of groundwater quality suggested that the copper leaching into the groundwater surrounding the ponds was insiginificant.

Sediments taken from the three commercial catfish ponds were studied for content, leachability, bioaccessibility, and speciation of sediment-bound Cu(II). Results showed that copper was concentrated in the top 10 cm of the sediments. Leachability tests based on the toxicity characteristic leaching procedure (TCLP) showed ~1-8% of sediment-bound copper was leachable, while the bioaccessible copper, determined following a physiological based extraction test (PBET) procedure, accounted for up to ~40-80% of total Cu. Becasue of the high redox potential in the surface sediments, acid volatile sulfide was not a significant sink for copper. Tests following a sequential extraction method revealed that the residual phase copper (i.e. Cu bound in the lattices of primary and secondary minerals) was the major Cu fraction in the ponds with acidic and calcareous sediments but carbonate-bound, Fe/Mn oxide-bound and organically bound Cu, as well as the residual fraction, seemed equally important in the pond with neutral sediment.

Effects of various soil fractions and soil compositions on the leachability and bioaccessibility of soil-bound Cu were investigated with three representative soils (calcareous, neutral, and acidic). The synthetic precipitation leaching procedure (SPLP) was used to assess the metal leachability, the PBET was used to assess the

bioaccessibility, and a selective dissolution approach was applied to fractionate the soil fractions. Data showed that soil carbonates played an important role in Cu desorption from soil. The leachability of Cu bound in carbonate-rich soils was less than that in soils with lower carbonate content. However, the bioaccessibility of copper in carbonate-rich soils was greater than that for soils with low carbonate content. Leachability and bioaccessibility of Cu in different particle size fractions fractionated on were found to be correlated with the carbonate contents in each fraction. Results also showed Fe/Mn oxides, organic matter and clay minerals are responsible for Cu retention under acidic leaching conditions, and clay minerals consistently showed the strongest affinity for Cu.

This study developed a new class of iron phosphate (vivianite) nanoparticles, prepared with sodium carboxymethyl cellulose (CMC) as a stabilizer, and tested the feasibility of applying the nanoparticles for *in-situ* immobilization of lead (Pb^{2+}) in soils. TEM measurements indicated that the mean particle size was about 8.4±2.9 nm (standard deviation). Batch test results showed that the CMC-stabilized nanoparticles can effectively reduce the TCLP leachability and PBET-based bioaccessibility of Pb^{2+} in the 3 representative soils. When the soils were treated with the nanoparticles at a dosage ranging from 0.61 to 3.0 mg as PO_4^{3-}/g-soil for 56 days, the TCLP leachability of Pb^{2+} was reduced by up to 95%, whereas the bioaccessibility of Pb^{2+} in the soils was reduced by 31~47%.

Style manual or journal used Water, Air and Soil Pollution

Computer Software used Microsoft Excel 2003, Microsoft Word 2003, Sigmaplot 8.0, and Visual MINTEQ 2.32.

ACKNOWLEDGMENTS

The author would like to express his sincere and profound gratitude to Dr. Dongye Zhao for his support, invaluable advice, guidance and patience throughout the duration of his studies. The author would also like to extend his great appreciation to the members of his advisory committee: Dr. Mark O. Barnett, Dr. Joey N. Shaw, Dr. Claude E. Boyd and Dr. Yucheng Feng for their constant support throughout this research. Many advices from my late committee member, Dr. Jim F Adams, were also unforgettable. Great thanks are due to Dr. Joey N. Shaw, Mr. Jinling Zhuang and Dr. Junchen Liu for providing and operating the related research facilities. Special thanks are also extended to Dr. Ming-Kuo Lee for his assistance in dissertation writing. The author would also like to express his deepest appreciation to his parents and family for their versatile and continuous support. The author has special appreciation to his wife, Miao Guo and his son Eric Liu for bringing a lot into his life that he stands too short to count. This work was partially funded by Auburn University Environmental Institute, USGS Alabama Water Resources Research Institute, Auburn University Highway Research Center and the Strategic Environmental Research and Development Program (SERDP) under the direction of Dr. Andrea Leeson.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I.

GENERAL INTRODUCTION

Contamination of rivers, lakes and reservoirs by heavy metals has been studied for many decades. However, there has been no detailed study documenting the fate and environmental impacts of copper applied in the catfish pond aquaculture. Because of the different environmental characteristics in the catfish ponds from other surface water bodies, resulting from more human interferences such as mechanical aeration, frequent water drainage and sediment removal, and heavy application of algaecides, it is necessary to research and assess the environmental consequences of the pond aquaculture practices, especially the heavy uses of Cu as an algaecide.

While heavy metals have been detected in thousands of sites, it remains highly challenging to remediate metal contaminated sites cost effectively. To address this issue, this study developed an innovative in-situ nanotechnology to immobilize metal cations such as lead and copper in soils and/or solid and hazardous wastes by use of a new class of stabilized iron phosphate nanoparticles. Compared to the commonly used phosphate sources such as water soluble phosphate or rock phosphate, the newly developed nanoparticles offer the advantages of high immobilization effectiveness and much less leaching of phosphate into the environment.

Overall, this dissertation focuses on the following research aspects:

Researching the transport of Cu among pond water, groundwater, pond sediment and the catfish body after the Cu was applied in the pond water as an algaecide, discovering the final fate of the Cu and evaluating the potential impact of Cu application on the ground water quality.

1. Researching the Cu speciation and environmental availability of Cu bound in the pond sediments and estimating the potential effects of Cu-laden sediments on the environment, animals and human.
2. Studying the influences of geochemical properties of the sediment on the environmental availability of the Cu and finding the best management practice for reducing the Cu contamination.
3. Synthesizing the iron phosphate nanoparticle and studying the effects of the nanoparticle on the leachability and bioaccessibility of soil-bound Pb.

The first chapter of this dissertation provides a general introduction and overview of the dissertation. Chapter II details the fate and transport of Cu in the catfish ponds through experimental investigation and field sampling. Research on experimental catfish ponds revealed that Cu applied finally accumulated in the pond sediment and little Cu was retained either in the water phase or in the catfish body. The Cu mass balance showed that no Cu had output from the ponds or entered the ground water with the pond seepage, suggesting that the possibility of ground water contamination caused by the Cu application was low. Field survey on three commercial catfish ponds with different ages and sediment properties also showed that only small amount of Cu was present in the pond water, the neighboring ground water and the fish tissues while the pond sediment retained most of the Cu applied in the ponds as the algaecide. Although Cu in the experimental pond sediment exhibited slowly downward immigration, probably caused by the bioturbation and/or colloid facilitated-transportation, field observations on the commercial ponds with many year Cu applications showed that Cu primarily accumulated only in the top (sediment) layer of the pond bottom. Therefore attention should be given on the behavior of Cu in the pond sediment.

Chapter III investigated the role of acid volatile sulfide (AVS) in metal binding, the simultaneously extracted heavy metals (SEM_{Cu}) content in the three commercial catfish pond sediments, and Cu speciation, leachbility and bioaccessibility in the sediments using a sequential extraction method (SEP), the toxic characteristic leaching procedure (TCLP), and the physiological extraction batch test (PBET). Due to the high redox potential in those fishpond bottoms, acid volatile sulfide was not a significant binding phase in the surface sediments, resulting in the molar ration of AVS to SEM_{Cu} less than 1 in most cases. The sequential extraction method results indicated that the residual phase was the major Cu fraction in the first two pond sediments but carbonate-bound, Fe/Mn oxide-bound and organically bound Cu, as well as the residual fraction, seemed equally important in the third pond. Toxicity characteristic leaching procedure showed only 1~8% of sediment Cu was leachable while bioaccessible Cu, evaluated by physiological based extraction test, accounted for up to 40~80% of total Cu. Aging seemed to play an important role in changing the Cu speciation and thus its availability in the sediments.

In addition to the total Cu concentration, sediment properties also played an important role in Cu availability. Chapter IV investigates the effects of soil geochemical composition such as soil carbonates, iron and manganese oxides, soil organic matter and soil clay minerals on the leachability and bioaccessibility of the soil-bound Cu, which were evaluated by synthetic precipitation leaching procedure (SPLP) and PBET, respectively. Experimental results revealed that soil carbonates showed different effects on Cu leachability and bioaccessibility: i.e. higher carbonate content resulted in the lower Cu leachability but higher bioaccessibility in soils, suggesting that carbonates could strongly retain Cu at moderately acidic environment but will completely lose their absorption capacity in extremely low pH ranges. Soil iron/manganese oxides, soil organic matter and clay minerals all exhibited Cu retention capacity to different extents and thus were able to reduce the Cu leachability and bioaccessibility in soils, with soil clay minerals and possibly some organic matter binding strongest with Cu even in the extremely acidic environment.

Chapter V presents the synthesis of a new class of iron phosphate ($Fe_3(PO_4)_2 \cdot 8H_2O$, vivianite) nanoparticles and the application of the particles in immobilization of the soil-bound lead. With the aid of carboxymethyl cellulose (CMC) as the stabilizer, the nanoparticles were successfully produced with average size of 8 nm. Batch test results showed that the CMC-stabilized nanoparticles can effectively reduce the TCLP (toxicity characteristic leaching procedure) leachability and PBET (physiologically based extraction test) bioaccessibility of Pb^{2+} in three representative soils (calcareous, neutral, and acidic) at a dosage ranging from 0.61 to 3.0 mg as PO_4^{3-}/g-soil. When the soils were treated with the nanoparticles for 56 days, the TCLP leachability of Pb^{2+} was reduced by 85~95%, whereas the bioaccessibility was lowered by 31~47%. Results from a sequential extraction procedure showed a 33~93% decrease of exchangeable Pb^{2+} and carbonate-bound fractions, and an increase of residual-Pb^{2+} fraction when Pb-spiked soils were amended with the nanoparticles. Addition of chloride in the treatment further decreased the TCLP leachable Pb^{2+} in soils, suggesting the formation of chloro-pyromorphite minerals. Compared to soluble phosphate used for *in situ* metal immobilization, application of the iron phosphate nanoparticles results in ~50% reduction in phosphate leaching into the environment.

CHAPTER II

FATE AND TRANSPORT OF COPPER APPLIED IN CHANNEL CATFISH PONDS

1. Introduction

In the past four decades or so, channel catfish *(Ictalurus punctatus)* farming in the U.S., especially in the central and southern U.S., has grown 80 fold. The water surface for catfish aquaculture in the United States has expanded from about 1,000 ha in the early 1960s (Boyd et al., 2000) to about 80,000 ha in 2001 (USDA, 2004). In 2004, the United States Department of Agriculture (USDA) reported that catfish growers in eleven selected states achieved a total sale of 480 million dollars (USDA, 2004). Since these practices involve heavy uses and discharges of environmentally intensive chemicals such as heavy metals and nutrients, concerns about the associated environmental impacts have been growing (Boyd et al., 2000)

A channel catfish pond differs from natural confined water bodies, such as a lake or a reservoir, in a number of aspects, including 1) it has a smaller water capacity and higher biomass density, 2) it receives elevated chemical loading (e.g. nutrients, pesticides/algaecides, and lime), and 3) it undergoes frequent human interferences (e.g. water drainage and refill, sediment removal, aeration, etc.). The related practices often result in environmentally intensive effluents and sediments.

Copper sulfate has been the most commonly used algaecide for about a century in the U.S. to control the off-flavor (e.g., “fishy taste”) problem caused by blue-green algae in channel catfish ponds (Riemer and Toth, 1970). In fact, Cu sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) has been the only algaecide approved by the U.S. EPA for catfish pond applications (USEPA, 2003). Typically, copper is applied to the fishponds at about 1% (w/w) of the total alkalinity of the pond water, and applications are made at intervals of 2-10 days from early summer to early fall (Boyd, 1990). Individual ponds typically receive about 50 kg ha^{-1} of $CuSO_4 \cdot 5H_2O$ or about 12.5 kg/ha of Cu each year. In 2001, the ~80,000 hectares of channel catfish ponds in the U.S. (mainly in Alabama, Arkansas, Louisiana, and Mississippi) received a total of 4,000,000 kg of $CuSO_4 \cdot 5H_2O$ or 1,000,000 k*g* of Cu (USDA, 2004). However, no detailed studies have been available pertaining to the potential adverse impacts on the human and environmental health.

Copper is a trace metal essential for the human body and for many enzyme systems. However, excessive exposure to high concentrations of Cu can result in adverse health effects. The maximum contaminant level (MCL) of Cu in drinking water is 1.3 mg/L (USEPA, 2002). Studies have shown that long-time ingestion of water containing Cu greater than the MCL can lead to liver or kidney damage (Zietz et al., 2003). Researchers have observed severe toxic effects of Cu on people or animals with weakened detoxification systems (Scheinberg, 1991). Recent evidence indicated that trace amounts of Cu in water, along with cholesterol, might play an important role in the etiology of Alzheimer’s disease (Sparks and Schreurs, 2003). In addition, free Cu may catalyze the formation of highly reactive hydroxyl radicals, which can result in oxidative damage to cells (Gaetke and Chow, 2003).

Among animals, ruminants are quite susceptible to copper toxicity. They are the only animals in which significant, and even lethal, copper toxicosis can occur without an inherited abnormality or the addition of dietary copper supplements. Copper toxicosis may develop in sheep taking forage with a normal copper content of 8-10 mg/kg, and this even more likely to occur if the molybdenum concentration in the diet is below 0.5 mg/kg (Scheinberg, 1991).

Although some fish and crawfish may survive at copper concentrations of 0.03-0.8 mg/L, copper exceeding of 0.1 mg/L in water is usually toxic to fish (Scheinberg, 1991). Strauss and Tucker (1993) reported a 96-h LC_{50} of 51-65 µg/L at 16 mg/ L total hardness and 1,084-1,880 µg/L at 287 mg/L total hardness for channel catfish. Copper may also be toxic to plants, affecting mainly the growth of the roots. Toxic levels of copper for young spring barley, ryegrass, rape and wheat are 19 to 21 mg/kg soil. Copper is also toxic to cauliflower, potato, carrot, lettuce seedling, and rice seedling (Owen, 1981).

A fishpond can be envisioned as a mini-ecosystem consisting of water, sediment, and biota (mainly fish). Upon application, Cu will transport within and distribute among these phases. Cu applied to a fishpond may find its way into the environment in one or more of the following pathways: 1) Seepage: Since the pond bottom and/or walls are water permeable to some degree, Cu applied in the pond system may leach into surrounding groundwater or soil along with the seepage water; 2) Fish accumulation: Fish can accumulate heavy metals including Cu primarily through dietary exposure (USEPA, 2000); 3) Water replacement, pond dewatering, and pond drying, which are common practices in pond aquaculture; and 4) Sediment removal: Sediment in aquaculture ponds is removed routinely to maintain adequate depth of the fishponds and to remove harmful agents accumulated in sediment such as nutrients and organic matter (Boyd, 1995). For intensive aquaculture, sediment may be removed after each crop (Boyd, 1995).

The overall goal of this study was to investigate the distribution and transport of Cu applied to channel catfish ponds through both a pilot-scale experimental study and field measurements at actual commercial ponds. The specific objectives of this study were to:

1. Carry out a thorough Cu budget study in a well-controlled pilot-scale experimental fishpond;
2. Quantify the dynamics and distribution of Cu in the pond system during the application season; and
3. Investigate the distribution of Cu in representative commercial catfish ponds.

2. Materials and Methods

2.1. THE EXPERIMENTAL POND

A pilot-scale experimental fishpond at the Auburn University Fisheries Research Unit in Auburn, Alabama, USA, was employed for the pilot study. **Figure 2.1** shows the plan view of the pond dimensions and locations of sampling points (i.e. A-E, 1-16). The average water depth was kept at 0.8~0.9 m during the study. All side walls were made of concrete. Feed water was introduced by gravity flow from a local reservoir as needed, which was filled with runoff water from a locally wooded watershed. The pond bottom was originally paved with compact native soil, characterized as Typic Kanhapdults (clayey, kaolinitic, and thermic). **Table 2.1** gives relevant physical and chemical properties of the native soil and the pond sediment. A 37 kw surface aerator was installed at 5 *m* from the wall (point E in **Figure 2.1**). Copper sulfate ($CuSO_4 \cdot 5H_2O$) was slug-fed in the pond at the aerator (Point E) on a weekly basis.

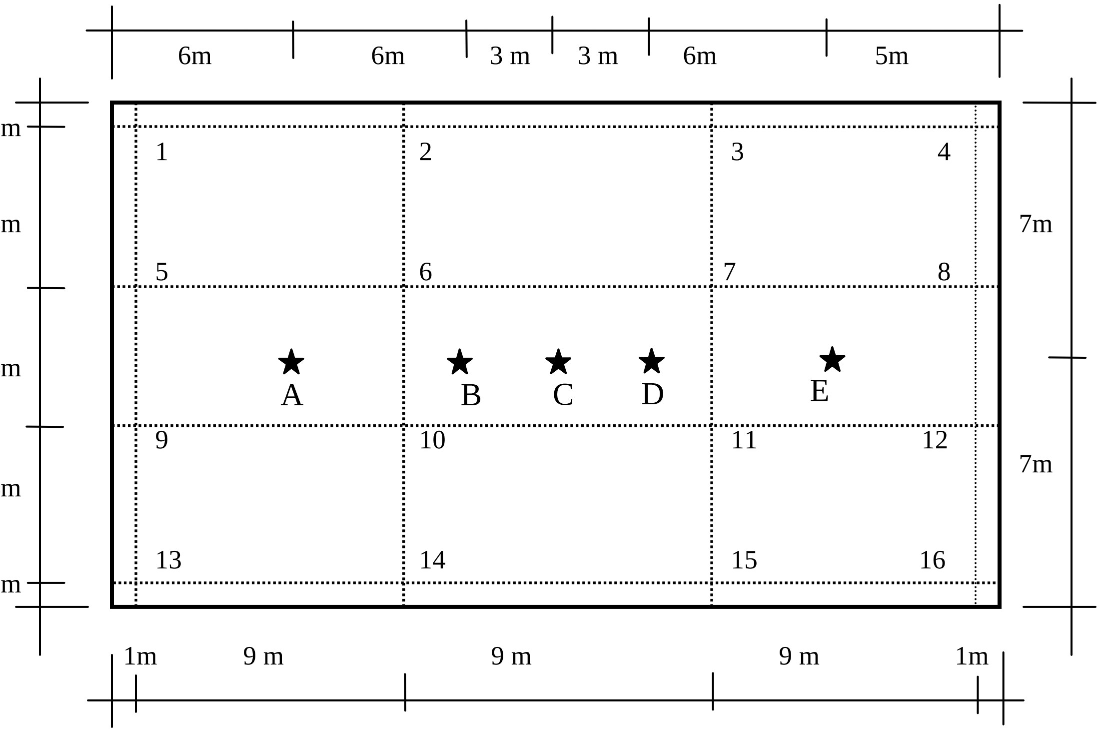


Figure 2.1 A plan view (not to scale) of the experimental pond and location of the water and sediment sampling points. Water and sediment cores were sampled at Points A-E to study the dynamic behaviors of Cu in water and sediment, whereas only sediment cores were taken at Points 1-16 to determine total Cu mass retained in the sediment.

A staff gauge that permits water level readings to the nearest 0.25 cm was installed in the pond. A class-A evaporation pan (Yoo and Boyd, 1994) and a standard rain gauge (Yoo and Boyd, 1994) were placed on the pond bank to monitor the water loss due to evaporation and water input from rain, respectively. A water flow meter (Trident# 8, Neptune Technology Group Inc.) was fitted on the inlet pipe for recording inflow rate. As a routine maintenance practice, lime was applied to the pond water on a yearly basis to enhance alkalinity and neutralize bottom soil acidity.

2.2. EXPERIMENTAL PROCEDURES

The experiment was initiated on March 11, 2003 by stocking the pond with ~300 channel catfish seedlings weighing 165 kg total. The fish were fed 3 times per week at 2.7 kg feed per feeding for the first two months. Then the feed quantity was increased to 5.4 *kg* until the end of the study season (September 26, 2003). The Cu content in the feed samples was determined to be about 10.8 mg/kg as Cu using EPA METHOD 3050B (USEPA, 1996).

From June 13 through September 26, 2003, copper sulfate ($CuSO_4·5H_2O$) was applied to the pond once a week at ~190 g per application. The amount of copper sulfate added was approximately equivalent to 1% of the total alkalinity (mg/L $CaCO_3$) of pond water (Boyd and Tucker, 1998). The surface aerator was operated as follows: a) it was first operated continuously for 24 hours (typically starting at 9 a.m.) immediately after each application of Cu sulfate to evenly distribute Cu in the pond water and to prevent the fish from being harmed by the shock loading of Cu; and b) after the application day, it was operated only nightly from 9 p.m. to 6 a.m. to compensate the high oxygen demand in the night time.

Before the first Cu addition, water samples, sediment cores and fish samples were analyzed for the background Cu concentrations. **Table 2.1** also presents important compositions of the pond water before Cu was added. Copper concentrations, pH, and dissolved oxygen (DO) in water after each copper application were monitored

TABLE 2.1

Pond water quality and soil property data for the experimental pond and the commercial ponds.

Catfish pond	Pond Water properties				Soil properties								
	pH	Cu (mg/L)	Organic carbon (mg/L)	Alkalinity (mg/L as $CaCO_3$)		pH	CEC (cmol/kg)	Cu (mg/kg)	Organic carbon (%)	Sand (%)	Silt (%)	Clay (%)	Clay* minerals
Experimental pond (30- years old)	7.81	0.002	3.46	57.2	Pond Sediment	6.6	14.5	28.9	2.5	30	33	37	(K)(M)(S)
					Native soil	6.3	6.25	18.3	1.6	49	30	21	(K)(M)(S)
Commercial pond P-1 (1-year old)	7.87	0.012	11.98	141.1	Native soil	7.8	34.85	23.2	1.2	15	29	56	(K)(M)(Q)(S)
Commercial pond P-5 (5-years old)	7.43	0.033	14.21	74.3	Native soil	6.9	14.17	25.6	0.9	18	28	54	(K)(M)(Q)(S)
Commercial pond P-25 (25-years old)	7.44	0.021	4.32	37.2	Native soil	4.3	8.77	13.5	0.6	69	4	27	(K)(M)(Q)(S)

*** K=Kaolinite, M=Mica, Q=Quartz, S=Smectite**

periodically. Throughout the experimental period, the water level in the pond, rainfall, evaporation, and inflow rate were recorded on a weekly basis. These data were used in the subsequent water balance calculation following the approach of Boyd (1982).

To study the dynamic behavior of Cu in the pond water, water samples were taken from locations A, C, and E of the pond (**Figure 2.1)** and at various time intervals after one application. Two operationally defined Cu species in water were considered in this study. The "dissolved Cu" (D-Cu) refers to Cu remaining in water after micro-filtering the water samples using a 0.22 μm PVDF Membrane (Millipore Co., Billerica, MA, USA), whereas the "total Cu" (T-Cu) was determined by analyzing water samples that were first acidified with 0.1 M HNO_3 to a $pH < 2.0$ and without the filtration treatment (Clesceri, et al., 1998).

To study the transient Cu accumulation in the sediment in response to the Cu additions, sediment cores were taken from locations A, B, D, and E (**Figure 2.1**) on a weekly basis. A manually-operated, 5-cm diameter core sampler (Wildlife Supply Company, MI; Model No.2424A15) was used to obtain sediment cores to ~22 *cm* deep. To map the spatial distribution of Cu in the sediment, sediment cores were also taken from Points 1-16 (**Figure 2.1**) at the beginning and the end of the pilot study, respectively. All sediment cores were dissected to 2-cm-thick cakes, and copper content in the cores was determined following EPA METHOD 3050B (USEPA, 1996). The bulk density of each sediment cake was measured separately following the approach by Munsiri et al. (1995).

Catfish of various ages (sizes) were sampled three times during the test. Copper content in the whole fish body as well as in various body parts (liver, gill, meat, and bone) was determined. Following a procedure described by Oldewage (2000), the fish samples were first freeze-dried for 72 hours, and then the samples were fine-ground to homogenous powders. Again, EPA METHOD 3050B (USEPA, 1996) was followed for digestion of the dried fish powders.

2.3. ANALYSES OF SEDIMENT, SOIL AND CHEMICALS

Copper in water or in acid digests was analyzed using either a flame atomic absorption spectrometer (FLAAS) (Varian SpectrAA 2220FS) or a graphite furnace atomic absorption spectrometer (GFAAS) (Perkin Elmer 3110). The detection limit is 0.02 mg/ L for the FLAAS, and 0.002 mg/L for the GFAAS. An Orion 520A pH meter was used to measure pH of water or soil suspension and YSI 57 DO meter for measuring DO. Alkalinity of the pond water was determined through the titration method (Clesceri et al., 1998).

Native soil samples were taken from Bt horizon (50~70 cm deep) in the vicinity of the pond for characterizing the soil properties. The sediment column of this pond was estimated to be 30 cm deep (Munsiri et al., 1995). Both soil and sediment samples were pulverized to pass a 2-mm sieve after drying at 105°C, and the subsequent soil measurements were based on the weight of air-dried samples. Soil pH was measured in 0.01 M $CaCl_2$ at 1:1 soil: solution ratio. The fraction of sand, silt, and clay was determined using the pipette method described by Gee and Or (2002). The total carbon content was analyzed following the Dumas method with a LECO CN-2000 combustion unit (LECO Corp., Joseph, MI) at 1050 °C. Soil clay minerals were identified with the XRD method using an X-ray diffraction unit (Siemens D5000 x-ray diffractometer) after the soil samples were treated with the procedure given by Kunze and Dixon (1986). The

CEC was determined with 1 N NH_4OAc buffered at pH 7.0 following the method described in Soil Survey Laboratory Methods Manual (USDA, et al, 1996).

2.4. DETERMINATION OF TOTAL COPPER RETAINED IN SEDIMENT

Total Cu content in the top 22-cm sediment layers was determined through volumetric integration of the Cu concentrations in each dissected layer. The planar distribution of Cu in each layer was mapped through interpolation based on the measurement of the 20 cores (Points 1-16, A, B, D and E in **Figure 2.1**). Eqn (2.1) gives the total mass of Cu retained in sediment:

$$M_S = \int_0^V C(x,y,z)\rho(x,y,z)dV \qquad (2.1)$$

where M_s (*kg*) is the total mass of Cu retained in the sediment of volume V, $C(x,y,z)$ (*mg* Cu kg^{-1} dry soil) is the Cu concentration at location (x, y, z), $\rho(x,y,z)$ (*kg* dry soil m^{-3} of wet sediment) is the bulk density of sediment at location (x, y, z), and dV (m^3 of wet volume) is the volume element at location (x,y,z).

Dividing the sediment core into finite number of layers (0.02 *m* each) along the depth and assuming that sediment bulk density is the same within each single layer, eqn (2.1) is approximated with

$$M_s = h_i \sum_{i=1}^{N} \rho_i (\int C_i(x,y) \Delta x \Delta y) \qquad (2.2)$$

where h_i is the thickness of layer i (0.02 m), N is the total number of layers, $C_i(x,y)$ is the Cu concentration at location (x, y) of layer i (mg Cu/ kg dry soil), ρ_i is the bulk density of sediment in layer i (kg dry soil m^{-3} of wet volume), and $\Delta x \Delta y$ is the area element at location (x, y) (m^2).

The finite-element method was employed to map the concentration distributions at a given layer. In brief, each layer of sediment was divided into 100×100 grid elements, each with a dimension 0.14 m × 0.29 m × 0.02 m. Cu concentration within a given element was considered to be homogeneous and was calculated by interpolation based on Cu concentrations measured at the 16 sampling points. The Interactive Data Language software (IDL 6.0, Research System Inc.) was used to calculate the Cu concentrations at all grid points through two-dimension linear interpolation. The net gain in Cu in the sediment phase (ΔM_s) was then calculated by:

$$\Delta M_s = M_{s,\,final} - M_{s,\,initial} \tag{2.3}$$

where $M_{s,\,initial}$ is the Cu mass in the sediment before the Cu was applied, and $M_{s,\,final}$ is Cu in the sediment at the end of the study season.

2.5. OVERALL COPPER BUDGET IN THE POND SYSTEM

The copper budget or mass balance in the pond system is described by Eqn (2.4):

$$M_1 + M_2 + M_3 - M_4 = \Delta M_w + \Delta M_s + \Delta M_f \tag{2.4}$$

where M_1 is the mass of Cu applied to the fishponds as algaecide (g); M_2 is the mass of Cu added to fishponds with rainfall, runoff and fill water (g); M_3 is the mass of Cu added as fish feedings (g); M_4 is the mass of Cu lost from fishponds due to water seepage (g); ΔM_w is the mass of Cu gained in pond water (g) at the end of the study season; ΔM_f is the mass of Cu accumulated in the fish body (g); and ΔM_s as defined in Eqn (2.3).

2.6 COPPER DISTRIBUTION IN COMMERCIAL CATFISH PONDS

Copper distribution in three commercial catfish ponds in the western Alabama was measured and compared. The ponds differ in their age and sediment characteristics (**Table 2.1**). Pond P-1 was a one-year old catfish pond and was built on 6 hectares of a calcareous soil; Pond P-5 was five-years old, and was based on 8 hectares of a neutral soil; whereas pond P-25 was 25-years old and on 2.5 hectares of an acidic soil. Multiple samples of the pond water, pond sediment, soil, and catfish were taken from those three ponds in September, 2003 and then analyzed for Cu following similar methods of sampling and analysis of those used for the experimental pond. **Table 2.1** gives salient water quality data and sediment properties for the three commercial ponds. Ground water samples were also taken from the monitoring wells located within 200 meters down gradient of the fishponds. The water samples were acidified with 0.1 M HNO_3 to a $pH < 2.0$ (Clesceri et al., 1998) on site before they were transported to the lab for analysis.

3. Results and Discussion

3.1. WATER BUDGET

Based on the weekly measurements, the weekly water seepage rate was calculated using Eqn (1.5) (Boyd, 1982),

$$\text{Seepage} = \text{Stage at time 1} - \text{Stage at time 2} + \text{inlet water} - \text{water output} + \text{Rainfall} + \text{Runoff} - \text{Pond evaporation} \tag{2.5}$$

where all terms were expressed in centimeters. The total runoff during the season was estimated at 2.3 cm (Boyd, 1982), which was evenly distributed over the 16 weeks. No water had been outputted from the pond during the season.

The calculation showed that the average seepage of the experimental pond over the experimental period was 0.5 cm/ d, i.e. about 2 m^3 or 0.6 % of the pond water seeped out of the pond daily.

3.2. DYNAMICS OF COPPER IN POND WATER

Figures 2.2a and **2.2b** show the observed dynamic concentration profiles of T-Cu and D-Cu, respectively, in the pond water sampled at three locations (A, C and E) following a representative Cu application. In all cases, steady state was reached in approximately 48 hours after each Cu addition. However, the level of T-Cu was much greater than D-Cu in all cases. For instance, at point E (where Cu was added), the peak concentration of T-Cu (approximately 600 μg/L) was over 1 order of magnitude greater than that for D-Cu (approximately 57 μg/L). These observations suggest that a primary fraction (approximately 90%) of Cu added to the ponds was rapidly (within minutes) associated with the suspended fine sediment particles. Consequently, these fine particulates play a governing role in facilitating the fate and transport of Cu. At steady state (after approximately 2 days), virtually all sediment-associated Cu settled in the sediment phase, leaving a steady amount (about 4 μg/ L) of D-Cu in the pond water.

The peak T-Cu concentration at Point A, which is about 18 m away from Point E, was approximately 175 μg/L, compared to only approximately 26 μg/L for D-Cu. While the D-Cu concentration peak reached Point A in less than 1.5 hours, the T-Cu peak did not arrive until 4.5 hours. Such a time lag again indicates the important role of suspended fine particles on the transport of the total Cu. To maintain a healthy water quality in aquaculture ponds, it has been a common practice to keep a relatively high pH (>7.0) and alkalinity through regularly liming the pond water. Boyd and Tucker (1998) attribute the

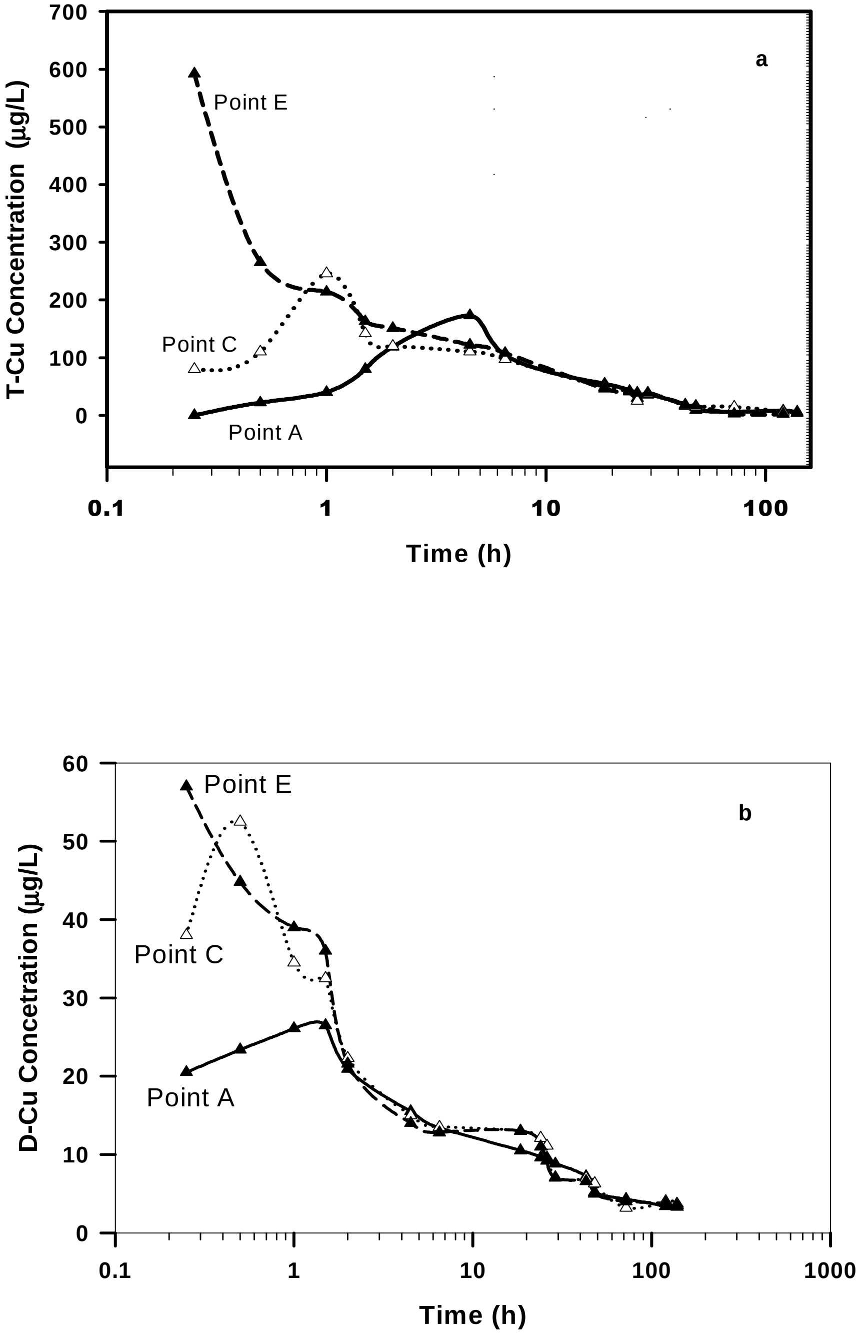


Figure 2.2. Transient change in concentration of total Cu (**a**) and dissolved Cu (**b**) in pond water at Points A, C and E following a Cu application.

low level of Cu in the pond water at steady state to formation of Cu precipitates such as malachite ($Cu_2(OH)_2CO_3$), tenorite (CuO), and cupric hydroxide ($Cu(OH)_2$) at the high pH and alkalinity. However, calculations based on the pond water chemistry showed that the saturation indices (SI) for those precipitates mentioned above were all less than 1, indicating that Cu^{2+} in pond water was under-saturated with any of those Cu precipitates. The result suggested that concentrations of copper in pond water were actually controlled by the sediment-particle adsorption process instead of the precipitation. These calculations were performed using Visual MINTEQ (ver. 2.32) with input data as follows: Cu^{2+} (total) = 4 μg/L, pH = 7~8, alkalinity =57.2 mg/L as $CaCO_3$.

Iron/manganese oxides, soil organic matter, clay minerals and soil carbonates are often considered as the major sorbing phases for heavy metals in the water column (Förstner and Wittmann, 1981). For example, Han et al (2001) analyzed the copper speciation using sequential extraction procedure in sediment samples from a catfish pond and found that more than 80% of copper was bound with soil organic matter, iron/manganese oxides and soil carbonates. However, for anoxic sediments, acid volatile sulfide (AVS) may become another important binding phase for metals sequestration (Di Toro et al., 1992; Ankley et al., 1993; Yu, et al. 2001; Saenz et al., 2003).

Figure 2.3 shows dynamic pH and DO profiles in the pond water measured at Point C in response to a pulse feed of copper at Point E. To contrast the cause-effect relation, the T-Cu profile is superimposed in **Figure 2.3**. According to Boyd (1995), pH and DO in aquaculture pond water typically exhibit a sinusoidal fluctuation pattern over a 24-h day-night period (as segmented between the two vertical dashed lines in **Figure 2.3**), which is attributive to the alternating respiration and photosynthesis of algae. Since the photo-

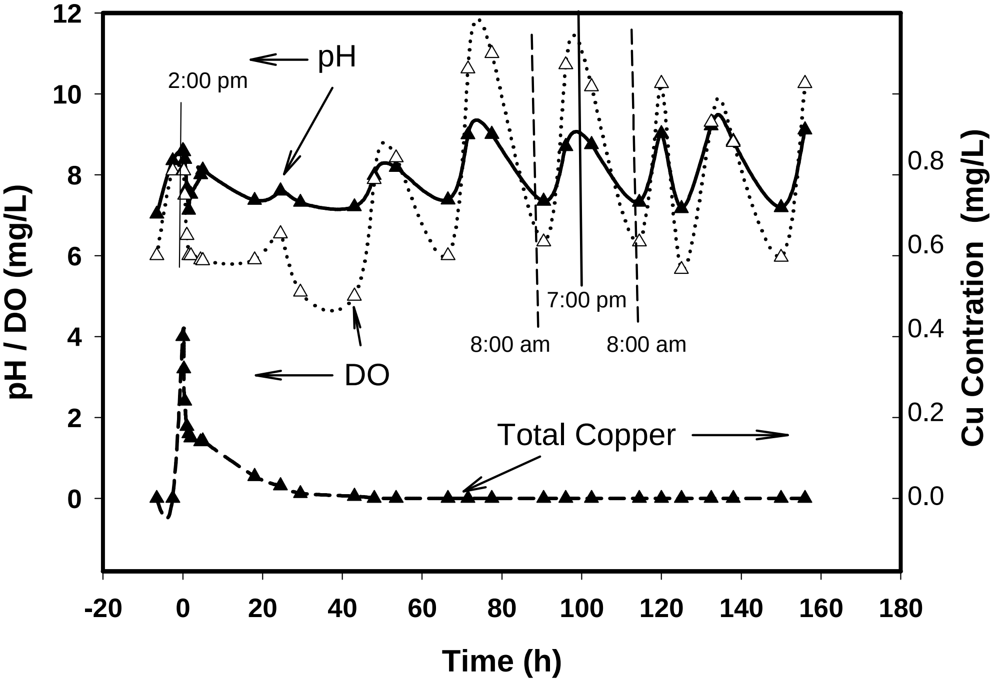


Figure 2.3. Dynamic profiles of DO and pH in pond water following a copper application. Copper added at t (time) = 0.

synthesis consumes dissolved CO_2 in water and produces DO, a coupled rise in pH and DO would be expected during the day. On the other hand, decaying and respiration of algae deplete DO and produce CO_2, resulting in night- time drop in DO and pH. **Figure 2.3** reveals that addition of copper caused an immediate DO upset and perturbation of the pH profile. For ~ 3 days after the copper addition, pH was dropped from its normal average value of ~9 to ~8 and remained stable, and DO was lowered to < 5 mg/L.

Evidently, the addition of copper had effectively disturbed normal metabolic activities of algae. However, due to the relatively rapid transport of copper from water to sediment, the pH and DO profiles bounced back to their normal tracks after 3 days, suggesting that the “algaecidal” effect of copper may not last for more than three days.

3.3. TRANSPORT AND ACCUMULATION OF COPPER IN SEDIMENT

Dynamic copper accumulation in the pond sediment was monitored through weekly measurements of Cu concentrations in sediment cores taken from 4 selected points (A, B, D, and E as shown in **Figure 2.1**). **Figure 2.4.a** shows the weekly changes in Cu concentration in various sediment layers of the sediment cores taken at Point E in response to the weekly Cu application (Note: sediment cores were taken approximately 12 hours before next Cu addition). **Figure 2.4.a** reveals that Cu concentrations in the top two layers displayed rapid and steady increasing profiles in response to the Cu addition. Rapid growth in Cu concentration in the 4-8 cm range was observed in about 3 to 4 weeks. At week 12, Cu in the deepest layer (14-16 cm) began to rise.

For comparison, **Figures 2.4.b** and **2.4.c** present similar dynamic accumulation profiles of Cu at Points A and D, respectively. Note that Point A is approximately 20 m away from Point E, where Cu is introduced; whereas Point D sits about 3 m from the center of the pond. Unlike Point E (where the aerator is located), Cu in the top three layers of Point A increased sharply after week 1, while Cu accumulation at the near-center Point D took place at a more moderate pace, and a clear time lag was evident. The different Cu accumulation profiles at these points suggest that Cu was unevenly distributed in the pond sediment. This also supports the assertion that Cu’s transport was

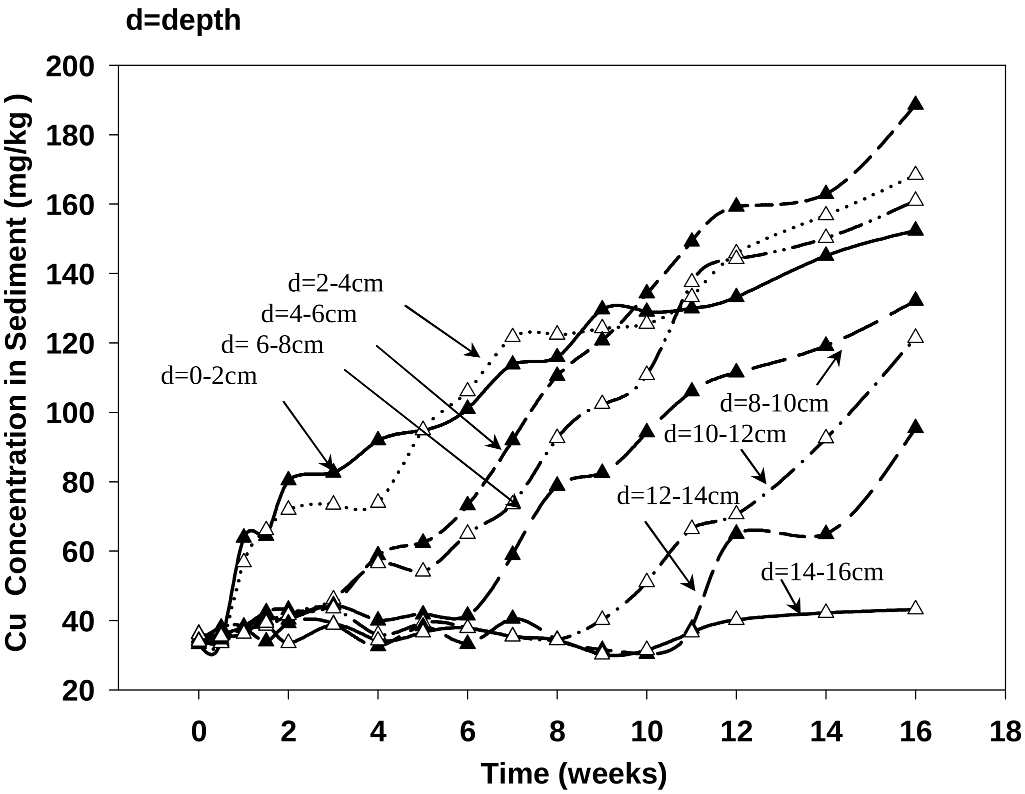


Figure 2.4a Accumulation of Cu in sediment at point E during the Cu application season.

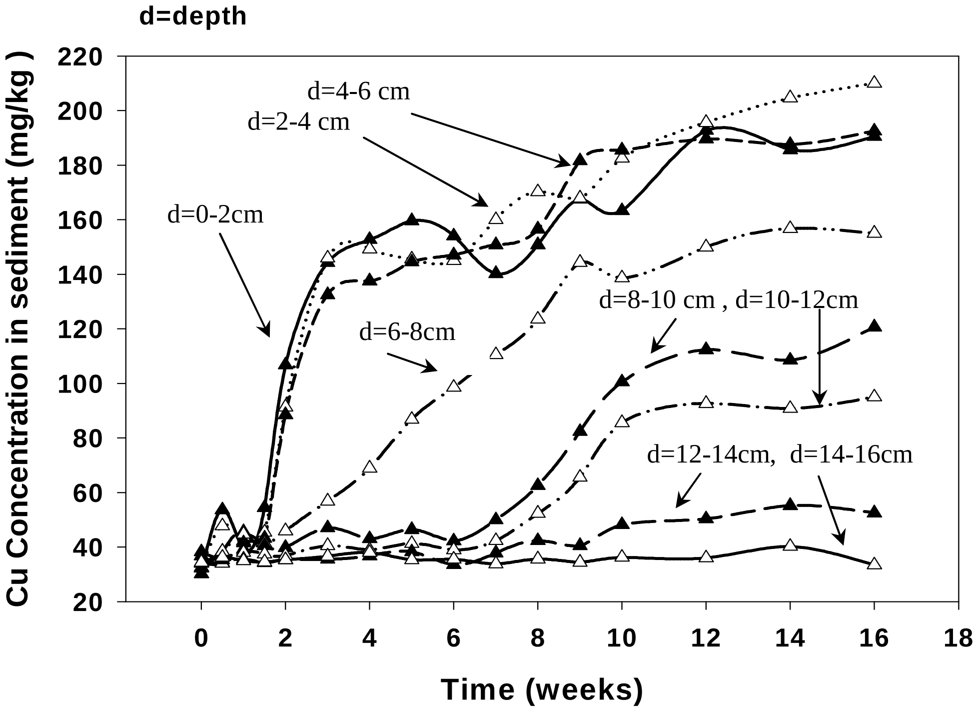


Figure 2.4b Accumulation of Cu in sediment at point A during the Cu application season.

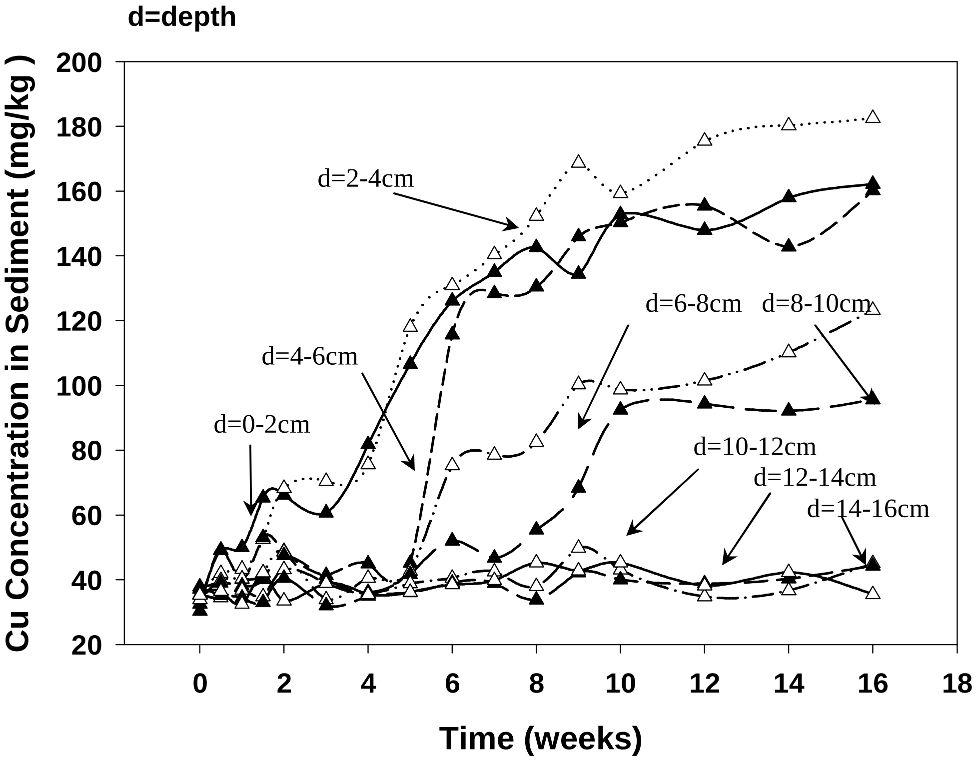


Figure 2.4c Accumulation of Cu in sediment at point D (c) during the Cu application season.

primarily facilitated by the transport of suspended sediments. Because sediment re-suspension and re-distribution are dependent on the pond hydraulics, the distribution of Cu is strongly affected by the aeration process. As more sediment particles settle in the area near the side walls than at the center, more Cu was observed at Points A and E than at Point D.

Figure 2.5 compares the vertical distributions of Cu in selected sediment cores (Point B) taken at weeks 0, 8 and 16 of the study. Similar concentration profiles were also observed for Points A, D, E, and 1-16 (data not shown). The initial Cu concentration in the sediment core ranged from 25 to 35 mg/kg. At the end of the Cu application season, the Cu concentration culminated to approximately 200 mg/kg, with most Cu accumulation occurring in the top 10 cm of the sediment. However, the concentration wave was able to reach 10 *cm* deep in the 16 weeks of study period.

Although **Figure 2.5.** indicates that the concentration front of Cu in the sediment cores was progressing downward in the sediment at an average velocity of approximately 0.14 cm/d, no significant Cu leakage was detected in the surrounding groundwater.

Munsiri et al. (1995) observed that the sediment accumulation rate were only 1.23 cm/year for 23-year-old ponds and 0.93 cm/year for 52-year-old ponds located in the same area as the research pond. This observation suggests that the observed fast downward migration of copper in the pond sediment were not due to the piling up of the new sediment.

Bioturbation (i.e. the disturbance or mixing of sediment layers or particles by biological activities) is a significant process at the water-sediment interface, and can play

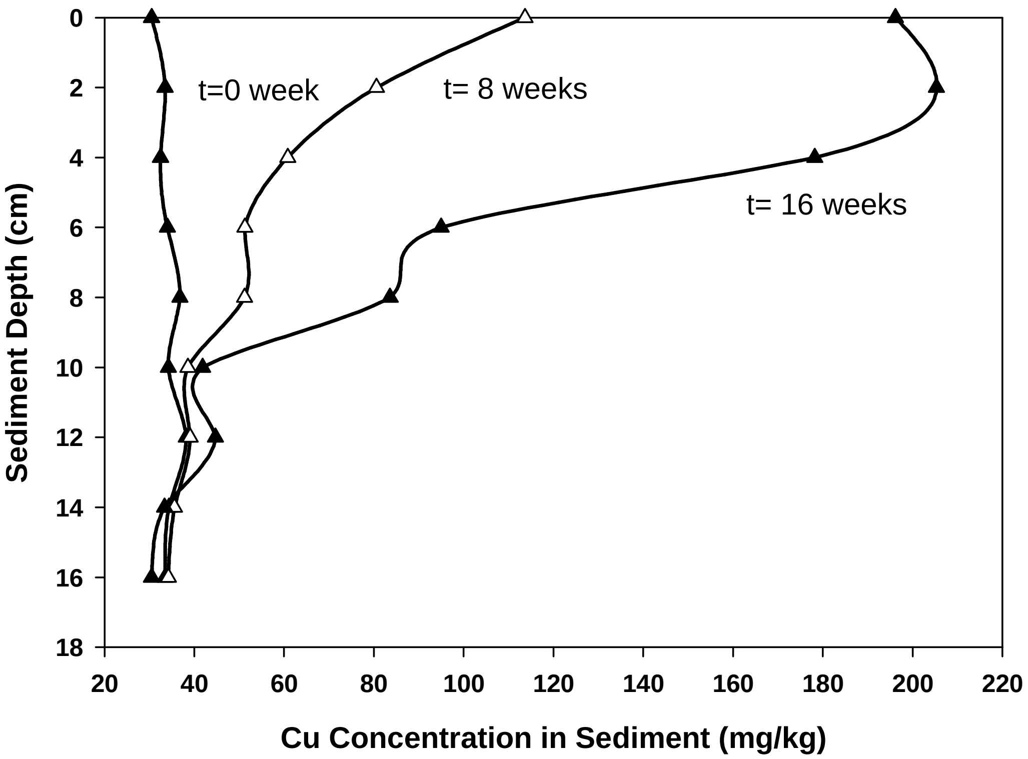


Figure 2.5. Vertical distributions of Cu in sediment at Point B at the beginning, middle and end of the study period.

an important role in accelerating the migration of copper into the deeper sediment layers. Infauna macroinvertebrates, which inhabit in nearly all fine-grained sediment deposits, were found to significantly affect the physical and chemical properties of sediment through their burrowing, feeding and locomotion activities (Matisoff, 1995). Matisoff (1995) reported that bioturbation of various aquatic organisms was able to facilitate the transport of sediment-bound heavy metals and radionuclides. In a study on the vertical distribution of radionuclides in a lagoon sediment off an old nuclear weapon testing site, McMurtry et al. (1985) found that a significant portion of the initial fallout disposition to the sediment surface had been mixed to depths of at least 1.5 m by activities of several species of callianassid shrimp after about 30 years. Wijnhoven et al. (2006) applied a simulated bioturbation to the top 2 cm of Zn-spiked soil columns, finding that the turbation caused a downward redistribution of soil-sorbed zinc and the effect was measurable at a soil depth of more than 15 cm in 15 days. Sun and Torgersen (2001) successfully modeled the fate and transport of ^{224}Ra in sediment by incorporating bioturbation process with adsorption/desorption reactions. Boyer et al. (1990) observed trenches constructed by burbot (*Lota lota*) in the deep waters of Lake Superior and concluded that the fish was capable of mixing surface sediment to a depth of 30 cm. Because many benthic species such as oligochaete worms and amphipods commonly observed in aquaculture ponds are known potent sediment movers (Boyd, 1995), bioturbation by fish and benthos may have played a key role in transporting Cu in the sediment. The relatively flat Cu concentration peak after 16 weeks (**Figure 2.5**) appears consistent with this scenario.

In addition, colloids-facilitated contaminant transport may also play a role in transporting Cu in the sediment. It has been reported that colloids can facilitate migration of strongly adsorbable contaminants such as pesticides (Sprauge et al., 2000), mercury (Lowry et al., 2004), lead (Kenchtenhofer, 2003), copper (Kanrathansis, 1999; Kenchtenhofer, 2003), zinc (Kanrathansis, 1999), and radio nuclides (Kersting et al., 1999) in the subsurface environment. The process can be affected by a number of processes, such as hydro-mechanic disturbance, preferential flow, water seepage velocity, pore water chemistry, soil macrospore structures, and soil properties. In this study, the periodic re-suspension of Cu-laden sediment particles due to the cyclic aeration and bioturbation promote the transport of colloids, which explains why the concentration wave of Cu was able to reach a depth of 10 cm within 16 weeks. However, colloids-facilitated transport would be much slower if bioturbation and mechanical mixing were absent. Because the bioturbation and mechanical mixing are most influential to the top sediment layers (< 10 *cm*) (Munsiri et. al., 1995), a much slower particle migration velocity is expected in the deeper and more consolidated sediment.

3.4. COPPER ACCUMULATION IN FISH

In addition to soil and groundwater contamination, possible Cu accumulation in fish is also a potential concern. Over the 16-week study period, a total of 356 kg of fish food was applied, and the gross fish weight grew from 165 kg to 346 kg in the pond. Results from fish analyses before and after the study period indicated that the Cu concentration in the catfish body decreased from 12.7±2.81 (standard deviation) mg/kg in the seedlings to 6.15±2.54 mg/kg in the adult fish during the season. The results revealed that the net gain of Cu in all fish was only 0.0324 g during the 16-week Cu application season. Oldewage

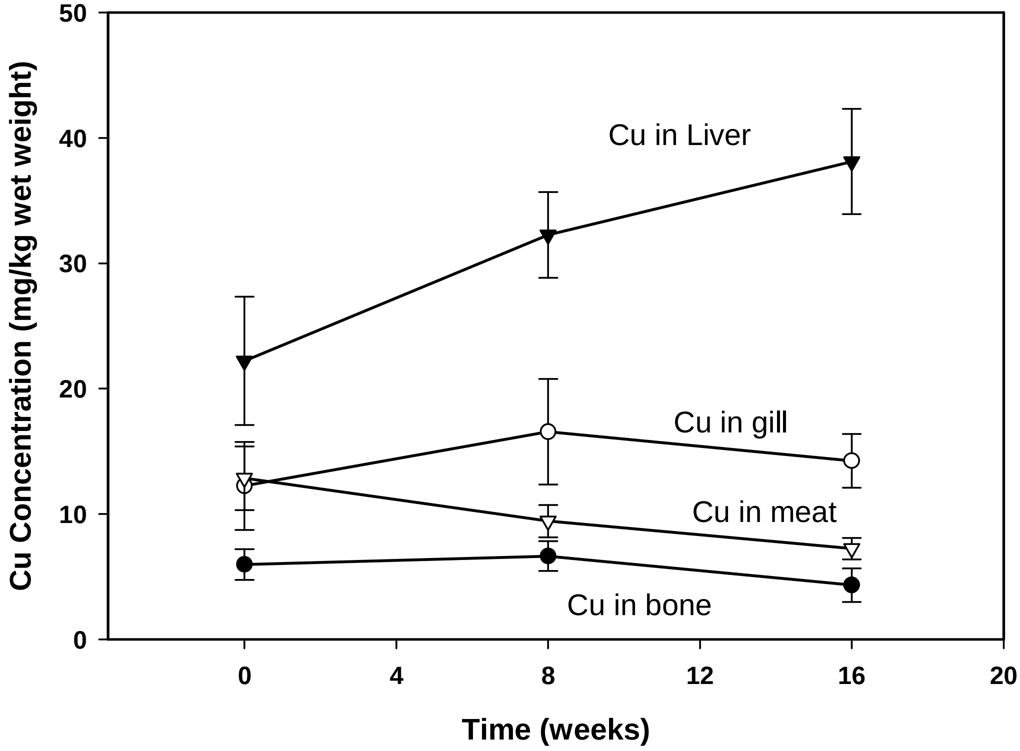


Figure 2.6. Changes in copper concentrations in selected fish tissues with time.

et al. (2000) studied the bioaccumulation of Cr, Cu, and Fe in fish *Clarias gariepinus* from a polluted river and observed that Cu concentration in fish tissues decreased with fish age. To further understand Cu distribution within a fish body, the Cu content in four different body parts (liver, gill, muscle, and bone) was also monitored at weeks 0, 8, and 16, respectively. **Figure 2.6** shows that the Cu concentration in the liver and gills far exceeds that in meat and bone. Moreover, while Cu concentration in meat decreased by nearly 50%, Cu in liver rose by nearly 75% over the 16 weeks, suggesting that Cu was removed for the body and accumulated in the liver over time.

Similar patterns of Cu accumulation in fish organs were also reported by others. For example, Kraeme (2005) reported that copper content in yellow perch (*Perca flavescens)* living in a metal-contaminated lake increased from 10 to 100 mg/kg in liver and from 5 to 15 mg/kg in gill after 50 days of exposure. Farkas (2003) observed the heavy metals accumulation in gill, liver and muscle of fish *Abramis brama L*. in a contaminated site followed very similar profiles as in **Figure 2.6**. Earlier, Thomann et al. (1997) showed that the accumulation of cadmium in fish liver reached its peak level after ~60 days of exposure to the metal.

3.5. OVERALL COPPER BUDGET

Based on the Cu measurements in each compartment of the fishpond system, the overall Cu budget was calculated using Eqn (21.4). Cu input to the fish pond due to runoff was neglected considering the small size of the watershed and well-maintained vegetation around the pond. Cu gain in the pond water (ΔM_w) was determined to be 0.72 *g* by comparing the steady-state Cu levels before and after the study period. Cu input to the pond from rainwater and feed water (M_2) was determined based on on-site measurements.

The combined contribution was 2.93 *g* Cu, of which approximately 1.54 *g* was due to rainfall and 1.39 *g* to feed water. As expected, the single most important Cu input to the pond system was due to the algaecide application, which totaled 754 *g*, i.e. M_1 = 754 *g*. Cu input from fish food (M_3) was determined to be 2.80 *g*.

Copper retained in the pond sediment was determined by comparing the change of Cu content in the top 22 *cm* of the sediment before and after the study period. Vertical and horizontal distributions of Cu in the sediment were determined based on measurements of 20 sampling points as shown in **Figure 2.1**. A numerical scheme based on the finite element method (The Interactive Data Language software) was used to interpolate the concentration distributions in each horizontal layer. Total Cu in the sediment was computed then by integrating the distributed Cu concentrations in each segment. **Table 2.2** summarizes the overall Cu budget calculation. Based on our weekly sampling and analysis of the neighboring groundwater, there was no appreciable Cu leaking into the aquifer during the study period. Therefore, the resultant negative seepage value in **Table 2.2** indicates that the overall Cu budget can be well balanced within an error of <6 % of Cu added in the pond. The results confirmed that virtually all Cu applied was retained in the top 16 cm of sediment within the relatively short 16-week period.

3.6 FIELD INVESTIGATION

To support the pilot observations, field data on copper in sediments were collected in three commercial fish ponds. **Table 2.1** indicated that the Cu concentration in the commercial ponds ranged from 0.012 to 0.033 mg/L despite the seasonal intensive application of copper sulfate. Again, saturation index calculation showed that the aqueous Cu in those pond water was under-saturated with all of Cu precipitates, suggesting the

Table 2.2.
Summary of Cu budget calculations.

Cu input (g)	Cu applied (M_1, g)	774
	Rainwater and fill water (M_2, g)	2.93
	Fish food (M_3, g)	2.80
	Total Input (g)	**780**
Cu accumulation (g)	In water (ΔM_w, g)	0.72
	In sediment (ΔM_s, g)	823
	In fish (ΔM_f, g)	0.03
	Total Accumulation (g)	**824**
Cu output (g)	Seepage (M_4, g)	**- 44**

sediment-particle adsorption process played a major role in controlling the Cu activity in pond water. Data in **Table 2.1** were applied in the chemical equilibrium calculation for each pond.

Figures 2.7a-c show the vertical distribution of Cu and the bulk density of the sediments in the three commercial fishponds. According to Munsiri (1995), a bulk density of 1.4 $g\ cm^{-3}$ was used to distinguish between pond sediment and bottom soil (i.e. the bulk density of sediment is less than 1.4 $g\ cm^{-3}$). Based on this definition, the depth of sediment in P-1, P-5, and P-25 was determined to be about 4, 14, and 10 *cm*, respectively. Below the respective depth is considered as the bottom soil.

Evidently, the peak concentrations for the younger ponds (P-1 and P-5) occurred at a depth of ~3 cm (**Figures 21.7a** and **2.7b**). In contrast, the peak concentrations for the 25-year old P-25 (**Figure 2.7c**) occurred at a depth of 5-6 cm. It is noteworthy that the peak concentrations for the actively used P-5 ranged from ~200 to ~250 mg/kg, which are much greater than in newly built P-1 (7.0-16 mg/kg) and the 25-years old P-25 (7.0-33 mg/kg). The peak concentrations for the newly built P-1 fell into the reported background concentration range of 4-18 mg/kg in typical U.S. soils (Thornton, 1976), possibly indicating that no copper has yet been applied to the pond. The much lower copper concentration in the more aged P-25 than in the 5-years old P-5 is attributed to the intermittent sediment replacement.

A closer examination of the individual Cu distribution profiles indicates that although the copper concentration wave went deeper for more aged ponds, the peak Cu concentration occurred within the sediment layer rather than the bottom soil regardless of pond age or pond soil properties. This observation agrees with the observed pilot-test results, and indicates that Cu transport is facilitated primarily by bioturbation and hydraulic mixing of the Cu-laden sediment particles. The results also reveal that the application of copper may not cause significant changes in Cu content in the bottom soil. Long-term groundwater monitoring showed that the Cu content in the groundwater under those three commercial catfish ponds were 0.014 mg/L for P-1, 0.000 mg/L for P-5, and 0.001 mg/L for P-25, respectively. These concentrations are the same as in the background water, confirming that nearly all copper applied to the ponds was retained in the pond sediments.

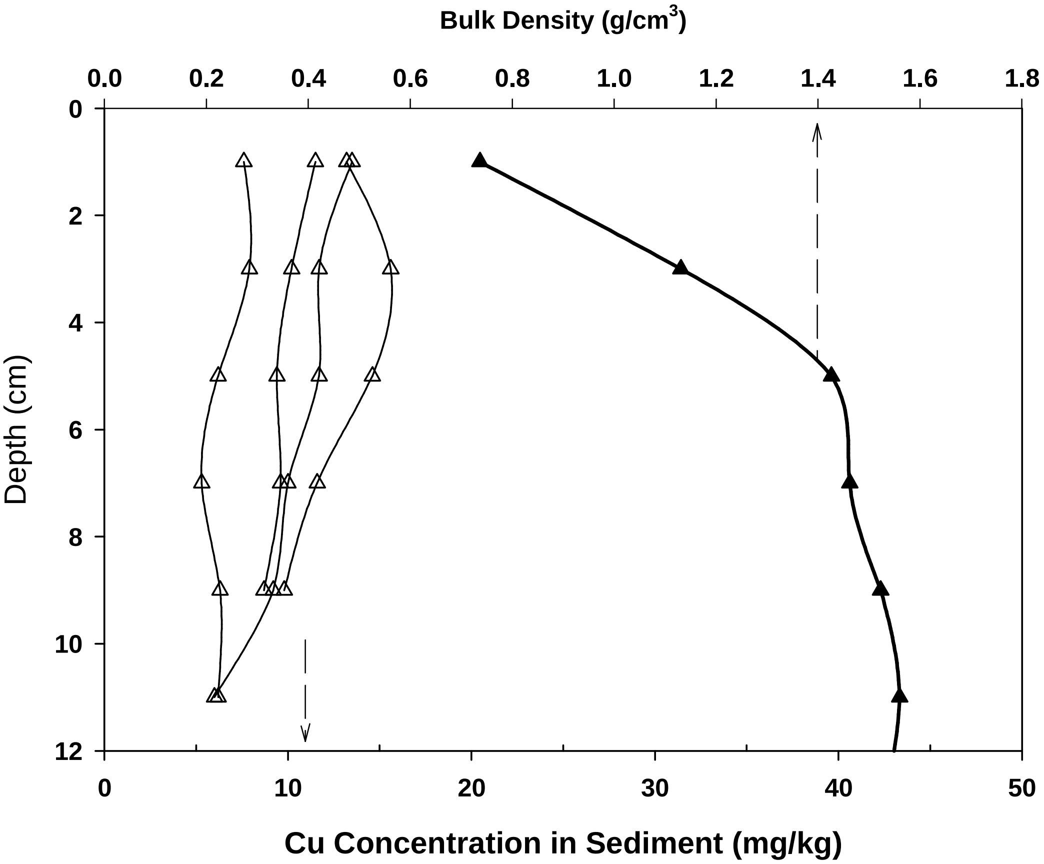


Figure 2.7a. Vertical distribution in Cu concentration and bulk density with the depth of the pond sediment / bottom soil for the 1-year pond.

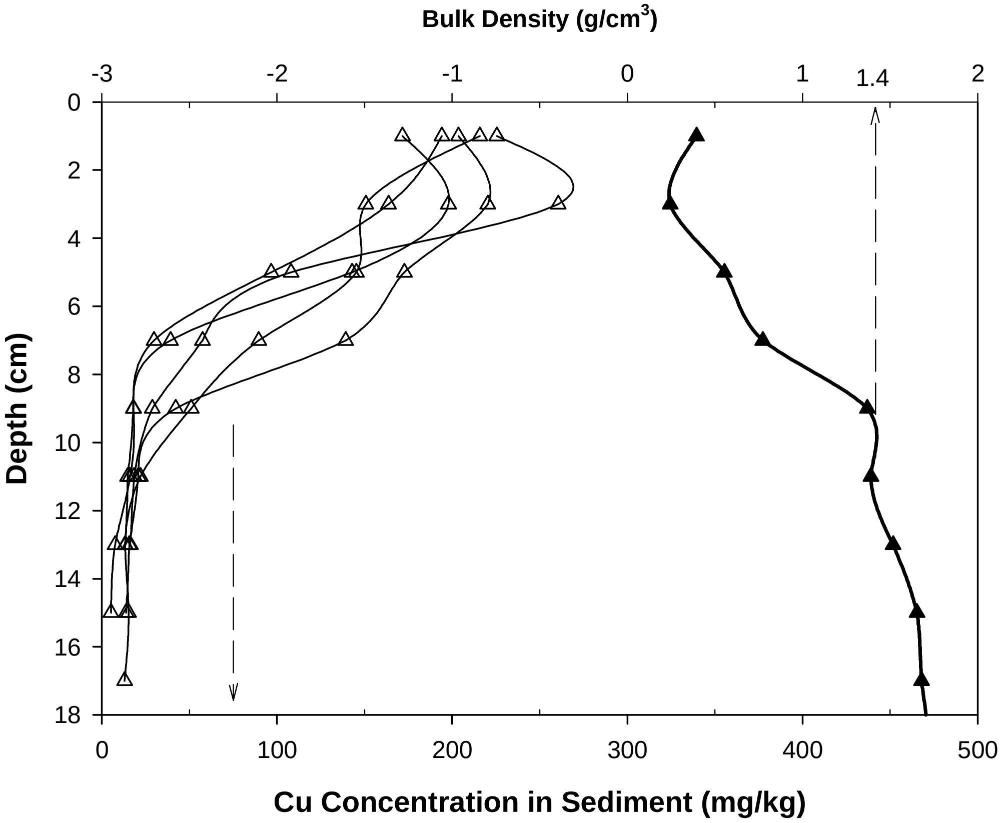


Figure 2.7b. Vertical distribution in Cu concentration and bulk density with the depth of the pond sediment / bottom soil for the 5-year pond.

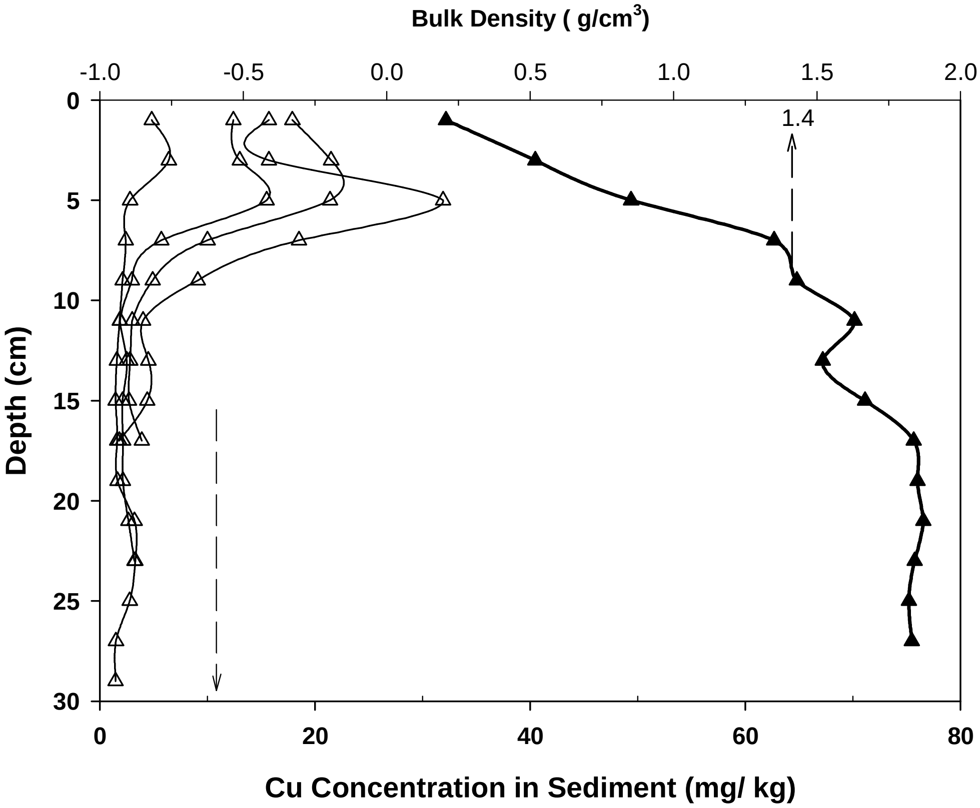


Figure 2.6c. Vertical distribution in Cu concentration and bulk density with the depth of the pond sediment / bottom soil for the 25-year pond.

TABLE 2.3.
Cu concentration in tissues of the catfish raised in commercial ponds.

fishpond	Weight (kg)	Cu concentration in fish tissues (mg Cu/kg dry weight)			
		liver	gill	meat	bone
P-1	0.59±0.46 (n=5)	8.64±2.26	5.07±3.21	0.88±0.41	0.29±0.22
P-5	0.80±0.55 (n=6)	12.79±9.13	3.00±0.79	0.57±0.42	0.14±0.16
P-25	0.40±0.08 (n=6)	34.67±21.73	3.36±1.82	0.67±0.53	0.19±0.30

Table 2.3 presents copper concentrations in various body parts of fish sampled from the three commercial ponds. Consistent with the pilot findings, livers contained the highest copper, ranging from 8.64±2.26 mg/kg (dry weight) to 34.67±21.73 mg/kg, followed by gill which contained 3.00±0.79 to 5.07 ± 3.21 mg/kg. Fish flesh and bone contained minimal amounts of copper: 0.57±0.42 to 0.88±0.411 mg/kg in flesh and 0.14±0.16 to 0.29±0.22 mg/kg in bone, which are much lower than the Food and Agriculture Organization (FAO) limit of 20.0 mg/kg (Abou-Arab et al., 1996).

4. Conclusions

Results from both the pilot pond and commercial ponds revealed the pivotal role of the suspended sediment particles on the overall fate and transport of Cu in both water and sediment phases. Because of the strong interactions between Cu and the suspended particles, more than 90% of Cu applied to the fish pond become rapidly (within 2 hours) and firmly associated with the suspended solids. Within approximately 2 days after its addition, over 99% of Cu added was transferred to the bottom sediment phase due to the adsorption process by the sediment particles

The regular pH and DO profiles in pond water were distorted in response to each application of copper sulfate. However, both pH and DO resumed to their normal patterns after three days, which corresponds to the typical duration when the copper's algaecidal effect was operative. Therefore, pH and DO levels may serve as an indicator for the activity of algae and other planktons.

A Cu budget study and direct groundwater monitoring suggested that virtually all Cu applied ended up in the sediment phase. Facilitated primarily by mechanic stirring and bioturbation, Cu was observed to migrate deeper into the sediment column. However, both pilot-experimental data and field observation indicated that nearly all Cu applied was actually concentrated within the pond sediment layer (<16 *cm*). The applied copper did not appear to be able to reach the bottom soils in either the higher Cu-loading pond (P-5 and the experiment pond) or the 25-year old pond (P-25). This observation was further supported by the observed extremely low Cu concentrations in monitoring wells located near the fishponds. The knowledge gained from this study may facilitate a sound assessment of the environmental impacts associated with copper application in fish ponds. Further, it may also provide a practical guideline for timely replacement and safe disposal of the pond sediments.

Both experimental and field data showed Cu accumulation in fish primarily occurred in the liver and gills. While the Cu concentration based on the total body weight decreased by nearly 50% during the season, Cu in the liver increased by nearly 75%. In contrast, Cu concentration in fish muscle was diluted by a factor of nearly 2. The measured Cu levels in fish may serve as a basis for formulating sound fish consumption advisories. In addition, caution should be taken towards the reuse and disposal of fish leftover (e.g. for cattle food) due to the elevated Cu contents in some of the fish organs.

CHAPTER III

THE LEACHABILITY, BIOACCESSIBILITY, AND SPECIATION OF CU IN THE SEDIMENT OF CHANNEL CATFISH PONDS

1. Introduction

The channel catfish *(Ictalurus punctatus)* has been farmed extensively in the central and southern USA for many decades. The water surface being used for catfish production in the United States has expanded from about 1,000 hectares (ha) in the early 1960s (Boyd et al., 2000) to about 80,000 ha in 2001 (USDA, 2004). One of the noteworthy chemicals often encountered in aquaculture practice is copper sulfate, which has been the most commonly used algaecide for about a century in the U.S. to control the off-flavor problem (e.g. fishy taste) caused by blue-green algae in channel catfish ponds (Riemer and Toth, 1970). As a matter of fact, copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is currently the only algaecide approved by the U.S. EPA for the use in catfish ponds (U.S.EPA, 2003). Typically, copper is applied to the fishponds at about 1% (w/w) of the total alkalinity of the pond water, and applications are made at intervals of 2-10 days from early summer to early fall. Individual ponds typically receive about 50 kg/ha of $CuSO_4 \cdot 5H_2O$ or about 12.5 kg/ha Cu each year. Based on these numbers, in 2001, the 80,000 ha of channel catfish ponds in the U.S. (mainly in Alabama, Arkansas, Louisiana, and Mississippi) received a total of 4,000,000 kg of $CuSO_4 \cdot 5H_2O$ or 1,000,000 kg of Cu

(USDA, 2004). According to Liu et al. (2006), nearly 100% of the applied Cu would transfer to the pond sediment. However, there have been few reports to evaluate the potential environmental problems associated with such a high amount of Cu accumulated in the sediments of channel catfish ponds although contaminated sediments in rivers, lakes and other surface water bodies have been fully studied for decades (Allen, 1995; Fröstner and Wittmann, 1981; Locat et al., 2003, Vdovic et al., 2006).

Cu contamination in the catfish pond sediment might be different from that in other water body sediments due to the following reasons:

1. Catfish ponds receive a high load of copper as mentioned above. Most of it accumulates in the sediment.

2. Pond sediment is frequently disturbed by aquaculture management activities. For example, in order to increase dissolved oxygen content in the water and reduce the accumulation of the toxic substances in the bottom, the pond sediment is often exposed to the air through pond water aeration, forced water circulation, and sediment drying and even tilling. Hence, pond sediment environments may differ from those in the lakes or reservoirs in terms of the anaerobic/aerobic conditions, which may affect the speciation and thus availability of Cu in the sediment.

3. Periodic drainage and sediment removal result in the frequent output of Cu-laden sediment to the surrounding water bodies or farmland, which makes environmental evaluation of Cu-laden pond sediment necessary.

Although Boyd et al. (2000) have proposed several best management practices for catfish aquaculture to prevent the Cu-laden sediment from entering the environment, assessment of the leachability, bioavailability, and speciation of sediment-bound Cu were not included in the paper. Han et al. (2001) have researched the Cu speciation in the sediments of commercial catfish ponds using the sequential extraction procedures (SEP), indicating the Cu was primarily bound with the carbonates, organic matter and iron oxides. Nevertheless, in Han et al.'s paper, sediment samples were exposed to the air during sampling, transporting and treatment and the anaerobic condition, under which the sediments usually existed, was not taken into account at all. This might have resulted in an underestimate of the importance of metal-sulfide binding in the anaerobic horizons characteristic of most natural in-place sediment.

Di Toro et al (2001) revealed that the toxicity of a heavy metal resulted from a competitive equilibrium in the solution among the heavy metal ion, other co-existing cations, organic and inorganic ligands, and the biotic ligand (the target organ) through the competitions between the heavy metal and the co-existing cations for the biotic ligand and between biotic ligand and other ligands for the heavy metal. Toxicity and mortality occur when the competing result, or the concentration of the metal bound to the biotic ligand, exceeds a threshold concentration (Di Toro et al, 2001). Therefore, if there are enough strong binding ligands such as sulfide and carbonate to quantitatively sequester the metal through forming precipitants, theoretically, there would be no or much less biotic ligand-available metal in the solution to exert the toxicity toward organisms. In this case, the metal is considered to be biologically unavailable and hence nontoxic. Thus, the availability of a heavy metal is controlled by strong metal-binding solid phases (Bull and Williamson, 2001). In freshwater sediment the major binding phases with Cu are carbonates, Fe/Mn oxides, organic matter, acid volatile sulfides (AVS) and clay minerals (Fang et al., 2005; Peng et al., 2004; Yu et al., 2001). Commonly used methods to assess the speciation of Cu in anaerobic sediment are sequential extraction procedures (Peltier et al., 2005; Sáenz et al., 2003; Yu et al., 2001) and acid volatile sulfides (AVS) / simultaneously extracted metals (SEM) method (Ankley et al., 1993; Berry et al., 1996; Fang et al., 2005; Hansen et al., 1996; Yu et al., 2001).

Another important factor controlling the availability of the soil/sediment bound Cu is the soil solution chemistry (Amonette, 2002), i.e., the properties of the environment in which the soil-bound chemical is present. For example, the availability of soil-bound Cu might be low in the natural water bodies such as lakes or rivers with a pH around 7. However, if this soil were swallowed accidentally by cattle or human beings, its availability would likely increase several times in the stomach where the solution pH is around 2. There are several methods available to evaluate the availability of soil/sediment bound metals under different solution environments such as the toxic characteristic leaching procedure (TCLP) and physiological based extraction test (PBET). The former is often applied to evaluate the leachability of solid waste in the natural environment (esp. in landfill) (Ghosh, 2004;U.S.EPA, 1992) and the latter to evaluate the bioaccessibility of contaminates in animal or humans' stomach (Fendorf et al., 2004; Ruby et al., 1996; Yang et al., 2002).

Generally speaking, the major purpose of this paper is to evaluate the leachability, bioaccessibility, and speciation of Cu in the catfish pond sediment. The detailed objectives are as follows:

1. to assess the role of sulfides in binding Cu in fishpond sediment using the AVS / SEM extraction method;

2. to evaluate the leachability of the Cu-laden sediment using the TCLP method;

3. to estimate the bioaccessibility of the Cu-laden sediment using the PBET method;

4. to study the speciation of Cu in different sediments using the sequential extraction procedure (SEP); and

5. to explore the relationship between Cu speciation and its environmental availability.

2. Materials and Methods

2.1. PONDS AND SAMPLING PROTOCOL

Three commercial catfish ponds located in West Alabama were selected as sampling sites based mainly on the pond age and sediment pH. Basic information about those three ponds is shown in **Table 3.1**. Sediment samples were collected in June, 2005. Five sediment cores were randomly taken from each pond following the procedures described by Munsiri et al. (1995), then on site each core was segmented as 2-cm thick cakes which were separately stored in capped 50-ml plastic cups. The specimen cups were then immediately placed into an airtight container which was flushed with nitrogen gas and transported on ice to minimize oxidation of the samples. Care was taken during the whole sampling procedure to minimize the exposure of the samples to the air. Upon arrival in the lab, samples were transferred to the refrigerator and the test began as soon as possible.

2.2. SEDIMENT AND SOIL ANALYSIS

The redox potential was determined by insertion of the platinum redox electrode (model 97-78, Orion Research Inc.) directly into the segmented cakes under an anaerobic chamber (Model 10, Sheldon Manufacturing Inc.) and the data were read from an expandable ion analyzer (model EA940, Orion Research Inc.). The sediment pH was measured in 0.01 M $CaCl_2$ in a 1 g: 1 mL suspension under anaerobic condition. Total Cu concentration in the sediment samples were determined using U.S. EPA method 3050B (1996).

Native soil samples were taken from Bt horizon (50~70 cm deep) in the vicinity of the pond for characterizing the soil properties. Soil samples were pulverized to pass through a 2-mm sieve after dried in the atmosphere. So, all of the subsequent measurements were based on the weight of air-dried samples. Soil pH was measured in 0.01 M $CaCl_2$ in a 1 g: 1 mL suspension. The fraction of sand, silt, and clay was determined using the pipette method described by Gee and Or (2002). The total carbon content was analyzed following the Dumas method with a LECO CN-2000 combustion unit (LECO Corp.) at 1050 °C. Soil clay minerals were identified with the XRD method using an X-ray diffraction unit (Siemens D5000 x-ray diffractometer) after the soil samples were treated with the procedure given by Kunze and Dixon (1986). The CEC was determined with 1 N NH_4OAc buffered at pH 7.0 following the method described in Soil Survey Laboratory Methods Manual (USDA et al., 1996).

2.3. AVS AND SEM MEASUREMENT

The AVS/SEM measurements followed procedures described by Allen et al. (1993). The AVS was liberated from 5 g of wet sediment using 6 M HCl under a flowing N_2 atmosphere. H_2S generated in the reaction was trapped in 0.5 M NaOH, and total sulfide released was determined by the methylene blue method (Clesceri et al., 1998). The

TABLE 3.1.

Pond water quality and soil property data for the commercial catfish ponds.

	Pond Water properties				Native Soil properties						
	pH	Cu (mg/L)	Organic carbon (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Soil pH	CEC (cmol/ kg)	Cu (mg/kg)	Sand (%)	Silt (%)	Clay (%)	Clay* minerals
1- year-old pond (P-1)	7.87	0.012	11.98	141.1	7.8	34.85	23.2	15	29	56	(K)(M)(Q)(S)
5- year-old pond (P-5)	7.43	0.066	14.21	74.3	6.9	14.17	25.6	18	28	54	(K)(M)(Q)(S)
25- year-old pond (P-25)	7.44	0.043	4.32	37.2	4.3	8.77	13.5	69	4	27	(K)(M)(Q)(S)

* K=Kaolinite, M=Maca, Q=Quartz, S=Smectite

remaining solution in the reaction vessel was filtered through a 0.45-μm filter and stored for the simultaneously extracted metals analysis.

2.4. TCLP AND PBET TESTS

The TCLP test was conducted according to U.S. EPA protocol (USEPA, 1992) by mixing 1 g of dry soil samples (or equivalent amount of the wet sediment) with an extraction solution at a liquid to solid ratio of 20. The extraction solution consisted of 0.1 M acetic acid and 0.0643 M NaOH with a pH of 4.93±0.08. The solid and leachant suspension was placed in a capped polypropylene bottle and tumbled at 30 rpm for 18 h. After extraction, the liquid was separated by filtration through a 0.45-μm filter. The dissolved metal concentration in the filtrate was then measured with the fraction of metal dissolved representing the leachability. The leachability of Cu was calculated in the following manner (Eqn 3.1):

$$The\ leachability\ value\ (\%) = \frac{(\,Cu\ concentration\ in\ extract,\ \ mg/L)(0.01\,L)}{(Cu\ concentration\ in\ soil,\ \ mg/g)(0.5\,g)} \times 100$$

………………………………. (3.1).

The physiologically based extraction test (PBET) used here followed the procedures described by Kelley et al. (2002): The extraction solution consisted of 30 g/L glycine (0.4 M) with the pH adjusted to 1.5 using HCl. An equivalent to 0.1 g of a sediment sample was placed in a 15 mL Teflon centrifuge bottle. Then 10 mL of 37 °C simulated gastric solution was poured in each bottle. After capping, each bottle was rotated in a temperature-controlled water bath at 37 ±2 °C for 1 h. After 1 h, the bottles were immediately removed and stood up right for approximately 5 min before taking a portion of the supernatant, which was then filtered with 0.45-μm filter. The dissolved metal concentration in the filtrate was measured with the fraction of metal dissolved representing the bioaccessibility. Similarly, the bioaccessibility of Cu was calculated in the following manner (Eqn.3.2):

$$The\ bioaccessibility\ value\ (\%) = \frac{(\ Cu\ concentration\ in\ extract,\ \ mg/L)(0.01\ L)}{(Cu\ concentration\ in\ soil,\ \ mg/g)(0.1\ g)} \times 100$$

…………………………………. (3.2).

2.5. SEQUENTIAL EXTRACTION PROCEDURES

The procedures employed in this study were based on those developed by Tessier et al. (1979) and Shuman (1985). Procedures for measurement of exchangeable, carbonate, residual Cu were modified according to Han et al. (2001). The Cu bound with Mn/Fe oxides was determined using hydroxylamine hydrochloride (Peltier, 2005). **Table 3.2.** shows the reagents and procedures used for each of the five extraction steps. For each step, a certain amount of extractant was added to the sediment sample, which was the equivalent of 1g dry weight, in a 50 mL Teflon centrifuge tube. After being treated with a procedure listed in table 2, the mixture was then centrifuged at 5000 x *g* for 10 min. The supernatant was decanted and filtered through a 0.45-µm filter. The filtrate was stored for Cu analysis. The sediment residue was retained for the next step. The same centrifugation–decantation procedure was used after each of the listed extractions. All the procedures were conducted in the anaerobic atmosphere. Iron and manganese concentration in the step 3 extract were also measured to estimate the amorphous iron oxides and manganese oxides contents in the samples. Those two components were supposed to be released in this step (Hettiarachchi, 2003).

2. 6. ANALYSIS OF CHEMICALS

Copper, iron, and manganese in water or in acid extracts were analyzed using either a flame atomic absorption spectrophotometer (FLAAS) (Varian SpectrAA 2220FS) or a graphite furnace atomic absorption spectrophotometer (GFAAS) (Perkin Elmer 3110), depending on the concentration. The detection limit is 0.02 mg /L for the FLAAS, and 0.002 mg/ L for the GFAAS. An Orion 520A pH meter was used to measure the pH of water or soil suspension. Alkalinity of the pond water was determined through the titration method (Clesceri et al., 1998).

TABLE 3.2.

Experimental conditions for sequential extraction procedures

Step	Extractant	Target
1	12.5 mL, 1 M NH_4NO_3 , pH 7.0, 0.5 h shaking.	Water soluble / exchangeable fraction (WE)
2	12.5 mL, 1 M Na-acetate, pH 5.0, 6 h shaking.	Carbonate-bound fraction (CB)
3	12.5 mL, 0.04 M hydroxylamine hydrochloride in 25% acetic acid, 6 h at 95 ^oC in water bath.	Fe/Mn oxide-bound fraction (OX)
4	1.5 mL of 0.01 M HNO_3 and 2.5 mL of 30% H_2O_2, 5 h at 85 °C in water bath, followed by an additional 1 mL of H_2O_2, 1 h at 85 °C in water bath, then 7.5 ml of 1 M NH_4NO_3 , 10 min shaking at room temperature.	Organic matter/sulfide-bound fraction (OS)
5	12.5 mL, 4 M HNO_3, 16 h at 80 °C in water bath.	Residual fraction (RS) (forms in lattices of primary and secondary minerals)

3. Results and Discussions

3.1. CHARACTERISTICS OF SEDIMENTS

The redox potential (E_h) and pH distributions in sediments of three commercial catfish ponds are shown in **Table 3.3**. Data showed that the redox potential in the sediment of those ponds ranged from about -0.15 to +0.35 volts with the lowest potential in the P-25 pond and the highest in the P-5 pond. According to Patrick et al. (2002), P-5 sediment was under a moderately reduced environment (E_h around +0.1~+0.35 V) while P-1 and P-25 were under reduced conditions (E_h about -0.15~ +0.1V). Highly reduced environments (E_h< -0.15 V), under which the reaction of reducing of sulfate to sulfides is dominant (Boyd, 1995), were not observed in those ponds. The sediment pH values were around 5.7 to 8.5, largely depending on the native soil pH, with minor fluctuations (less than 1 pH unit) along the depth. The redox potential and pH are important parameters to determine the speciation of soil components. According to the Eh-pH diagram for SO_4^{2-} /S^{2-} redox boundary provided by Morse and Rickard (2004), the theoretical redox potential to reduce SO_4^{2-} to S^{2-} was below -0.3 V for P-1 (pH 8.3~8.5), below -0.18~ -0.23 V for P-5 (pH 6.7-8.0) and below-0.1~-0.15 V for P-25 (pH 5.9~6.4). These results agreed with Boyd's conclusion mentioned above (1995) that Eh< -0.15 V for sulfate reduction. Comparing these theoretical values with the measured redox potential in those ponds (**Table 3.3**), a conclusion can be made that significant amount of AVS should be observed only in P-25.

Table 3.3 also exhibits the profiles of some major sediment compositions which are relevant to Cu speciation according to Tessier's method (1979). Data show that the P-1

TABLE 3.3

Chemical characteristics of the sediments at different sites and depths

segment cm	pH	Redox (E_h, volt)	Carbonates (% $CaCO_3$)	Organic Matter (% Organic C)	Am.Fe-Oxides (% Fe_2O_3)	Mn-Oxides (% MnO_2)
P-1						
0-2	8.35	0.12	52.31	0.23	0.12	0.01
2-4	8.31	0.17	54.20	0.23	0.18	0.01
4-6	8.40	0.15	49.25	0.49	0.16	0.01
6-8	8.30	0.15	52.43	0.26	0.12	0.01
8-10	8.28	0.17	49.93	0.31	0.20	0.01
10-12	8.45	-0.13	47.56	0.25	0.16	0.01
12-14	8.35	-0.14	48.83	0.23	0.30	0.02
P-5						
0-2	7.89	0.31	11.28	0.57	0.80	0.06
2-4	7.91	0.33	9.29	0.79	0.77	0.09
4-6	7.77	0.32	9.35	0.63	0.65	0.09
6-8	7.32	0.32	7.76	0.61	0.72	0.13
8-10	7.14	0.32	6.20	0.51	0.55	0.07
10-12	7.06	0.32	4.46	0.45	0.59	0.10
12-14	6.70	0.27	2.60	0.31	0.38	0.02
14-16	6.85	0.15	3.50	0.39	0.17	0.04
P-25						
0-2	5.86	-0.08	0.34	0.69	0.15	0.02
2-4	5.88	-0.13	0.74	0.29	0.16	0.04
4-6	5.84	-0.09	0.84	0.29	0.11	0.02
6-8	5.73	-0.13	0.75	0.41	0.08	0.02
8-10	5.88	-0.09	0.76	0.21	0.07	0.01
10-12	6.35	-0.11	0.68	0.48	0.08	0.01
12-14	6.33	-0.14	0.76	0.35	0.12	0.01
14-16	6.37	-0.14	0.58	0.42	0.08	0.01
16-18	6.40	-0.17	0.76	0.32	0.07	0.01

had highest carbonate contents in the sediment while the P-5 was highest in organic matter, amorphous iron oxides and manganese oxides contents. According to Yu et al. (2001), Fe oxides could be considered as the main binding phase for metals in slightly anoxic sediments (E_h was as low as -0.13 v) although those are redox sensitive. The P-25 also contained the high organic in the top 2 cm of the sediment. Among those components, only organic matter exhibited a wide variation along the sediment depth in those three ponds, suggesting that there was an external source of organics while iron, manganese and carbonate were basically from the native soils. The organic matter originated from the fish food, fish manure and the plant debris.

Cu concentration profiles (**Figure 3.1**) showed that the Cu added as an algaecide mostly concentrated in the top 10 cm layer of the sediment. The P-5 pond had the highest Cu content in its sediment with peak concentration as high as 200 mg/kg (**Figure 3.1a**). The P-25 and P-1 pond shared the relatively low sediment Cu with respective peak concentrations of 25 and 11 mg/kg, which are almost 1-order of magnitude less than that in the P-5 (**Figure 3.1b**). The peak concentration for the newly built P-1 fell into the reported background concentration range of 4-18 mg/kg in typical U.S. soils (Thornton, 1976), possibly indicating that no copper has yet been applied to the pond. The copper concentration in the more aged P-25 is much lower than that in the 5-year-old P-5 because the sediment has been replaced at least once in this pond. Cu concentration profile in P-25 (**Figure 3.1a**) displayed a sudden decrease from 5 cm deep to the sediment surface following a constant increase from ~15cm to 5 cm, which contradicts the constantly increasing trend of Cu from 15 cm to the surface in P-5 (**Figure 3.1b**). This observation suggests that the replaced sediment in the P-25 was about 5 cm deep.

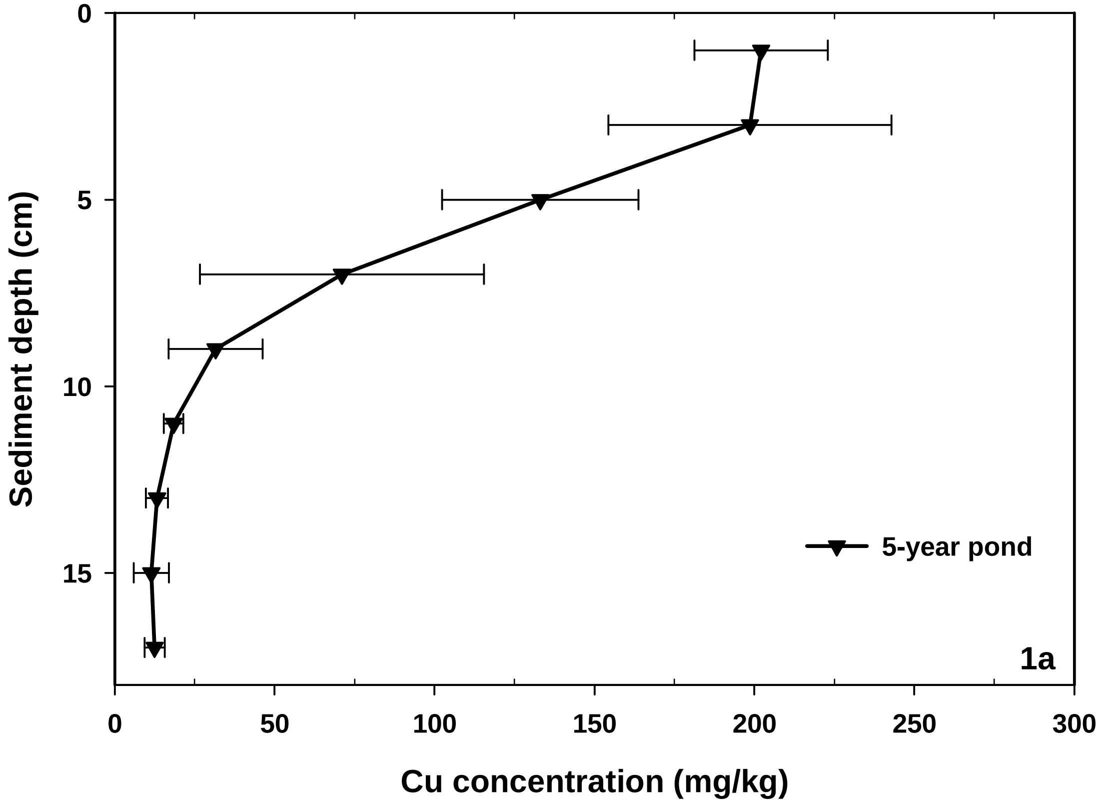


Figure 3.1a. Spatial distributions of Cu in the 5-year pond sediment.

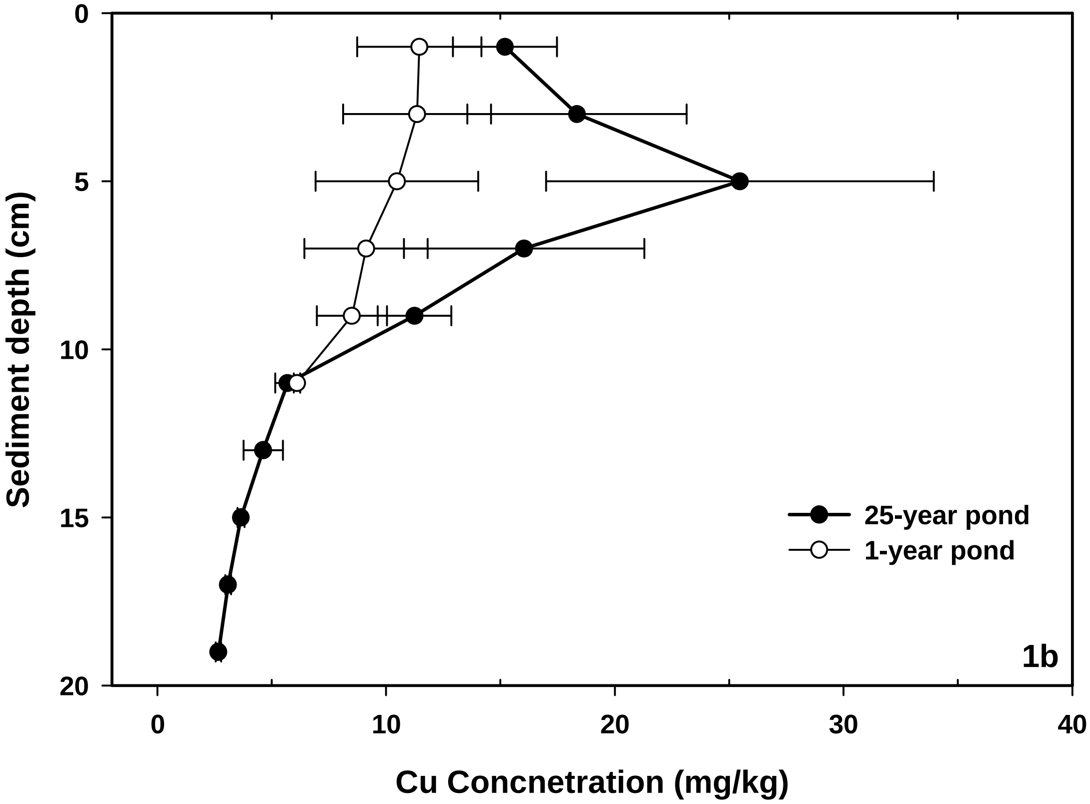


Figure 3.1b. Spatial distributions of Cu in the 1-year pond and 25-year pond sediments.

Figure 3.2 exhibits the acid volatile sulfide (AVS) and simultaneously extracted Cu (SEM_{Cu}) profiles in those three ponds. Within our expectation, the concentrations of AVS in the sediments were as low as less than 0.1 μ mole/ g dry soil for the P-1 and P-5 ponds and for top 10 cm of the P-25 pond. And about 0.4 μ mole/ g dry soil was present in P-25 sediment below a depth of 10 cm. Those observed levels of AVS were lower than those in the lake, river or other sediments reported by a few authors. For example, Ankley et al. (1993) reported that the AVS levels in two lake sediments (one in Washington and the other in Michigan) were about 0.3 ~ 4 μ mole/ g dry soil and 0 ~11.6 μ mole/ g dry soil. The sediments in West Bearskin Lake of Minnesota were observed containing 1.4~ 20.4 μ mole/ g AVS (Leonard et al., 1999). And AVS concentration of 4~13 μ mole/ g was believed to be the normal AVS range for unpolluted freshwater lakes (Leonard et al., 1993; Nriagu and Soon, 1985).

As a matter of fact, one of the best management practices in pond aquaculture is to maintain oxidized conditions at the pond bottom because the reduced environment may develop ferrous ions, nitrites, sulfides and partly decomposed organics which are toxic to the fish and other aquatic animals (Boyd, 1995). Avnimelech and Zohar (1986) also found that fish growth was inhibited when anaerobic zones existed at the sediment water interface. In addition to frequent aeration, practices such as water circulator usage, chemical oxidants (i.e. nitrate) application (Boyd, 1995) and pond bottom drying and tilling are the commonly used methods to enhance the dissolved oxygen concentrations at the pond bottom. Meanwhile, bioturbation is also a notable process to introduce oxygen to the deeper sediment (Boyd, 1995), resulting in the oxidation of metal sulfides in sediment (Peterson et al., 1996, Van Den Berg et al., 1998).

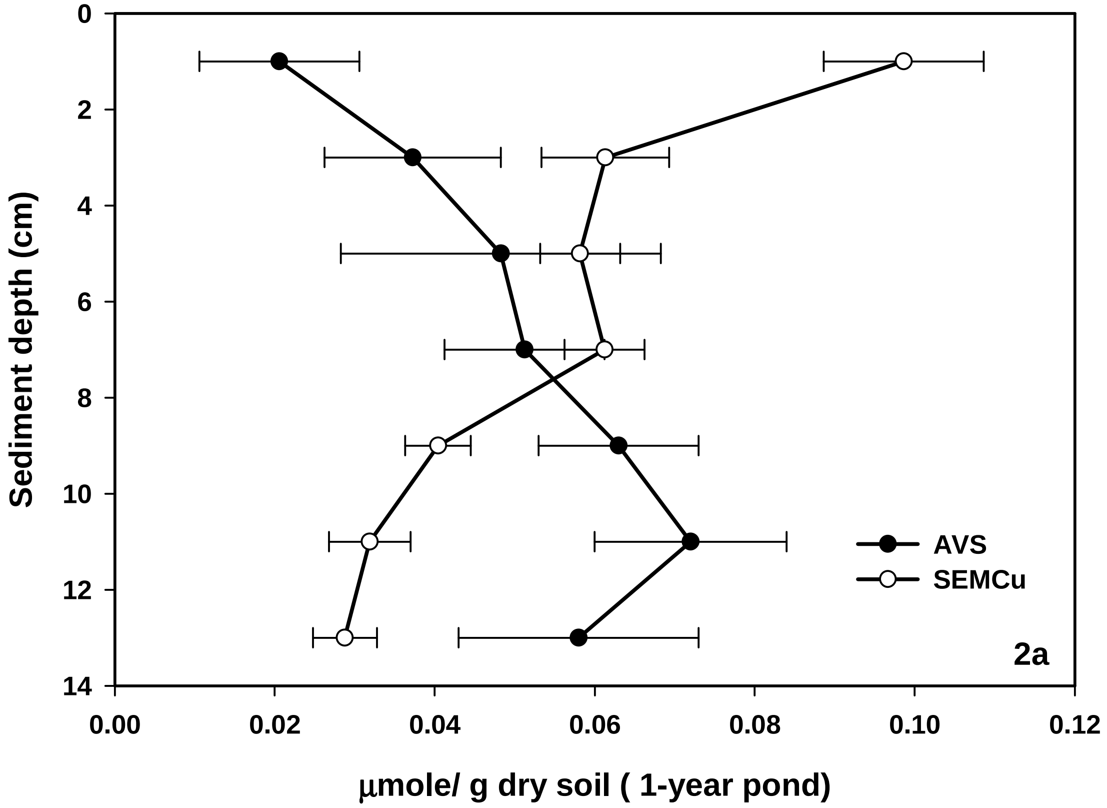


Figure 3.2a. Spatial distributions of acid volatile sulfide (AVS) and simultaneously extracted Cu (SEM_{Cu}) in the 1-year pond sediment.

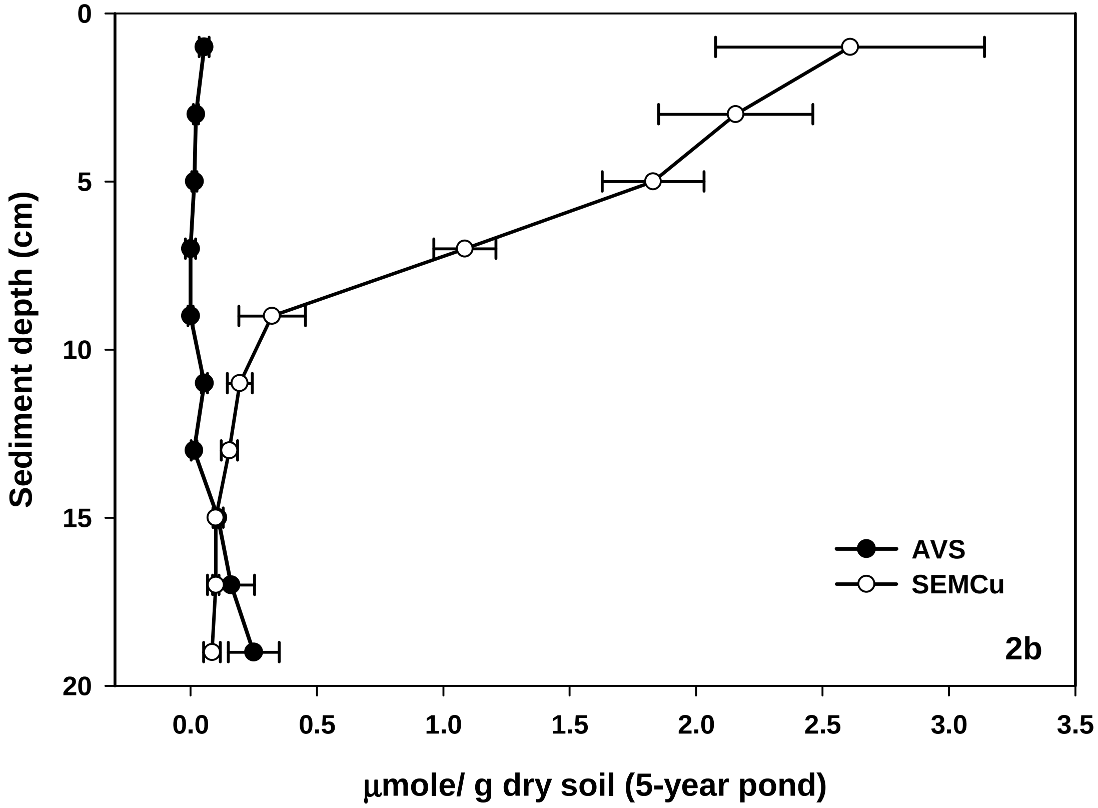


Figure 3.2b. Spatial distributions of acid volatile sulfide (AVS) and simultaneously extracted Cu (SEM_{Cu}) in the 5-year pond sediment.

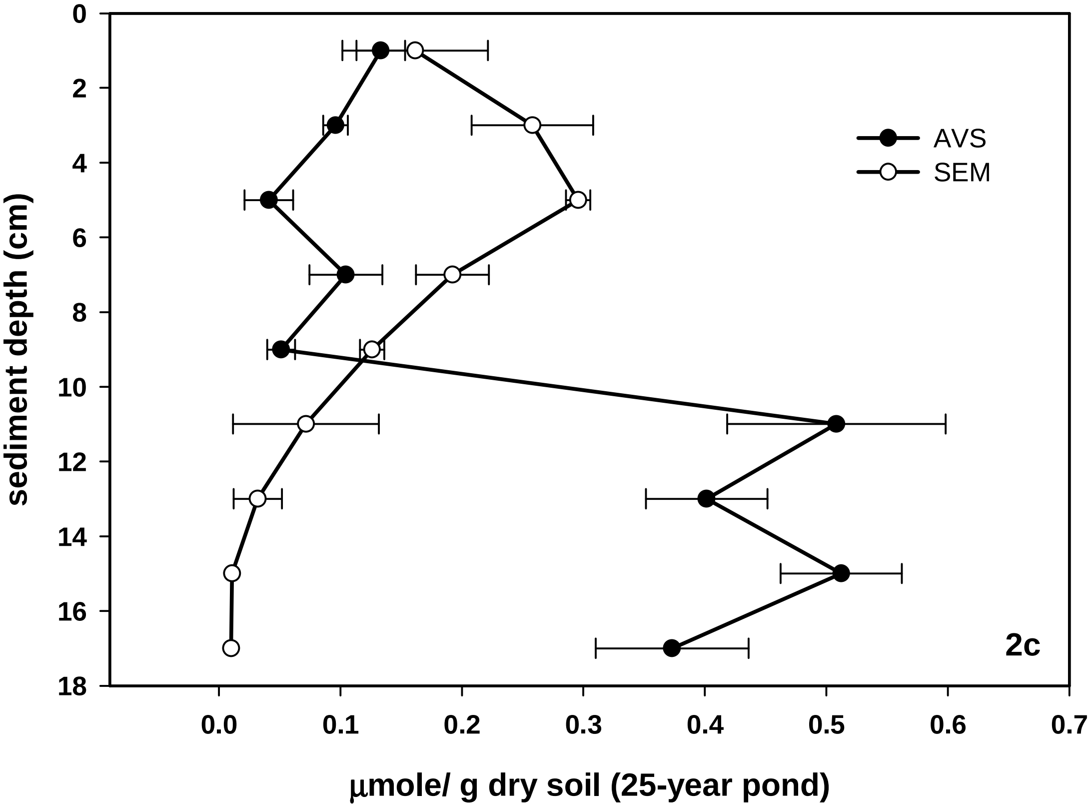


Figure 3.2c. Spatial distributions of acid volatile sulfide (AVS) and simultaneously extracted Cu (SEM_{Cu}) in the 25- year pond sediment.

Figure 3.2 displays that simultaneously extracted Cu ranged from 0.01~ 0.3 μmole/g dry soils for P-25, 0.03~0.1 μmole /g dry soil for P-1 and 0.10~2.6 μmole /g dry soil for P-5, largely depending on the total Cu concentration in the sediments. At most cases, pond sediments contained AVS / SEM_{Cu} < 1; except in deeper layers such as below 8 cm for P-1, below15 cm for P-5, and below10 cm for P-25. But the absolute concentration of SEM_{Cu} was highest in P-5.

In summary, from the redox potential viewpoint, sediments in aquaculture ponds were not so reduced as those in other fresh water bodies such as lakes, rivers, and reservoirs since the former is subject to much of human's interference. Therefore AVS concentration is expectedly much lower at the fishpond bottom than other water bodies, resulting in the molar ration of AVS to SEM_{Cu} less than 1 in most cases, which was also observed by other authors (Leonard et al., 1999; Yu et al., 2001). Those authors concluded that AVS/SEM was less than 1, implying that acid volatile sulfide was not a major binding phase for metals in the sediments. They believed that the AVS/SEM value did not support the idea that sediment metals were toxic to the benthic organisms because other binding phases such as carbonates, iron oxides and organic matter existed to reduce the toxicity of those metals.

3.2. TCLP AND PBET EXTRACTION RESULTS

Figure 3.3 showed the TCLP leachability of Cu in those three pond sediments. The leachable Cu was low with the concentration in TCLP extract about 0.0 ~0.6 mg/L and the leachable fraction only accounted for < 10% of soil-bound Cu. P-1 exhibited the lowest Cu leachability among the three ponds and showed a uniform leachability of about

2% throughout the sediment depth, still reflecting the Cu source was from the original soils. The P-25 pond sediment showed the highest leachability of 3%~9% of the Cu, suggesting the soil acidity was responsible for the higher Cu leaching. P-5 showed an intermediate leachability of 1~3%. The calcareous soil leached less Cu because the high carbonate content consumed partly the TCLP extract acidity, resulting in less hydrogen ions remaining to replace the soil-bound Cu. About 1.0 ~1.5 increase in pH value of the TCLP extract from the initial 5.00 was observed in TCLP test for P-1 sediment samples. For P-5 samples, final pHs were about 5.0~ 5.5. Accordingly, acidic soil leached more Cu due to all protons in the TCLP solution participating in Cu leaching so that the final pHs of TCLP extract were identified with or even lower than the initial value in the leaching test on P-25 samples. The P-5 pond and P-25 pond showed decreased tendency of Cu leachability with the increase of the sediment depth.

Generally speaking, the TCLP leachable Cu in those ponds sediments was lower than the Maximum Contamination Level (MCL) of Cu for the drinking water (1.3 mg/L) set by U.S.EPA (2002). This implied that the aquaculture pond sediments were environmentally safe with extremely low possibility of contaminating the surface or ground water bodies even if the Cu concentration was as high as more than 200 mg/kg and the pond had been actively managed for 5 years. The 25-year pond was difficult to evaluate because the sediment had been partly removed.

Tack et al. (1999) studied the potential leachability of heavy metals in land-disposed dredged sediment using diluted nitric acid (pH 4), finding that only 5% of the Cu in the sediment (210 mg/kg) was potentially leachable. Based on their research, the authors

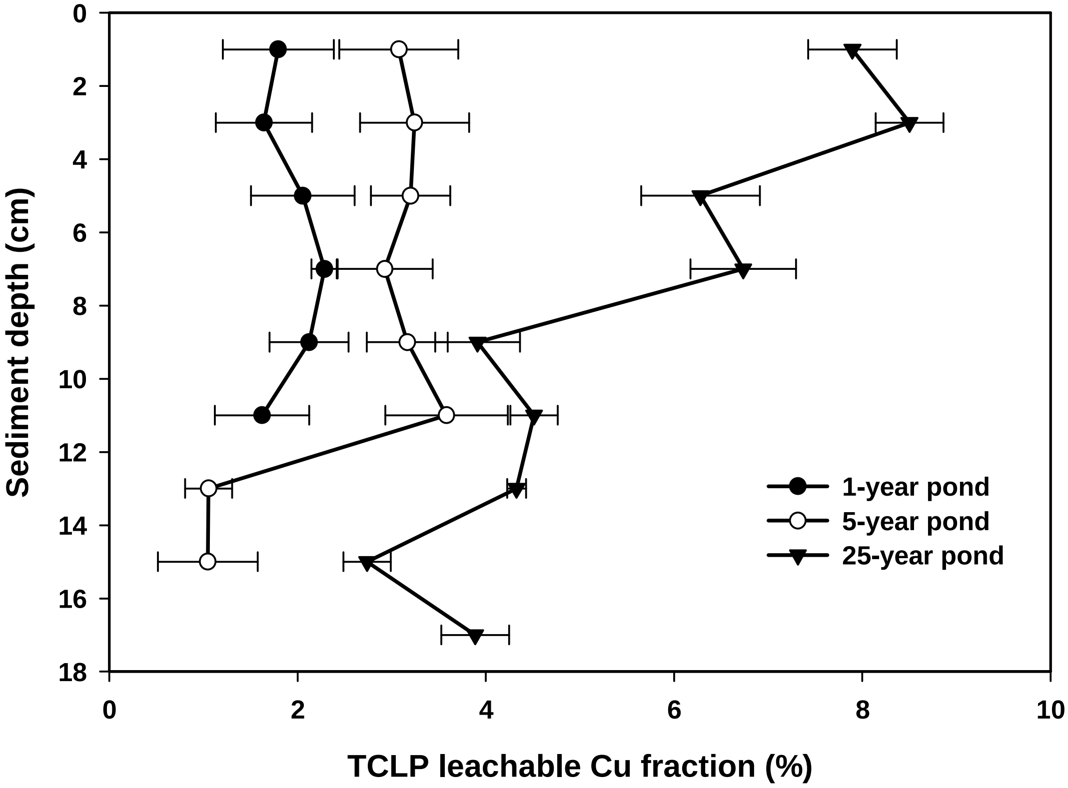


Figure 3.3. Spatial variations of the TCLP leachable Cu in the pond sediments

made a similar conclusion that contamination of the surrounding soils and waters by leachable metals (Cu and Pb) in the sediments was minimal. However, careful environmental impact assessment is still necessary before the Cu-laden sediment is disposed in the field at a large scale because besides pH change, other environment factors such as redox changes, presence of organic complex agents, microbial activity and plant growth may increase the availability of sediment-bound heavy metals as well (Förstner, 1981).

PBET test was based on the gastrial condition of higher animals such as human and cattle and the PBET leachable fraction of soil-bound chemicals were defined as bioaccessible metals in the soils by Ruby et al. (1996). This method has been applied to measure the bioaccessibility of Pb, As and Cr in soils (Ruby et al., 1996; Stewart et al., 2003; Yang et al., 2002). Experimental data (**Figure 3.4**.) indicated that a significant percentage of soil-bound Cu was released from the sediment under PBET test, resulting in the Cu concentration in the PBET solution as high as 1.7 mg/L. P-5 pond sediment contained the highest Cu and released 55~85% bound Cu with Cu strength in the extract of 0.1~1.7 mg/L, 25-year pond sediment showed the similar behaviors with 50%~65% of bound Cu bioaccessible, resulting in a maximum 0.15 mg/L Cu in the extract. The percentage of bioaccessible Cu also exhibited a decreasing tendency with the increase of the sediment depth for those two ponds. The 1-year pond contained a relative lower percentage of (38~60%) bioaccessible Cu. Those results suggested that major Cu could be leached out in humans or animal stomachs and the acute or chronic health problems may occur when the individual keeps taking in the Cu-loaded sediment. For instance, some Cu-sensitive animals such as sheep and young calves (Bremner, 1979) may be harmed by Cu when they are feeding on the ranch where the disposed pond sediment was applied as fertilizer. It was reported a long time ago that Cu poisoning occurred in sheep under natural grazing conditions in some parts of Australia, where Cu-bearing rocks have resulted in high Cu content in soils and plants (Bremner, 1979).

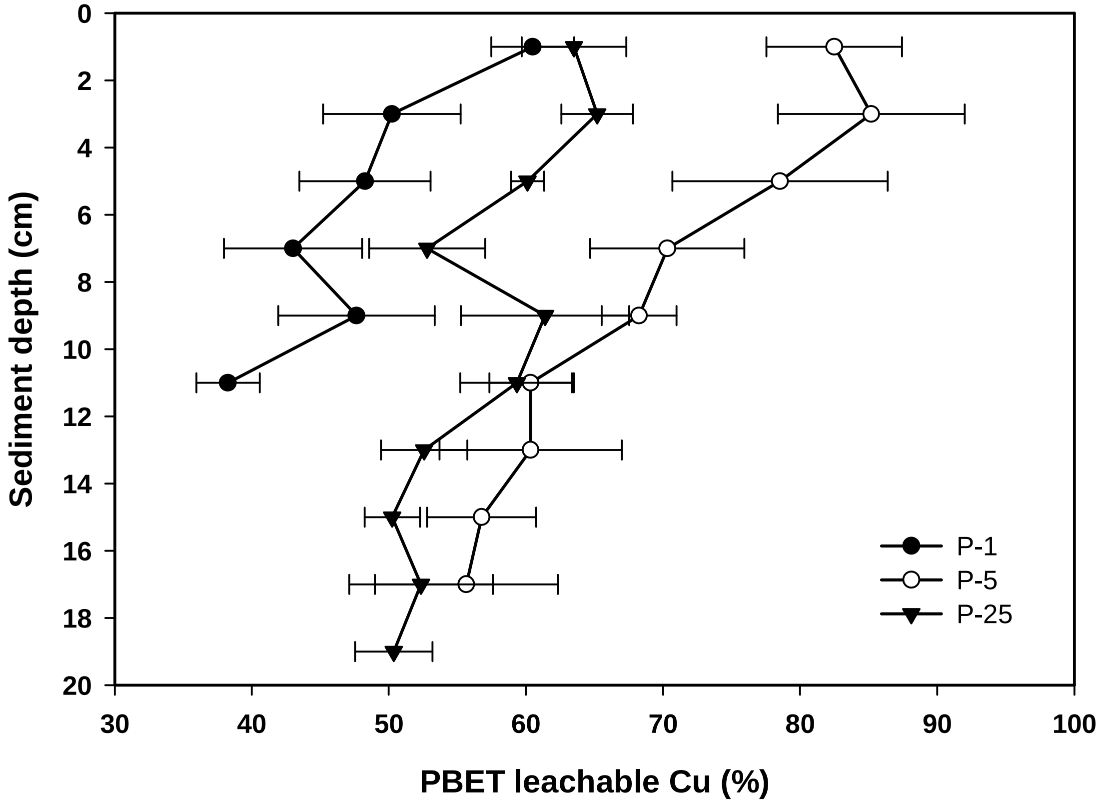


Figure 3.4. Spatial variations of bioaccessible Cu (PBET leachable) in the pond sediments.

3.3. CU SPECIATION IN THE SEDIMENT

In order to probe the relationship between Cu availability and Cu binding mode (speciation) in the sediment, sequential extraction procedures were conducted on those sediment samples. **Figure 3.5** shows the related results. In the 13-cm-deep sediment of P-1 (**Figure 3.5a**), the residual phase (RS) was observed as the major binding phase, accounting for 53~63% of total copper, and the next one was carbonate-bound fraction (CB) ranging from 15~22%, Cu bound in the Fe/Mn oxides (OX) and soil organic matter/ sulfides (OS) exhibited a similar percentage, about 7~17%; only a very small fraction (about 1~5%) of Cu was present in the water soluble/ exchangeable form (WE), implying the high affinity of Cu with soil. It is prominent that the distribution of Cu fractions exhibited an even fashion along the sediment depth in this pond, again confirming our previous observation that Cu in P-1 sediment basically originated from the native soil and thus its content and speciation did not show much spatial variation. Sequential extraction procedure was also conducted on a sample of native soil (Bt horizon) in the vicinity of P-1. The result (**Figure 3.5a**, bottom) showed that Cu speciation in the native soil was similar to that in the sediment (with high percentage of RS), further supporting the conclusion that the origin of the Cu in the P-1 was the native soil. Similar results were also found in two field sediment samples of Spain by Sáenz et al. (2003) and they also attributed the observed higher percentage (> 60%) of RS Cu to the mineralogical origin of the metal in those sampling sites.

In contrast, significant variation of each Cu fraction with sediment depth was observed in the 5-year pond (**Figure 3.5b**): Within the top 5 cm of sediment layer, the major phases were soil carbonate-bound (CB) and organics/sulfides bound phases (OS),

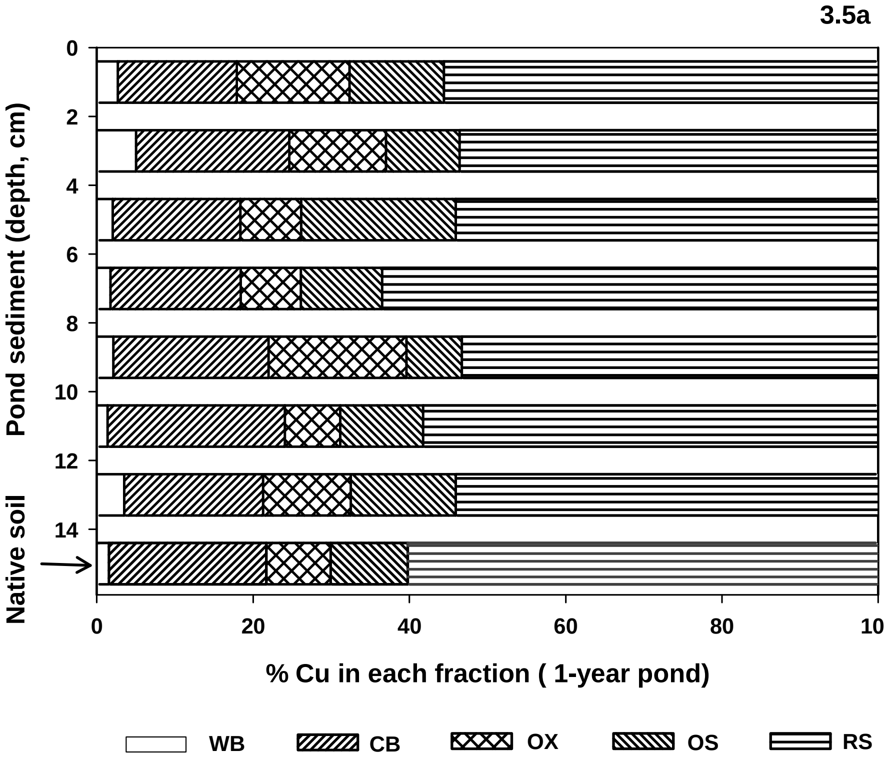


Figure 3.5a. Spatial variations of Cu speciation in the 1-year pond sediment. (WE: water soluble/ exchangeable fraction; CB: carbonate -bound fraction, OX: iron/manganese oxide - bound fraction; OS: organic matter/ sulfide bound-fraction; RS: residual fraction)

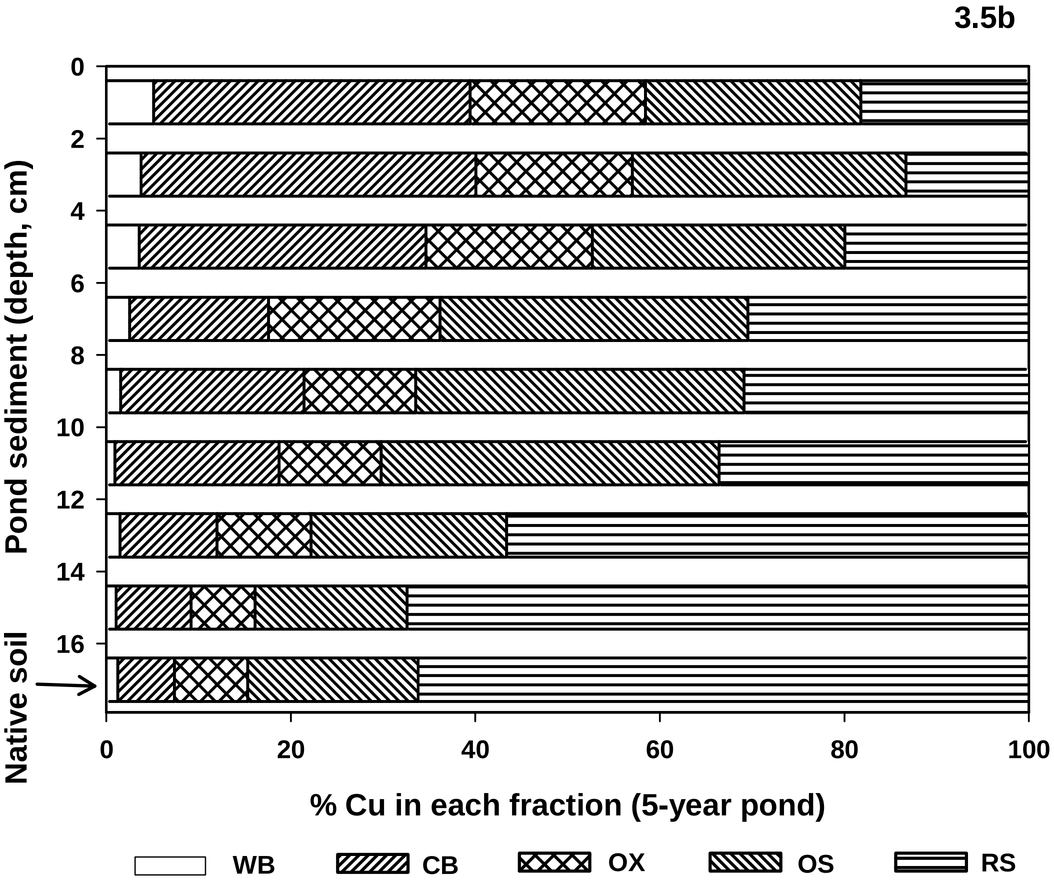


Figure 3.5b. Spatial variations of Cu speciation in the 5-year pond sediment. (WE: water soluble/ exchangeable fraction; CB: carbonate -bound fraction, OX: iron/manganese oxide - bound fraction; OS: organic matter/ sulfide bound-fraction; RS: residual fraction)

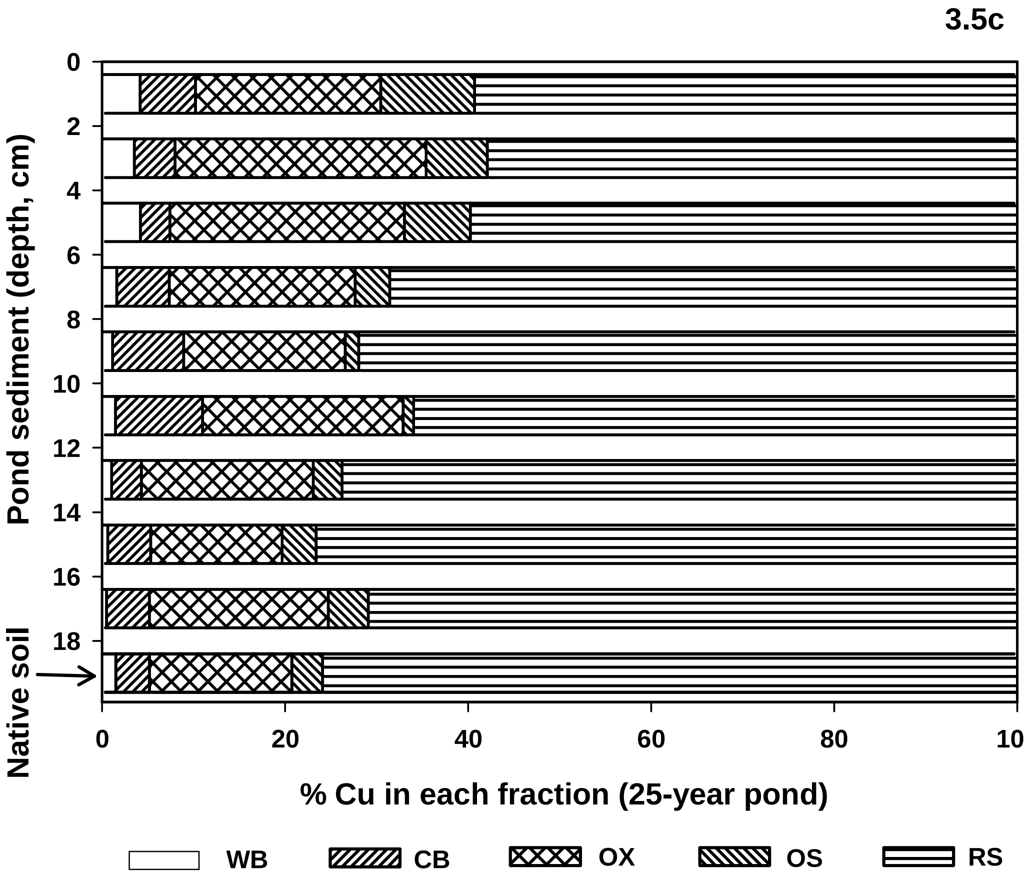


Figure 3.5c. Spatial variations of Cu speciation in the 25-year pond sediment. (WE: water soluble/ exchangeable fraction; CB: carbonate -bound fraction, OX: iron/manganese oxide - bound fraction; OS: organic matter/ sulfide bound-fraction; RS: residual fraction)

together accounting for about 60% of total Cu where 31~36% attributed to CB and 23~29% to OS. Then the residual phase shared a percentage of about 18% with the Fe/Mn oxides phase. In the deeper layer of 7-11 cm, significant decrease of CB fraction and simultaneous increase of the RS were observed where CB reduced by 50% to about 18% and the RS increased by 80% to 32%. Meanwhile a small percentage growth in the OS fraction was also found, implying the transformation of the CB to the OS and RS occurred in the deeper sediment. A reduction of OX fraction was found as well, but not until in the layer below 9 cm. The WE fraction, although not a major binding phase, also showed a lessening tendency in the shared percentage along the depth. Below 13 cm, RS became the major phase in the sediment accounting for more than 60% of total Cu, which increased by 96% from layers 5-7 cm and by 260% from the layers 1-3 cm. In summary, this 5-year-old pond, which was actively operated, showed a decreased tendency of the easily-available phases and increased the refractory phases of Cu along depth in the sediment.

As a matter of fact, in catfish pond aquaculture, Cu is applied periodically to the fishponds in soluble form as $CuSO_4•5H_2O$ and nearly 100% of added Cu was then absorbed on the sediment particles which finally settle down on the pond bottom (Liu et al., 2006). Although the downwards migration of Cu with soil particles in the sediment column may occur due to particle-facilitated transport such as mechanical mixing and bioturbation, generally speaking, Cu in the deeper layers was applied earlier than that in the surface. Therefore, the spatial change of Cu speciation in the sediment actually reflected the aging effects on the Cu speciation in the solid phase. In other words, freshly applied Cu, which accumulates on the top sediment layers, is primarily bound with the easily-available phases such as the WE, CB and OX phases while the Cu residing in the deeper sediment layers, which was applied earlier and experienced aging, bound more with the RS, suggesting a transformation of the easily-available forms of Cu to the residual forms with time because the latter is more thermodynamically stable in the natural environment. As a result, significant transformation was observed in the lower layers, i.e. with longer aging (**Figure 3.5b**).

The SEP result of the native soil near P-5 exhibited a similar distribution of Cu fractions to that in the 15-cm-deep sediment layer of P-5 (**Figure 3.5b**) with more than 60% of RS, suggesting that this layer and below was bottom soil (i.e. native soil) due to their similar Cu speciation patterns. Higher percentage of RS Cu in the native soil reflected the geological time of aging. Similarly results and conclusions also found for the relationship between bottom sediment layers (13-17 cm) in P-25 and the native soil near this pond (**Figure 3.5c**). Likewise, aging effect is also a good explanation for Cu speciation observed in P-1 sediment and the native soil (**Figure 3.5a**).

Effects of aging on the speciation and availability of heavy metals in soil/sediments has been observed and studied by many authors. For example, Fendorf et al. (2004) ascribed the bioaccessibility of the soil-bound lead, arsenic and chromium decreasing with aging to the temporal changes of binding styles of these contaminants in the soils. Another noteworthy work was done by Peng et al. (2004): they studied the bioavailability of Cd and Zn in a radiotracers (^{109}Cd and ^{56}Zn)-spiked sediment sample using digestive fluid extraction method, finding that a higher percent of spiked metals (40% for ^{109}Cd and 8% for ^{56}Zn) was extracted than the native metals (2.2% for Cd and 0% for Zn). The authors suggested that the spiked metals were bound with the more easily available fraction than the native metals and aging probably was the cause. Fan and Wang (2003) also provided evidence that fraction of Zn in the CB phase gradually decreased and increased in the OX phase in ^{56}Zn–spiked samples as sediment aging increased. Although several mechanisms have been proposed for the temporal changes of the binding modes of heavy metals in soil, sediment and other geomedia (Fendorf et al., 2004), it has been widely accepted that aging increases the binding strength and decreased the availability of the absorbates. The apparently spatial changes of Cu speciation in P-5 provided another evidence for this conclusion.

For the 25-year pond sediment, the major fractions in the entire sediment column were OX and RS accounting for 80~90% where RS about 60~70%. Such a high amount of RS fraction, according the hypothesis above, was due to the longer time aging than in the 5-year pond. The WE, CB and OS fractions were minor and each only accounted for less than 10%. Even so, the fractions also showed the shift of the active phases to the refractory ones from the top layer to the bottom layer. The RS fraction increased from 60% at the surface to 71% at 17 cm deep, while other fractions, except the CB, exhibited reduced percentages at greater sediment depths. The CB did not change much with the sediment depth. Pond management information showed that the sediment in this pond had been removed at least once, which suggested that the copper found in the sediment surface had undergone aging for a few years and thus RS was the major binding phase for Cu in this sediment.

Besides aging, Cu concentration in soil may also affect its speciation. Yu et al. (2004) reported that increase of Cu amendment levels resulted in a linear increase of the mobile fraction of Cu in two soil samples after a 7-week incubation. This conclusion may not be applicable to explain our results because Cu in each sediment sample in our research was subject to different aging time which was mostly longer than 1 year. Moreover, their results showed the residual fraction only accounted for less than 5% of total Cu regardless of the initial Cu concentrations, which suggests the aging time (50 days) was not long enough for Cu to reach equilibrium with each binding phase. Fan et al. (2002) also reported Cu speciation was affected by the total concentration through study on 14 surface marine sediment samples. They concluded that the organic fraction of Cu in the sediment increased while the OX and RS fractions decreased, with increasing total Cu concentration. Aging effect was not considered in this paper, either.

Additionally, geochemical composition was also reported affecting the speciation of metals in soils/sediments. Fan et al. (2002) found in the marine sediments that significant correlations between the organic matter content and the metal concentrations in the organic matter phase existed for Cu and Pb. In our case, however, highest carbonates content in P-1 did not result in the highest CB fraction of Cu present in this pond, suggesting that aging effects also played a significant role in the metal speciation and more research is needed to elucidate the effects of initial Cu concentration and sorption phase level on the Cu speciation with controlled aging time.

3.4. CORRELATION BETWEEN CU SPECIATION AND ITS LEACHABILITY AND BIOACCESSIBILITY

Through careful comparison of the Cu speciation profiles with those of the TCLP and PBET in three fishponds, respectively, it can be observed that TCLP leachable Cu fraction was approximately equal to (P-1 and P-5) or slightly larger than (P-25) the water soluble/exchangeable fraction of Cu in the sediment while PBET leachable fraction was equal to or slightly larger than the sum of the first 4 fractions (WE + CB+ OX + OS). This finding suggested that TCLP procedure can leach all exchangeable Cu and part of the carbonate-bound fraction and PBET can dissolve all the first 4 fractions and part of the residual fraction, implying that Cu leachability or bioaccessibility are highly correlated to the Cu speciation in the sediment. i.e. exchangeable Cu is the leachable fraction in the natural environment and all the first four fractions are bioaccessible fractions which could be dissolved in the animal stomachs. Similar results were also reported for Cd in spiked sediment that there was a significant relationship between the extractable metal and its distribution in the exchangeable phase (Fan and Wang, 2003). Marschner et al. (2006) found that the relative bioavailabilities of Pb in liver, kidney, and total uptake were highly correlated to Pb in the easily reducible Mn oxides through conducting an experiment on in vivo bioavailability of soil-bound Pb using the minipig dosing study. They concluded that reductive processes in the intestine might be more relevant for Pb bioavailability than the initial solubilization in the acidic stomach.

Moreover, the decreasing tendency of Cu leachability and bioaccessibility along the sediment depth (**Figure 3.3 and 3.4**) can be well explained by the spatial decrease of mobile phases (WE and CB) and increase of inert fractions (RS) (**Figure 3.5**) with the increase of the sediment depth in those three ponds. As discussed earlier, the Cu in deeper sediment layers experienced longer aging than that in the shallower layers, which permitted more Cu to convert from the easily available fractions to the more stable ones (**Figure 3.5**), resulting in the lower Cu leachability and bioaccessibility in the bottom sediment. Therefore, aging can effectively reduce the availability of Cu in the sediment through changing the Cu speciation.

4. Conclusions

In order to evaluate the environmental impact of Cu application in aquaculture, distribution, speciation, and availability of Cu in the sediment of three commercial catfish pond were studied using various extraction methods. Experiment data showed that AVS was not a major binding phase for sediment Cu due to deepened oxidizing layer in the pond bottom caused by the aquaculture activities, and thus resulting in the $AVS/SEM_{Cu} < 1$ in most cases.

TCLP extracted less than 10% of sediment Cu, suggesting that release of Cu in the natural environment is not significant. However, bioaccessible fraction Cu may amount up to 40~85% of Cu in the sediment, implying hazardous effects may occur if the sediment particle were taken up by domestic animals, especially Cu-sensitive species such as sheep and young calves and care should be taken in disposing of the dredged sediment.

Sequential extraction procedure provided evidence that Cu is mostly bound with residual phase for the 1- and 25- year ponds and CB, OX, OS and RS are the major species in the 5-years pond. Although aging, total Cu concentration and content of the binding phases may all have impacts on the Cu speciation, only the aging effect can successfully explain all the speciation changes with different pond sediments or with different sediment layers. Comparing the TCLP results and PBET results with sequential extraction data, it was found that TCLP leachable Cu was primarily in the water soluble/exchangeable fraction plus a small portion of carbonate-bound fraction, while except for the residual fraction, the PBET extracted nearly all the Cu species out. This information revealed leachability and bioaccessibility of soil-bound Cu was governed by the Cu speciation in the soil, and reduction in leachability of heavy metals in soil (e.g. by liming) may not result in simultaneous reduction in bioaccessibility.

CHAPTER IV

INFLUENCES OF VARIOUS SOIL FRACTIONS ON THE LEACHABILITY AND BIOACCESSIBILITY OF CU(II) IN SOILS

1. Introduction

Copper (Cu) is one of the most commonly detected heavy metals in soil and water. Anthropogenic sources of copper include applications of Cu-containing compounds such as pesticides, algaecide, or preservatives in agricultural and industrial practices, and, direct discharge of Cu-laden wastes from mining, farming, and manufacturing operations. Copper has been found in more than 906 of the 1,647 sites on the National Priority Lists (ATSDR, 2004). The accumulation of Cu in the environment threatens ecological and human health (Scheinberg, 1991).

Sorption and desorption in soils play an important role in the physical-chemical and biological availabilities of heavy metals including Cu in the natural environment. It has been well recognized that soil sorption reduces the biological availability, and thus, mitigates the associated environmental and health risks. In addition, desorption process is also extremely important in assessing toxicity of heavy metals sorbed in soils (Sparks, 1995; Scheidegger and Sparks, 1996). However, detailed information pertaining to sorption and desorption mechanisms as associated soil compositions is lacking.

It is well recognized that availabilities of heavy metals adsorbed on soils can be impacted by solution chemistries such as pH (Scheidegger and Sparks, 1996; Haughey et al., 2000). For example, it as reported that soil-sorbed metals became more mobile under the conditions of acid rain (pH = 4.0) (Park, 1987) or leachate produced in the municipal landfills (pH = 5.0) (Ghosh et al., 2004). However, despite the known heterogeneities of soils, our knowledge on the impacts of individual soil compositions remains very limited.

To measure the physical-chemical availability or leachability of soil-bound heavy metals including Cu, two standard leaching procedures are typically employed: the SPLP (Synthetic precipitation leaching procedure; USEPA Method 1312, 1994) and TCLP (Toxicity characteristic leaching procedure; USEPA Method 1311, 1992). The SPLP extraction simulates acid precipitation (rain or snow) conditions. USEPA recommended this method for estimating the potential *in situ* leachability of soil-bound metals in the field and assess the risk of groundwater contamination by contaminated soils (USEPA, 2001). The SPLP was also used to assess the potential leachability of heavy metals in the landfill environments (Townsend et al., 2004). TCLP is also a widely applied method in estimating the leachability of heavy metals and/or other contaminants in soils or solid wastes. TCLP employs an extracting fluid mimicking the conditions in municipal solid waste landfills where high levels of organic compounds are present. For copper, it was reported that TCLP may overestimate the leachability because of the high complexation ability of Cu with the organic acid of the TCLP fluid (Townsend et al., 2004). Based on studies on lead extraction from soils, Cao et al. (2005) concluded that compared to TCLP, SPLP offered better agreement with modeling predictions over a wide range of soil pH and contaminant concentrations, and is considered more realistic. Others proposed to replace TCLP with SPLP for testing leachability of contaminants in solid wastes (Cao et al., 2005, Kingham and Semenak, 2000). In this study, SPLP was used to quantify the leachability of Cu in soils.

To measure the so-called bioaccessibility of heavy metals, an *in vitro* physiologically based extraction test (PBET) method has been used (Ruby et al., 1996). The bioaccessibility is defined as the maximum fraction of the dose orally ingested that becomes available for absorption into the human body. Therefore, PBET reflects an extreme exposure scenario where Cu-contaminated soils find their way to invade into human or animal digestion systems where harsh environmental conditions (e.g. pH less than 2.0) may cause substantial desorption of sorbed metals. Different from other leaching tests such as TCLP and SPLP, PBET incorporates physiological parameters from the target species, and thus, is more suitable for toxicity assessment (Furman et al., 2006). PBET-based bioaccessibility of soil-bound lead, arsenic, and chromium has been studied by a number of researchers (Ruby et al., 1996, 1999; Yang et al., 2002, 2003, 2005; Stewart et al., 2003a, b; Fendorf et al., 2004). There has been no reported study on the bioaccessibility of soil-bound Cu.

In addition to solution chemistry, many other factors such as soil type, soil particle size, soil compositions (Furman et al., 2006; Stewart et al., 2003a, b; Yang et al., 2002; Zhang et al., 2003) can alter the leachability and bioaccessibility of soil-sorbed heavy metals. Although it is well recognized that the ubiquitous metal-sequestering properties of soils may significantly lower the bioaccessibility of heavy metals, current action levels set by state regulators concerning the bioaccessibility of heavy metals in soils do not consider effects of specific soil properties (Stewart et al., 2003a).

The overall goal of this study was to investigate the effects of different soil fractions (sand, silt and clay) and chemical compositions (soil carbonates, Fe/Mn oxides, organic matter and clay minerals) on the leachability and bioaccessibility of the soil-sorbed copper and to elucidate the sorption/desorption mechanisms. The results may facilitate prediction of availabilities of metals in soils. The specific objectives of this study were to

1. Test and compare the SPLP-leachability of Cu(II) bound to various soil fractions;
2. Evaluate the bioaccessibility of Cu(II) sorbed in various soil fractions via PBET; and
3. Identify major factors governing the leachability and bioaccessibility of Cu(II) in soils.

2. Materials and Methods

2.1 MATERIALS

All chemicals (analytical or higher grade) were purchased from Fisher Scientific Company (Pittsburgh, PA, USA). Solutions were prepared with deionized water (18 MΩ-cm) from a Milli-QTM Water System. Three top soils (up to ~25 cm) representing acidic (AS), neutral (NS) and calcareous soils (CS) were collected at three sites in western Alabama, USA, where catfish aquaculture has been widely practiced and copper sulfate has been applied as algaecide for decades. The soil samples were first air-dried and passed through a 2-mm sieve. Sieved samples were then analyzed for some salient physical and chemical properties as shown in **Table 4.1**. Soil pH was measured in 0.01 M $CaCl_2$ in a 1:1 soil: solution (g: mL) suspension (Thomas, 1996). Elemental analysis was conducted following USEPA method 3050B (USEPA, 1996) and using an inductively coupled plasma-optical emission spectrometer (Varian, Vista-MPX, Palo Alto, CA, USA). The content of sand, silt and clay was determined following the pipette method (Gee and Or, 2002). Total nitrogen and total carbon were analyzed following the Dumas method

TABLE 4.1

Salient physical and chemical properties of soils used in study

items	Acidic soil (AS)	Neutral soil (NS)	Calcareous soil (CS)
pH	4.36	6.93	7.85
[a]CEC7 (meq/100g soil)	10.77	18.17	34.85
Fe (g/kg)	8.41	30.39	11.15
Mn (g/kg)	0.57	0.374	0.161
Cu (g/kg)	0.013	0.055	0.023
OM (%)	0.6	0.9	1.2
S (%)	0.0065	0.0090	0.0208
N (%)	0.04	0.06	0.11
C (%)	0.34	0.52	0.70
$CaCO_3$ equiv. (%)	0.15	1.15	48.5
Sand (%)	69	18	15
Silt (%)	4	28	29
Clay (%)	27	54	56
$CaCO_3$ equiv. (%) in sand	0.15	1.20	47.00
$CaCO_3$ equiv. (%) in silt	0.20	1.30	49.68
$CaCO_3$ equiv. (%) in clay	0.10	3.45	55.98
[b]Clay minerals (%)	K(2.8) M(39.0) Q(39.5) S(18.7)	K(33.9) M(19.1) Q(18.8) S(28.2)	K(22.4) M(38.8) Q(21.7) S(17.1)
Mineral in sand fraction (%)	Quartz (100%)		

a. cation exchange capacity at pH 7.0

b. K= kaolinite, M=micas, Q=quartz, S= smectite with a LECO CN-2000 combustion unit (LECO Corp., Joseph, MI, USA) at 1050 °C, and organic carbon was measured using the same method except that the soil samples were first treated with 4 N H_2SO_4 to dissolve free carbonates before the dry combustion.The content of carbonates was calculated from the difference between total carbon and organic carbon. Soil sulfur was determined using a LECO SC-432 sulfur analyzer (LECO Corp., Joseph, MI, USA). Soil clay minerals were identified with the XRD method using X-ray diffraction unit (Siemens D5000 x-ray diffractometer) after the soil samples were treated following the procedure by Kunze and Dixon (1986). The content of clay minerals was estimated by the respective intensity of diffracted X-ray. The cation exchange capacity (CEC) was determined with 1 N NH_4OAc buffered at pH 7.0 following the method described in the Soil Survey Laboratory Methods Manual (USDA et al., 1996).

2.2. SOIL FRACTIONATION

To investigate the effects of soil properties on the availabilities of soil-bound Cu, soil samples were fractionated physically and chemically. **Figure 4.1** shows the schematic of the steps in fractionating the soils. The physical or size-based fractionation of each soils was carried out following the procedure used by Whittig and Allardice (1996). First, 10 g of an air-dried soil sample was added into 50 ml of DI water in a 90 ml polyethylene centrifuging tube. The slurry was then shaken overnight on a shaker at 120 oscillations per minute. Then, the slurry was centrifuged at $157\times g$ for 3.5 min (25°C). The colloids in the supernatant were collected by drying the supernatant, and defined as the clay fraction with a size of <2 μm. These procedures were repeated 6 times to achieve complete separation of the clay fraction. Then, a #270 sieve (opening, 0.053 mm) was used to separate the silt (2-53 μm) and sand (>53 μm) fractions from the slurry remaining in the centrifuge bottle. The three fractions were then air-dried and stored for subsequent adsorption/desorption tests.

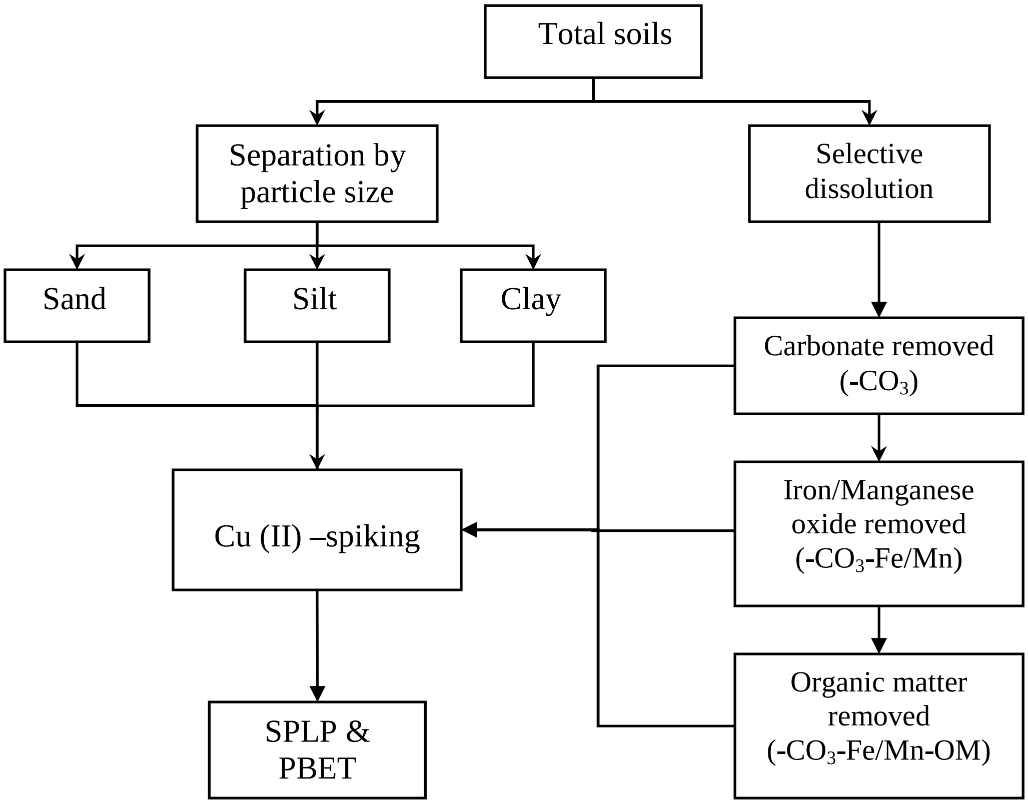


Figure 4.1. Schematic of soil fractionation.

The chemical fractionation of soils consists of a series of selective dissolution steps, each of which removes an operationally defined class of compositions including carbonates, Fe and/or Mn oxides, and soil organic matter (SOM). Soil carbonates in the soils were removed using 1 M NaOAc at pH 5.0 and at a soil-to-solution ratio of 1 g to 40 mL for a period of 5 h under continuous agitation (Tessier et al., 1979). For the calcareous soil, the treatment was repeated five times because of its much higher content of carbonates. After the treatment, the slurry was centrifuged at 3000 *g* and the supernatant decanted. The residual soils were then washed three times with 0.01 M $CaCl_2$, and then air-dried for subsequent sorption/desorption experiments or for further dissolution treatments.

Samples of the de-carbonated soils were further treated with 0.04 M $NH_2OH \cdot HCl$ in 25% (w/w) HOAc to remove Fe and Mn oxides at a soil to solution ratio of 1 g to 20 mL (Tessier, et al., 1979). For each soil, the soil-solution mixture was digested for 6 hours in a hot water bath kept at 96±3°C with intermittent mixing. Upon cooling, the mixture was centrifuged at 3000 *g* and the supernatant discarded. The treated soil was then washed 3 times with 0.01 M $CaCl_2$ and air-dried for subsequent experimental uses. While the treatment was proven to be effective for extracting Mn from soils (Shuman, 1982) and for amorphous Fe oxides (Frampton and Reisenauer, 1978), it was found to be less effective for extracting crystal Fe oxides or Fe associated with clay minerals (Hettiarachchi et al., 2003).

Portions of the above treated soils were further treated with sodium hypochlorite (NaOCl) to remove SOM (Shuman, 1983). One of the advantages of using NaOCl instead of other oxidizing agents such as H_2O_2 is that NaOCl can not only effectively remove

SOM, but also cause minimal destruction to the soil inorganic phases (Anderson, 1963). Each soil sample was mixed with 0.7 M NaOCl (adjusted to pH 8.5 with HCl) at a soil-to-solution ratio of 1 g to 20 mL. The mixtures were then digested at 90±3 °C in a water bath for 2 h with intermittent mixing. Upon cooling, each mixture was centrifuged at 3,000 *g* and the supernatant removed. The residual soils were washed twice with 30 mL (each) 0.01 M $CaCl_2$ to remove the residual NaOCl in the sample, and then air-dried for subsequent experimental uses.

For typographical convenience, the following notations are used for denoting the soil fractions in this work:

Total Soil = untreated original soil;

Soil-CO_3 = soil with carbonates removed;

Soil-CO_3-Fe/Mn = soil with carbonates and Fe/Mn oxides removed; and

Soil-CO_3-Fe/Mn-SOM = soil with carbonates, Fe/Mn oxides and SOM removed.

2.3. LOADING CU(II) TO SOILS

Cu(II) solutions with various concentrations (5 to 30 mg L^{-1}) were prepared in 10^{-3} M $CaCl_2$ solution at an initial pH of 6.0±0.1. Various soil samples or fractions were added to the Cu(II) solutions separately at a soil-to-solution ratio of 1 g/L in a set of 50-mL glass vials with Teflon-lined screw caps. The mixtures were shaken and equilibrated for ~4 days at 25 ± 2 °C, and then centrifuged for 10 min at 3000 *g*. The supernatant was filtered using a 0.45-μm membrane filter, and the copper concentration (C_e) in the filtrate was analyzed using an atomic absorption spectrophotometer. The amount of copper adsorbed

(Q) was calculated from the difference between the initial and final solution concentration. All the experiments were performed in triplicates to assure data quality.

2.4. COPPER LEACHABILITY AND BIOACCESSIBILITY TESTS

To test the leachability and bioaccessibility of copper in various soil fractions, copper leaching tests were carried out following the SPLP (USEPA Method 1312) and the PBET procedure, respectively. The SPLP extraction fluid was prepared by adding a mixture containing 60% (w/w) of sulfuric acid and 40% (w/w) of nitric acid to DI water until the pH was 4.20 ± 0.05. The same method was employed by USEPA (1994) to determine the leachability of Cu(II) in soils from a site in western Alabama. Typically, the leaching test was initiated by adding 0.5 g of a Cu-loaded soil sample into 10 mL of the extraction fluid in a centrifuging tube. Then, the slurry was mixed on an end-to-end rotator operated at 30 rpm for ~20 h, and then centrifuged at 3000 *g* for ~10 min. The supernatant was then removed, filtered using 0.45-μm membrane filters, and analyzed for Cu(II). The percentage of Cu leached was then calculated with eqn (1),

$$\% \; Cu \; leached \; by \; SPLP = \frac{(\, Cu \; Concentration \; in \; extract, C_e, mg \; L^{-1} \,)(\, 0.01L \,)}{(\, Initial \; Cu \; Concentration \; in \; soil, Q, mg \; g^{-1} \,)(\, 0.5g \,)} \times 100\% \tag{1}$$

In addition, a more biochemically oriented method, i.e. PBET, was employed to evaluate the in vitro bioaccessibility of soil-sorbed Cu(II). This method employs an extraction solution of glycine with pH 1.5 to mimic the conditions in the gastric system and small intestines. PBET was conducted following the procedures described by Kelley et al. (2002). First, 0.1 g of air-dried soil sample was mixed with 10 mL of the extracting liquid in 15 mL high-density polyethylene vials placed on an end-to-end rotator in an incubator at 37 ± 2°C. After 1 h, the vials were centrifuged, supernatant filtered with 0.45-μm membrane filter, and analyzed for Cu(II) with an atomic absorption spectrophotometer. The bioaccessibility of Cu(II) was then calculated by:

$$Bioaccessibility\,(\%) = \frac{(\,Cu\ Concentration\ in\ extract, C_e, mg\ L^{-1}\,)(\,0.01L\,)}{(\,Initial\ Cu\ Concentration\ in\ soil, Q, mg\ g^{-1}\,)(\,0.1g\,)} \times 100 \tag{2}$$

All leachability and bioaccessibility tests were performed in triplicates to ensure data quality.

3. Results and Discussions

3.1. SPLP LEACHABILITY OF COPPER IN VARIOUS SOILS OR SOIL FRACTIONS

Figures 4.2 and **4.3** show that there exists a linear correlation between the Cu concentration in the SPLP fluid and the initial concentration of Cu in the soils. McBride et al. (2000) also observed that Cu distribution between an extractant (0.01M KNO_3) and agricultural soils that were subjected to sewage sludge application followed a linear relationship. Table II summarizes the results of the linear regressions. These correlation equations may facilitate estimation of the relative leachability of Cu(II) (in percentage of Cu leached) for a given soil. USEPA (2001) recommended applying SPLP results to estimate the concentration of the contaminants in the groundwater. Therefore, the correlation equations may provide a simple means for estimating the possible Cu levels in the affected groundwater.

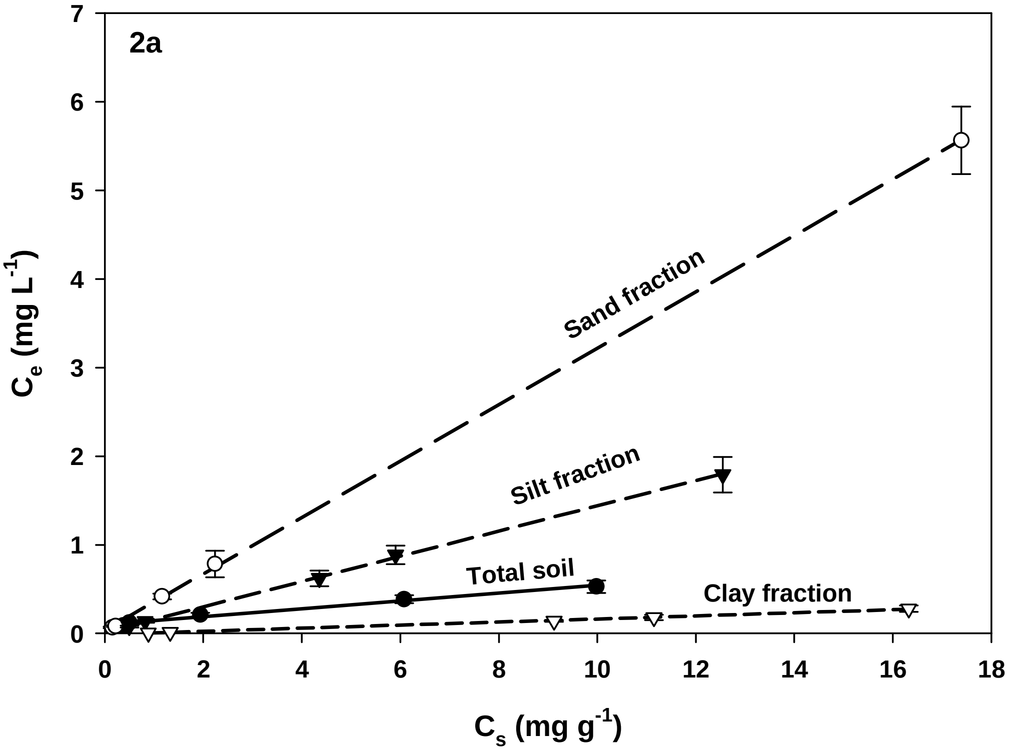


Figure 4.2a. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in calcareous soil or its sub-soils fractionated on particle size (sand, silt and clay).

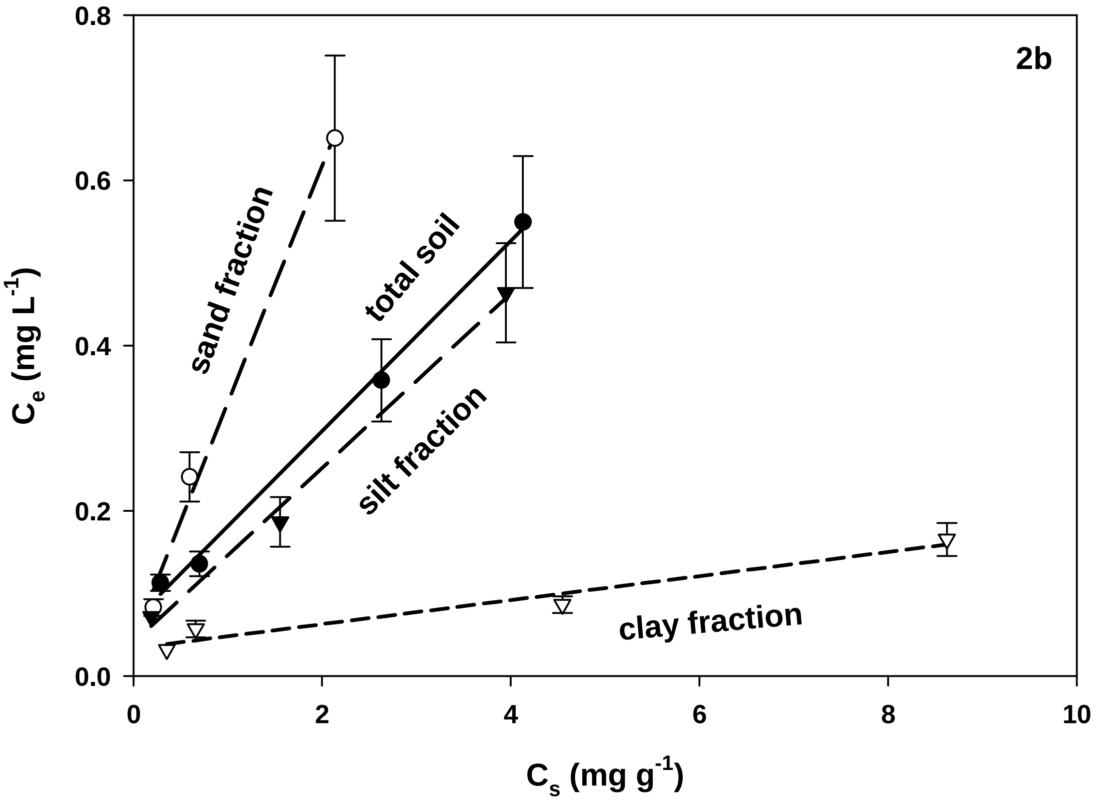


Figure 4.2b. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in neutral soil or its sub-soils fractionated on particle size (sand, silt and clay).

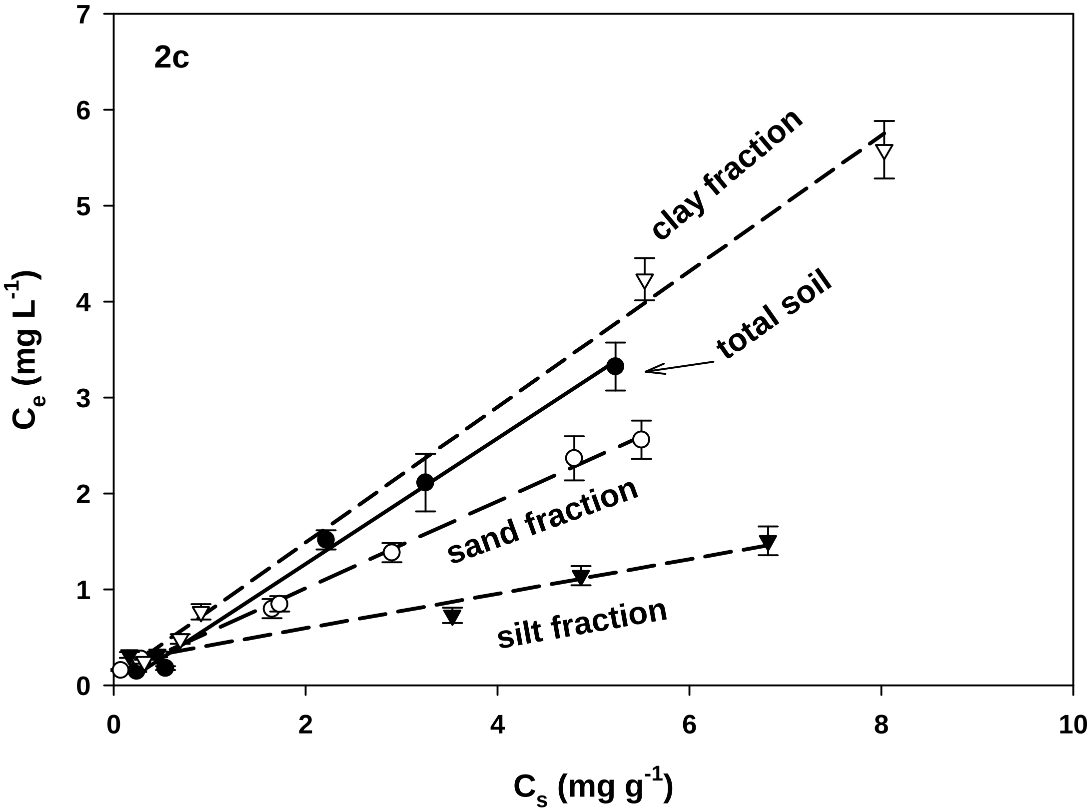


Figure 4.2c. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in acidic soil or its sub-soils fractionated on particle size (sand, silt and clay).

TABLE 4.2

Correlating SPLP-leachable Cu concentration with total Cu in soils

Soils	Fractions	Lineal regression result	% Cu leached at 1mg g^{-1} Cu in soil
Neutral soil (NS)	Total	C_e=0.12Q+0.07, R^2=0.996	0.36
	Sand	C_e=0.29Q+0.04,R^2=0.993	0.66
	Silt	C_e=0.11Q+0.04, R^2=0.994	0.29
	Clay	C_e=0.01Q+0.03, R^2=0.955	0.10
	-CO_3	C_e=0.15Q+0.09, R^2=0.993	0.48
	-Fe/Mn - CO_3	C_e=0.49Q-0.07, R^2=0.952	0.85
	-Fe/Mn - CO_3-OM	C_e=0.11Q-0.07, R^2=0.934	0.08
Acidic soil (AS)	Total	C_e=0.65Q-0.04, R^2=0.995	1.22
	Sand	C_e=0.45Q+0.10, R^2=0.997	1.11
	Silt	C_e=0.18Q+0.2, R^2=0.977	0.83
	Clay	C_e=0.71Q+0.07, R^2=0.996	1.56
	-CO_3	C_e=0.72Q-0.21, R^2=0.975	1.01
	-Fe/Mn - CO_3	C_e=0.72Q+0.08, R^2=0.998	1.60
	-Fe/Mn - CO_3-OM	C_e=0.01Q+0.01, R^2=0.902	0.03
Calcareous soil (CS)	Total	C_e=0.04Q+0.10, R^2=0.986	0.29
	Sand	C_e=0.32Q+0.04, R^2=0.999	0.71
	Silt	C_e=0.14Q+0.02, R^2=0.999	0.31
	Clay	C_e=0.02Q-0.01, R^2=0.998	0.01
	-CO_3	C_e=0.20Q+0.18, R^2=0.995	0.76
	-Fe/Mn - CO_3	C_e=0.13Q+0.09, R^2=0.971	0.44
	-Fe/Mn - CO_3-OM	C_e=0.04Q+0.09, R^2=0.916	0.25

Table 4.2 shows that the slopes of the correlation equations for total soils are in an order of CS (0.04) <NS (0.12) < AS (0.65), suggesting that the CS and NS leached less Cu(II) than the AS. Calculation based on the 1.0 mg g^{-1} initial soil Cu (Q) indicated that 1.12% of Cu leached from the AS under the SPLP, 0.36% and 0.29 % from the NS and CS, respectively (**Table 4.2**). Those numbers agree with observations reported by others that Cu had high affinity with soil and desorption from soil under natural environment was minor (e.g. Hogg, et. al., 1993; Itami and Yanai, 2006). The CS and NS leached less Cu(II) than the AS because of their relatively higher pH , which inhibited the Cu(II) leaching power of the SPLP liquid.

The calcareous soil is characterized with high alkalinity and high carbonates content (primarily calcium carbonate) (Deng and Dixon, 2002). Cu can be chem-sorbed to the soil in the form of hydroxycarbonate ($Cu_2(OH)_2CO_3$, malachite) and/or through formation of a surface-solid solution of $Cu_xCa_{1-x}CO_3$ on the surface of calcium carbonate (Papadopoulos and Rowell, 1989; Madrid and Diaz-Barrientos, 1992). A recent study (Lee et al., 2005) revealed that surface-bound humic acids had little influence on Cu(II) uptake by calcite, suggesting that surface-bound organic matter played a less important role in sorption of Cu(II) when calcite is present. In contrast, for soils of low carbonates content (e.g. the NS and AS), iron/manganese oxides, organic matter and clay minerals play a more profound role in metals uptake (Förstner, 1981). In addition, the pH buffering capacity of NS and AS is also much lower than that of CS. As a result, the Cu leachability for NS and AS is greater than for CS. Due to the different pH buffering capacity for the three soils, the final pH of the soil-SPLP solution mixtures was 7.36 ± 0.10 for CS, 6.23± 0.16 for NS and 4.05±0.23 for AS.

Regarding the particle size effect (**Figure 4.2**), the Cu leachability follows the order of Sand > Silt > Clay for NS and CS. For AS, however, the leachability order was changed to: clay > sand> silt. Although there has been no report on the effects of soil particle size on the SPLP leachability of Cu, researchers have observed that there exists a certain correlation between soil-bound Cu extractable by weak extracting solutions and the particle size. For example, through examining the water-soluble heavy metals including Cu in a sandy soil, Zhang et al. (2003) found that silt and clay fractions released 2~4 times less water soluble Cu than sand fraction. Similar results were also observed for Cd, Co, Cr, Pb and Zn. In addition, others (Yu and Klarup, 1994) also reported that for AS the leachability in the clay and silt fractions was greater than that in the sand fraction. However, the ultimate mechanism governing the SPLP leachability of Cu appears to be the carbonates content in each fraction (**Table 1**). Evidently, soil fractions containing higher carbonates showed a lower SPLP leachability for all cases regardless of the particle size. This observation suggested that chemical composition especially carbonates content rather than particle size is the key factor governing in the SPLP leachability of Cu.

For the de-carbonated fraction and the original soil (**Figure 4.3)**, the leachability change was much less profound for the NS compared to that for CS, and almost intact for AS. This observation again reveals the strong correlation between soil carbonate content and the SPLP leachability of Cu. Because of the different carbonates content, the removal of carbonates resulted in the greatest change in Cu leachability for CS, and least for AS. Rodriguez-Rubio et al. (2003) compared the desorption rate of Cu bound in a calcareous

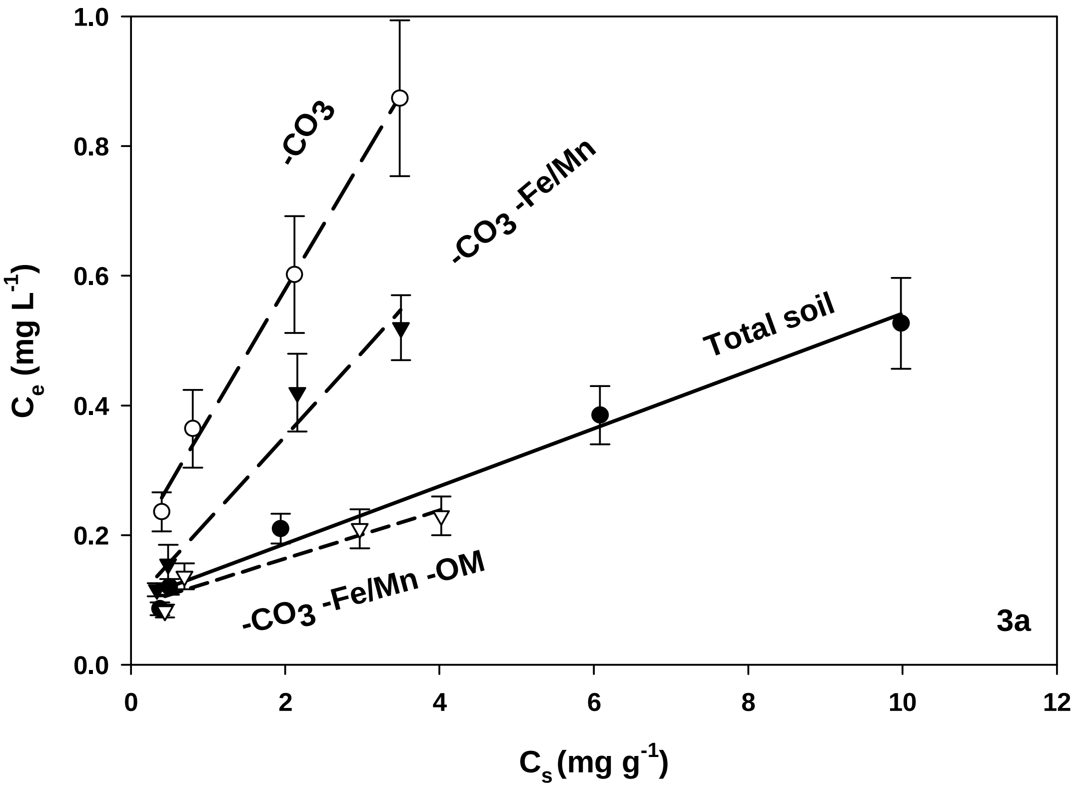


Figure 4.3a. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in calcareous soil or its chemically fractionated sub-soils.

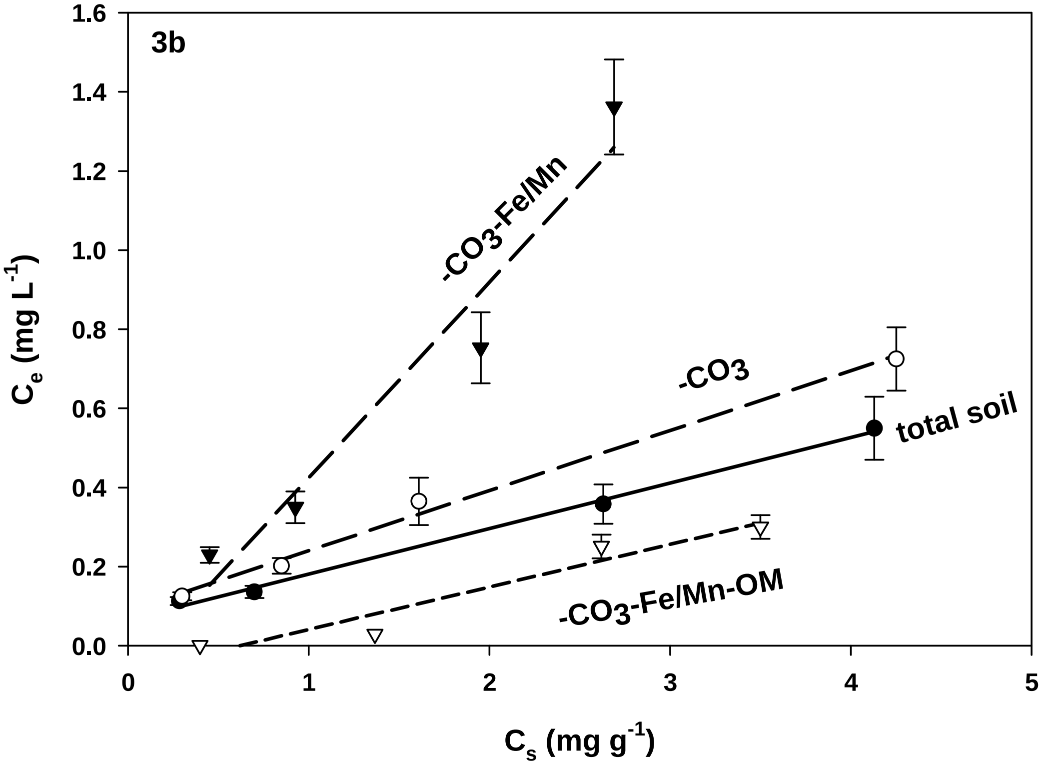


Figure 4.3b. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in neutral soil or its chemically fractionated sub-soils.

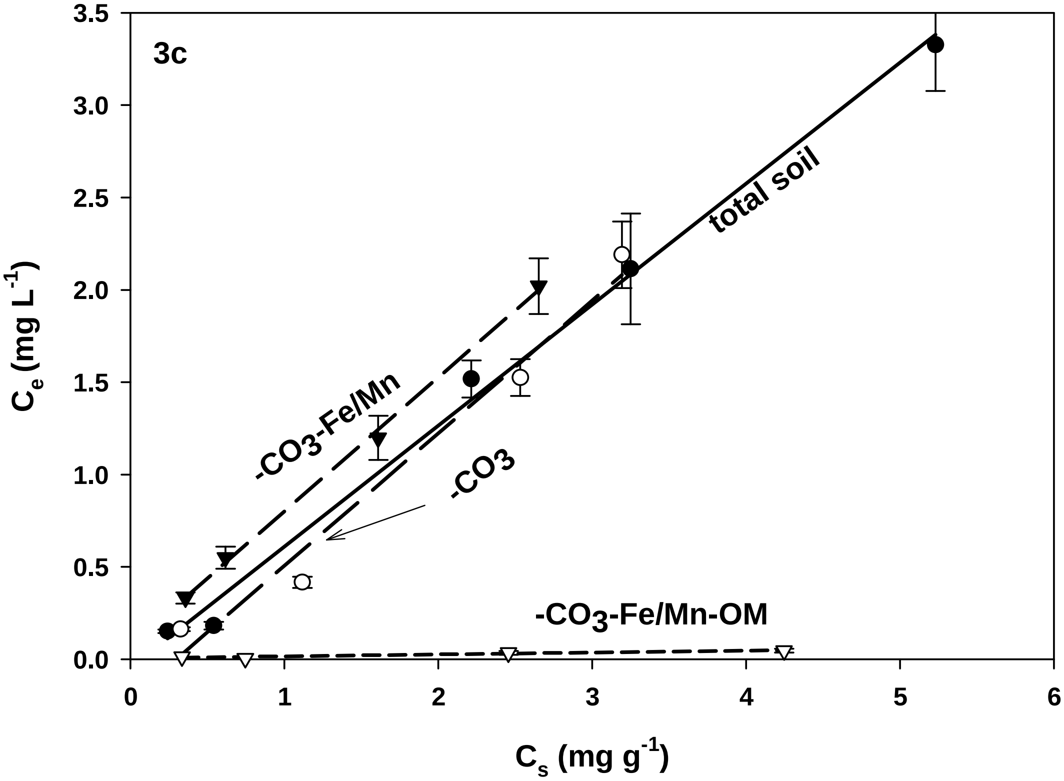


Figure 4.3c. SPLP-leachable Cu concentration (C_e) as a function of total copper initially loaded in acidic soil or its chemically fractionated sub-soils.

soil before and after the soil carbonates were removed and found that the equilibrium concentration of the Cu in the solution phase (0.01 M Ca^{2+}) dramatically increased from less than 0.1 mg L^{-1} to 6.8~9.7 mg L^{-1} after the removal. Madrid and Diaz-Barrientos (1992) reported that removal of carbonates caused a strong decrease in retention of Cu,Zn and Cd by various calcareous soils, but the treatment had little effect on the metal uptake for soils with lower carbonates content.

For non-calcareous soils or decarbonated fractions such as AS, NS, CS-CO_3, NS-CO_3 and AS-CO_3, the fractions of iron/manganese oxides and soil organic matter play important roles in heavy metals retention. As a result, the SPLP leachability of Cu was all greater for these soils compared to the calcareous soil. However, in all cases, the SPLP leachable Cu accounted for only less than 2% of total Cu initially in the soils, indicating that iron/manganese oxides and soil organic matter also offered a strong affinity towards Cu.

After the fraction of Fe/Mn oxides was further removed, the Cu leachability was increased for the fractions of NS-CO_3-Fe/Mn and AS NS-CO_3-Fe/Mn, indicating the important role of Fe/Mn oxides for sorption of Cu when high concentrations of carbonates are absent (**Figures 4.3b** and **4.3c**). However, for CS, the Cu leachability decreased upon removal of the carbonates and iron/manganese fractions. This difference suggests that the Fe/Mn fraction plays different roles in sorption of Cu for the three different classes of soils. Nonetheless, for all the three 3 soils, in the Cu leachability were reduced after soil organics were subsequently removed, the residual soil minerals offered the greatest affinity for Cu. This observation also indicates that Cu is preferably retained by soil fractions such as carbonates and Fe/Mn oxides that are more easily accessible for metals sorption but may not necessarily offer the strongest affinity. In other words, the leachability or availability of Cu is controlled by the accessibility of the sorption sites and by sorption kinetics rather then thermodynamics. However, for more aged contaminated soils, sorbed Cu ions may be transferred from relatively weaker sites to the interior stronger sites, resulting in much reduced leachability. The preferable uptake of metals by carbonates was also reported by Lee et al. (2005).

Similar phenomenon was also observed by other investigators. For example, Hinz and Selim (1999) researched sorption-desorption kinetics of Zn and observed that the removal of organic matter and iron oxides resulted in quadrupling Zn retention compared with the untreated soil. Researchers claimed that the removal of organic matter and iron coating on the clay mineral renders more reactive sites of minerals available, resulting in increased sorption capacity and affinity (Ghabru et al., 1990, Hinz and Selim, 1999, Young and MacDonald, 1998).

Kaolinite, micas, quartz and smectite comprised the major clay mineral fraction of the three soils used (**Table 4.1**). These minerals serve as the key sorbents for binding with Cu after other soil components were removed. Although kaolinite, micas and quartz were widely known as minerals with relatively low cation exchange capacity, they can offer great affinity towards heavy metals especially Cu ions, for which sorption process involves both ion pairing and specific interactions such as inner sphere complexation. According to a study by Itami and Yanai (2006), kaolinite offered a similar Cu adsorption capacity compared with montmorillonite at pH 6.0; however, the Cu desorption rate for kaolinite was much slower. Benjamin and Leckie (1980) demonstrated that at pH > 7.0 and at an initial Cu concentration of 3.1 mg L^{-1}, Cu in solution can be effectively precipitated on α-SiO_2 as Cu hydroxides. Spectroscopic studies of metal ion uptake on quartz and amorphous silica suggested that metal ions interact strongly with oxygen atoms of the Si tetrahedral through innersphere complexation (O'Day et al., 1996). Evidently, the clay minerals may play important roles in Cu sorption and leachability when they are exposed for Cu binding. However, the specific compositions of clay minerals may vary with different source soils (**Table 4.1**). As shown in **Figure 4.3**, the residual fraction of AS showed the greatest affinity compared to the other two soils, which may be attributed to the much greater quartz content for AS (**Table 4.1**).

3.2. PBET BIOACCESSIBILITY OF COPPER IN VARIOUS SOIL FRACTIONS

3.2.1. Cu bioaccessibility and initial Cu concentration

According to the definition given by Ruby et al. (1999), the oral bioaccessibility of soil-bound Cu is the fraction desorbed from soil matrix at pH about 1.5 simulating in vitro gastric functions and having the potential to cross the intestinal wall (Hamel et al., 1998).

Experimental data in **Figure 4.4** reveal that that the bioaccessible Cu (C_e) was linearly related to the total Cu (Q) for all three soils. Yang et al. (2003) also observed similar relationship between the bioaccessible Pb and the total Pb concentration in soils, with a constant ~80% bioaccessibility independent of the total Pb concentration. Stewart et al. (2003b) observed that the bioaccessibility of Cr(III) and Cr(V) was barely affect by their total solid phase concentrations.

Table 4.3 summarizes the best-fitted linear equations that correlate the bioaccessible Cu (C_e) and total Cu content (Q) in the form of $C_e = aQ + b$ (a, b: constants). Accordingly, the Cu bioaccessibility (C_e/Q) can be expressed as $a + b/Q$, which implies soil Cu bioaccessibility may be regarded as a constant (a) and being independent of the total Cu concentration only when the term b/Q is negligible i.e., when the initial total concentration (Q) is high enough.

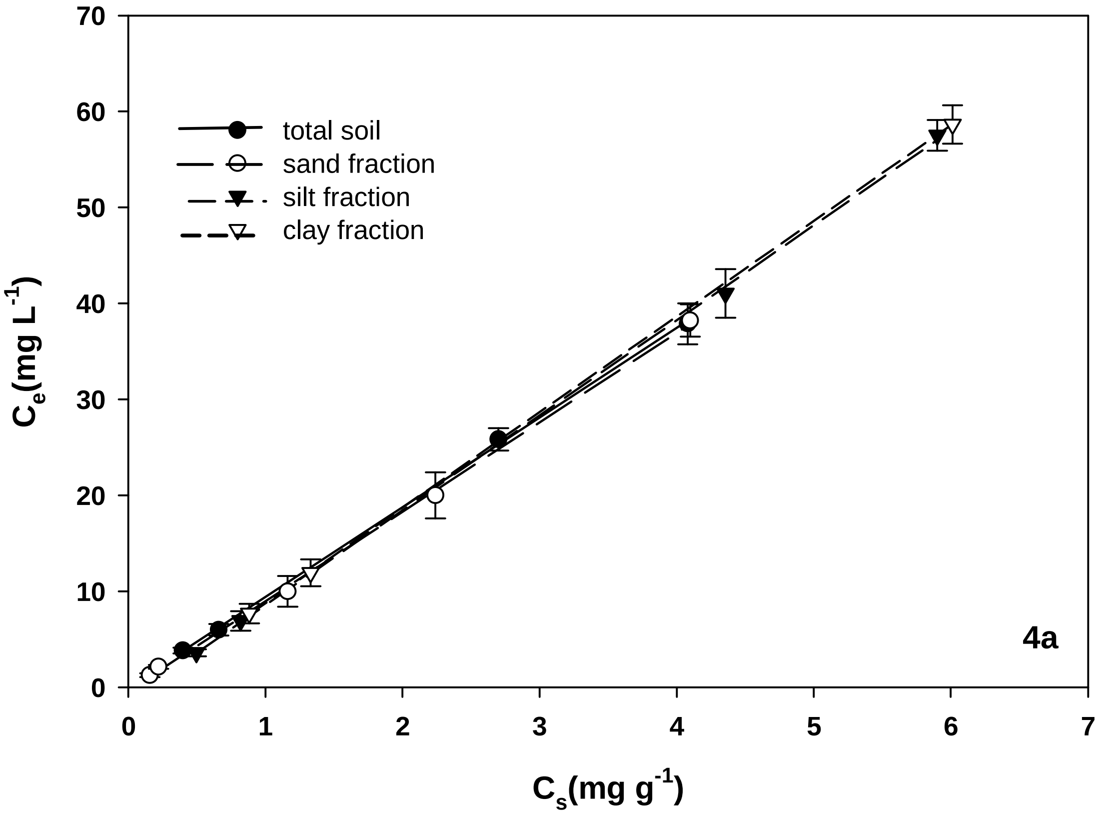


Figure 4.4a. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in calcareous soil or its sub-soils fractionated on particle size.

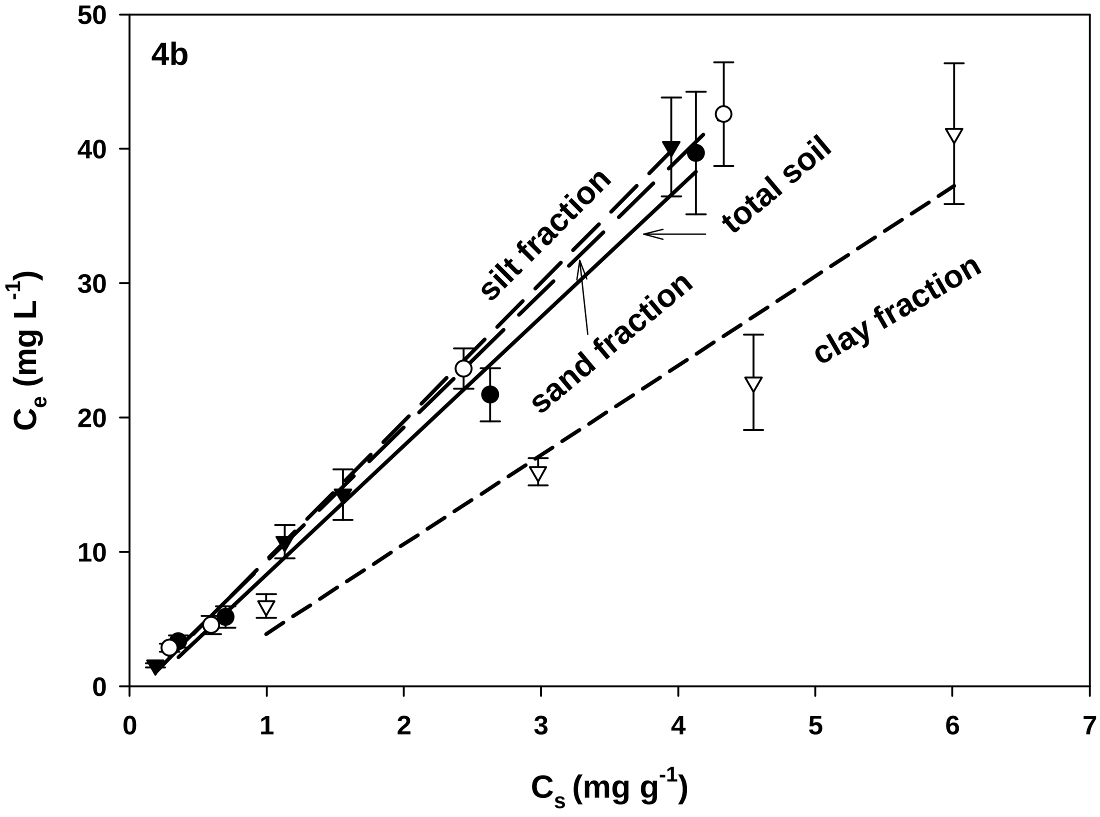


Figure 4.4b. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in neutral soil or its sub-soils fractionated on particle size.

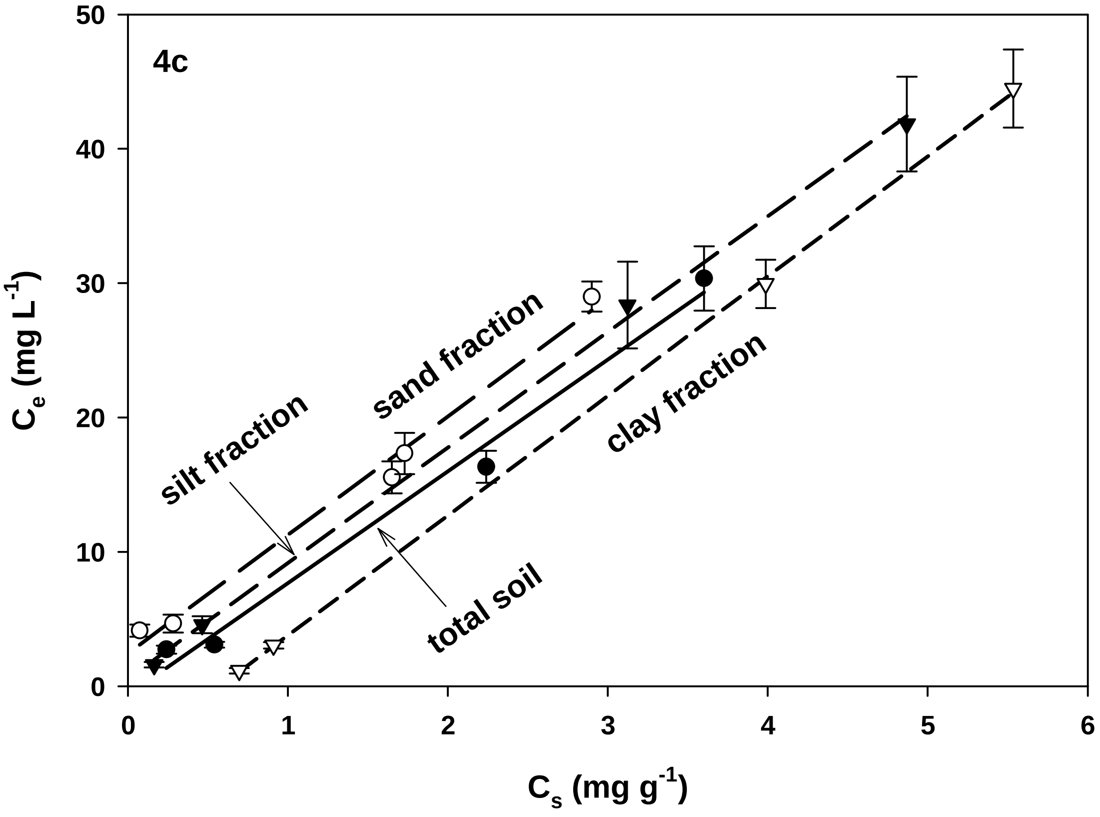


Figure 4.4c. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in acidic soil or its sub-soils fractionated on particle size.

TABLE 4.3

Correlating PBET-leachable Cu concentration with total Cu in soils

Soils	Fractions	Lineal regression result	% Cu leached at 1 mg g^{-1} Cu in soil
Neutral soil (NS)	Total	C_e=9.57Q-1.25, R^2=0.990	83.30
	Sand	C_e=9.97Q-0.68, R^2=0.999	92.94
	Silt	C_e=10.32Q-0.92, R^2=0.999	93.95
	Clay	C_e=6.65Q-2.75, R^2=0.955	39.04
	-CO_3	C_e=6.87Q-0.65, R^2=0.995	62.21
	-Fe/Mn - CO_3	C_e=8.66Q-1.41, R^2=0.9906	72.503
	-Fe/Mn - CO_3- OM	C_e=6.87Q-2.42, R^2=0.9805	44.46
Acidic soil (AS)	Total	C_e=8.31Q-0.62,R^2=0.9960	76.87
	Sand	C_e=8.80Q+2.46, R^2=0.9897	100.00
	Silt	C_e=8.60Q+0.57, R^2=0.9990	91.64
	Clay	C_e=8.91Q-5.12, R^2=0.9998	37.85
	-CO_3	C_e=8.10Q-1.44, R=0.9909	66.61
	-Fe/Mn - CO_3	C_e=9.99Q-0.73, R^2=0.9991	92.63
	-Fe/Mn- CO_3- OM	C_e=2.43Q +0.56, R^2=0.9714	29.93
Calcareous soil (CS)	Total		
	Sand	C_e=9.79Q+0.12, R^2=0.9998	100.00
	Silt		
	Clay		
	-CO_3	C_e=7.98Q+0.05, R^2=0.9995	80.28
	-Fe/Mn - CO_3	C=8.34Q, R^2=0.9999	83.37
	-Fe/Mn - CO_3- OM	C_e=7.16Q+0.14, R^2=0.9961	72.99

Results from **Table 4.3** indicate that the bioaccessibility of Cu is in general dependent on the initial total Cu concentration in the soils although the dependence lessens as the total Cu concentration increases.

3.2.2. Effects of soil compositions on Cu bioaccessibility

Based on data in **Figure 4.4** and **Table 4.3**, the PBET-based bioaccessibility of Cu in CS was nearly 100%, compared to 73% for NS, and 68% for AS. These results agreed with the observation by Ruby et al. (1999) that mineral phases formed under acidic conditions tend to be more stable under the acidic conditions of stomach and hence less bioaccessible. In contrast, mineral phases formed under alkaline conditions are more prone to dissolution under the acidic conditions of stomach. This is reasonable considering that Cu in the calcareous soil exists predominantly in malachite and/or azurite precipitations and/or surface-solid solution on the surface of calcite (Papadopoulos and Rowell, 1989; Madrid and Diaz-Barrientos, 1992), and these Cu species are easily dissolved under the extremely acidic conditions (also see section 3.3). For soils with low carbonate contents, Cu was bound with soil iron/manganese oxides, organic matter, and silicates minerals, which are more resistant to acid dissolution than Cu carbonates or hydroxides. As a result, the bioaccessibility of Cu bound in NS and AS was much lower. Note that the final pH in all PBET tests was in the range of 1.5~2.0.

Cu in sand, silt, and clay fractions of CS was nearly 100% bioaccessible (**Figure 4.4a**), indicating that Cu in those fractions also bound with soil carbonates or carbonate-hydroxyls. This is not surprising again considering the preferential binding of Cu with soil carbonates over other soil compositions (Lee et al., 2005). The bioaccessibility was

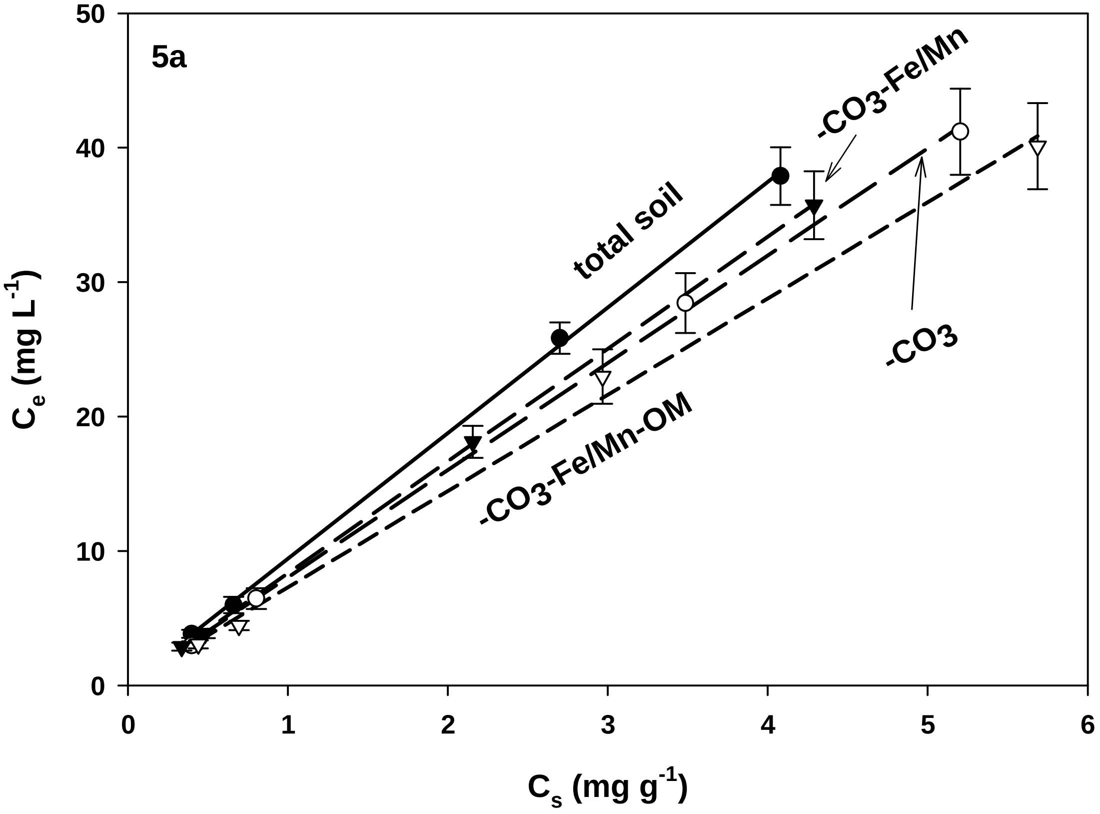


Figure 4.5a. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in calcareous soil or its chemically fractionated sub-soils.

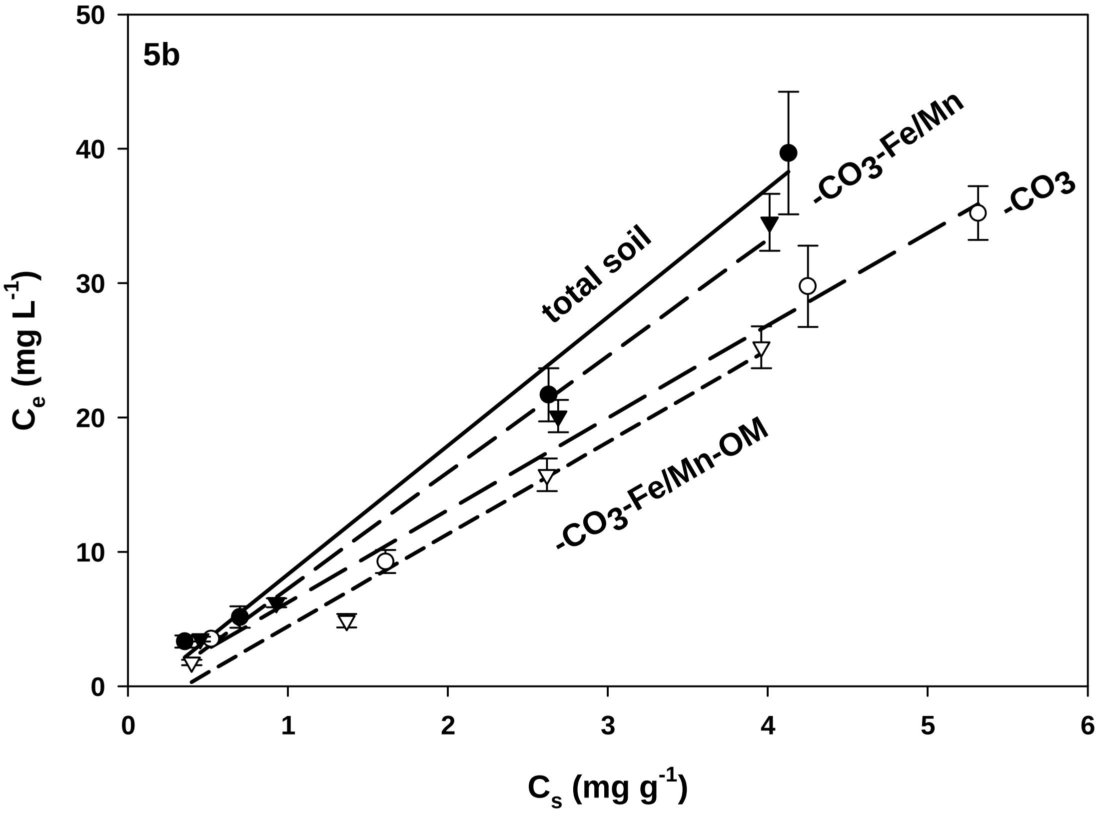


Figure 4.5b. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in neutral soil or its chemically fractionated sub-soils.

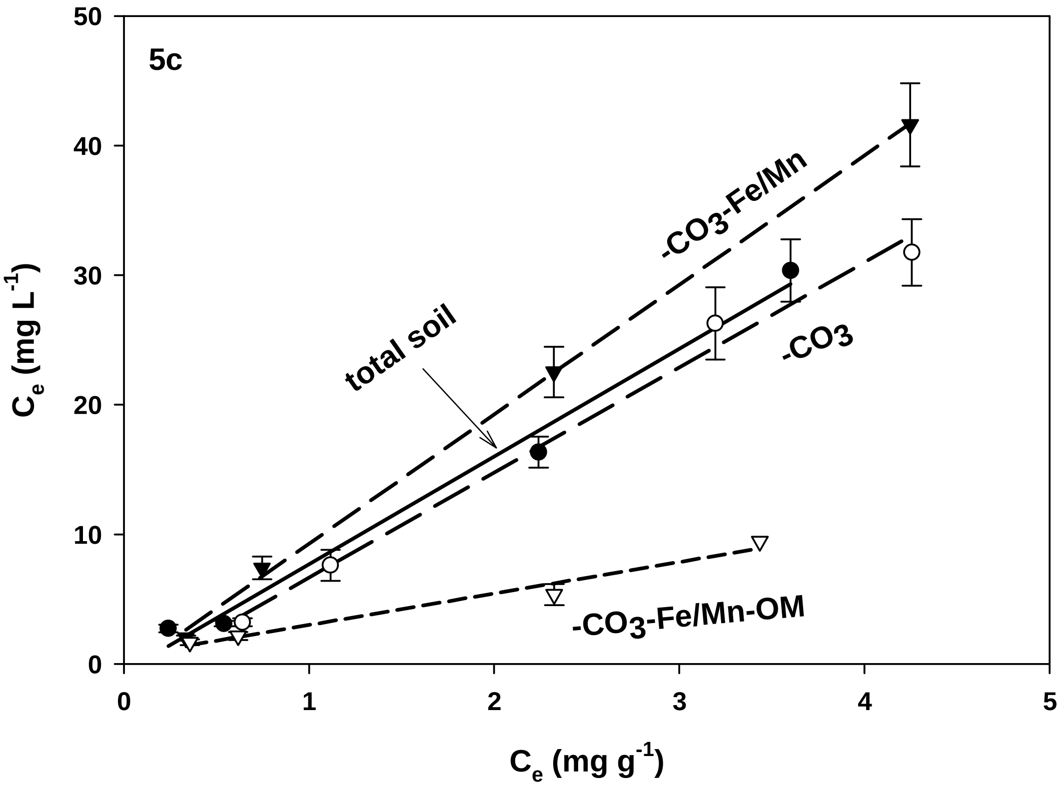


Figure 4.5b. PBET-leachable Cu concentration (C_e) as a function of total copper initially loaded in acidic soil or its chemically fractionated sub-soils.

reduced after the soil carbonates were removed from CS (**Figure 4.5a**), as reflected by a reduction in the slope from 9.57 for the original soil to 6.87 for the de-carbonated fraction (**Table 4.3**). When the soil Fe/Mn oxides were subsequently removed, the bioaccessibility was increased slightly (the slope was increased from 6.87 to 8.66), implying that unlike soil carbonates the presence of soil Fe/Mn oxides tends to reduce the Cu bioaccessibility. Cu bound in the soil minerals showed the least bioaccessibility. It was well-known that Cu sorbed tightly in silicate minerals such as quartz which is rather resistant to acid dissolution (Tikhomolova and Urakova, 2002).

The bioaccessibility of Cu from the NS also showed the similar trend when the soil carbonates, Fe/Mn oxides, and organic matter were sequentially removed, indicating the similar effects of soil components under the extremely acidic environment. For AS, the carbonates content was so low that the Cu bioaccessibility remained nearly unchanged after the soil was treated with the carbonate dissolution procedure.

The bioaccessibility of Cu bound in various fractions of CS and in the original soil was nearly identical (**Figure 4a**). In contrast, the bioaccessibility of Cu bound in the clay fraction of NS and AS was lower in the silt and in sand (Figure 4b-4c). . These observations indicate that in the absence of high concentrations of carbonates small-sized soil fractions offer greater affinity for Cu. Studies by Schmidt and Kögel-Knabner (2002) showed that a major part of organic matter in predominantly inorganic soils is usually found in the silt and clay fractions, suggesting that the Cu might be strongly bound by organic matter in the finer fractions. These results confirmed that chemical compositions rather than particle size are the key factors governing the chemical and biological availabilities of Cu in various fractions of soils.

Compared to SPLP, PBET applies a much stronger leaching agent with a much lower pH (<2.0) and a 5-times smaller soil-to-solution ratio (1g: 100 ml for PBET and 1g: 20 ml for SPLP). The experimental data showed that the PBET-based bioaccessibility of soil-bound Cu was more than 60 times higher than the potential leachability under the conditions of acid precipitation. This observation indicates that ingestion of metal-contaminated soils, especially calcareous soils, is likely the primary health treat.

It should be noted that the commonly used pH 1.5 used in PBET tests reflects the lowest pH value in human stomach (Yang et al., 2002, 2003, 2005; Stewart et al., 2003a, b). The actual pH in human stomach varies diurnally, ranging from approximately 2 (fasting) to 4 to 5 after a meal (Ruby et al., 1996). Yang et al. (2003) observed that the bioaccessibility of soil-bound Pb decreased from 81.1% at pH 1.5 to only 11.1% at pH 4, indicating the strong effect of pH on the bioaccessibility. Therefore, the bioaccessibility of Cu in this study reflects the worst-case scenario.

3.3 DISSOLUTION OF VARIOUS SOIL COMPOSITIONS UNDER SPLP AND PBET CONDITIONS

Natural soils are composed of various minerals, which may behave differently in sorption of metals. In addition, these minerals may undergo dissolution under the SPLP and PBET conditions and may alter the solution chemistry (e.g. pH). To probe the relative chemical stability of common soil minerals and to elucidate the associated effects on Cu availabilities, equilibrium calculations were performed using Visual Minteq (version 2.32) under the SPLP and PBET conditions. For SPLP, the following input parameters were assumed: $H^+ = 10^{-4.2}$ M, and mineral-to-solution ratio = 1 g: 20 mL; while for PBET, pH = 1.5, and mineral-to-solution ratio = 1 g: 100 mL.

Table 4.4 lists the minerals considered and gives the equilibrium pH and percentage of a mineral dissolved under the SPLP or PBET conditions. Cupric ferrite ($CuFe_2O_4$) was also included in Table because it can be a possible form of soil-bound copper (Lindsay, 1979). Under the SPLP conditions, the dissolution loss of all minerals was negligible (<0.012), and only 0.24% of the cupric ferrite was dissolved. In contrast, only Goethite (or crystal Fe) and quartz survived the PBET conditions, with all other minerals being completely dissolved by the PBET liquid. With respect to pH, the presence of carbonates increased the pH of the SPLP solution from ~4 to 9.56, thereby greatly diminishing its Cu extracting power. However, pH for the PBET solution remained intact for all cases.

These results reveal that the reduced SPLP leachability of Cu in the calcareous soil was attributed to the elevated pH in the SPLP extracting solution resulting from the high content of soil carbonates. However, this pH effect became absent under the PBET conditions. In fact, 100% of soil carbonates was dissolved under the PBET conditions, which accounts for the much greater bioaccessibility of Cu in the calcareous soil compared to the other two carbonates-deficient soils. In other words, carbonates would not offer any sorption capacity for Cu under the PBET conditions. While all soil components may play a role in retaining Cu under the SPLP conditions, only quartz and crystal Fe oxides can serve as the effective sinks for Cu under the PBET conditions.

Because of the complex nature of SOM, no calculations were performed for SOM. However, it is well known that some members of SOM such as humic acids and humins

TABLE 4.4

Dissolution of various soil minerals under SPLP and PBET conditions and their effects on final pH

Soil components	Representative minerals	Formulas	SPLP solution			PBET solution		
			Conc'n (M)	pH_f	Dissolved. (%)	Conc'n (M)	pH_f	Dissolved. (%)
Malachite	Malachite	$Cu_2(OH)_2CO_3$	0.226	6.14	0.012	0.045	1.50	100
Azurite	Azurite	$Cu_2(OH)_2(CO_3)_2$	0.178	6.36	0.012	0.036	1.50	100
Cupric ferrite	Cupric ferrite	$CuFe_2O_4$	0.209	5.38	0.24	0.042	1.5	100
Carbonates	Calcite	CaCO3	0.500	9.56	0.033	0.100	1.50	100
Amorphous Fe	Ferrihydrite (aged)	$Fe_5HO_8*4H_2O$ (aged)	0.104	4.00	0.000	0.021	1.50	100
Crystal Fe	Goethite	FeOOH	0.562	4.00	0.000	0.112	1.50	3.014
Kaolinite	Kaolinite	$Al_2Si_2O_5(OH)_4$	0.194	4.71	0.002	0.039	1.50	100
Macas	[a]Muscovite	$Si_3Al_3KO_{10}(OH)_2$	0.126	4.78	0.001	0.025	1.50	100
Smectite	[a]Mg-montmorillonite	$Mg_{0.2}(Si_{3.81}Al_{1.71}Fe(III)_{0.22}Mg0_{.29})O_{10}(OH)_2$	0.135	7.83	0.007	0.027	1.50	100
Quartz	Quartz	SiO_2	0.8335	6.81	0.012	0.167	1.50	0.210

a. minerals were not included in Visual Minteq database, and calculations were carried out using methods by Lindsay (1979)

b. only mineral dissolution were considered in the geochemical modeling. All the calculations were based on the assumption that various minerals maintain thermodynamic equilibrium with fluids are insoluble in acid solutions and can strongly complex with metal ions (Deng and Dixon, 2002). Thus a fraction of Cu complexed with those SOM may not be leachable or bioaccessible. Based on experimental studies of influence of soil properties on the Cr(III) bioaccessibility, Stewart et al. (2003a) observed that the Cr(III) bioaccessibility was generally lower in soils with high SOM content, and they claimed that organic mattercould form strong bonds with Cr(III) enough to resist dissolution under the PBET conditions.

The results in **Table 4.4** may also provide some useful guidelines for remediation of soils contaminated with heavy metals. For example, organic matter, iron/manganese, lime, clay minerals, and phosphates have been applied as a soil amending agent to immobilize heavy metals in soils or sediments (Basta et al., 2001). While most of these materials are able to offer strong affinity for copper uptake, the materials themselves must be resistant to acid dissolution if they are used to reduce the bioaccessibility. For example, lime may reduce the SPLP leachability of heavy metals, but may not be effective for decreasing the PBET bioaccessibility.

4. Conclusions

This study investigated different roles of various soil compositions (carbonates, Fe/Mn oxides, SOM, and residual soil minerals) in sorption of copper in three soils (calcareous, neutral and acidic). The results revealed that soil chemical compositions rather than particle size are the key factors governing the chemical leachability and bioaccessibility of Cu in soils.

The presence of carbonates in soils was able to increase the pH of the SPLP solution, and thus, diminished its extracting power. As a result, carbonates can greatly reduce the SPLP leachability of Cu. However, while carbonates can bind with Cu more favorably than other soil compositions, carbonates themselves are vulnerable to complete dissolution under the PBET conditions. As a result, Cu in soils of high carbonates is much more bioaccessible than in soils of low carbonates content.

In all cases, the residual soil minerals showed the greatest affinity for Cu, i.e. the lowest leachability and bioaccessibility regardless of soil types. However, these strongest binding sites appeared to be least available compared to those of other soil compositions such as carbonates and Fe/Mn oxides. The effects of Fe/Mn oxides and organic matter varied depending on types of soils and the extracting procedures. For CS, removal of Fe/Mn oxides resulted in a decrease in the SPLP leachability but little change in PBET bioaccessibility. For NS and AS, however, removal of Fe/Mn oxides increased both SPLP leachability and PBET bioaccessibility. Between these two fractions, the SOM appears to offer stronger affinity, and the presence of these two fractions prevented Cu from accessing to the stronger sites in the residual soil minerals. Overall, the copper accessibility of the four soil fractions follows the following sequence: carbonates > Fe/Mn oxides > SOM > residual minerals; however, in terms of copper affinity, the sequence is just reversed, i.e. residual minerals > SOM > Fe/Mn oxides > carbonates.

The information gained from this study may be useful for evaluating long term environmental fate and availabilities of heavy metals in various soils. Knowledge on the effects of various soil compositions may also facilitate engineered processes for immobilization of heavy metals in soils.

CHAPTER V

REDUCING LEACHABILITY AND BIOACCESSIBILITY OF LEAD IN SOILS USING A NEW CLASS OF STABILIZED IRON PHOSPHATE NANOPARTICLES

1. Introduction

Lead is a widespread contaminant in soils and groundwater (Wittmann, 1981; Ewers and Schlipköter, 1991). Lead has been ranked the second most hazardous substance in the U.S. by the Agency for Toxic Substances and Disease Registry (ATSDR) and the U.S. Environmental Protection Agency (USEPA) (ATSDR, 2005). In 1999, lead was identified as a major hazardous chemical at 47% of the 1,219 Superfund sites on the USEPA's National Priorities List (USEPA, 1999). Current remediation technologies for contaminated soil remediation are rather costly (Berti and Cunningham, 1997) and/or often environmentally disruptive. Consequently, innovative remediation technologies for controlling lead-poisoning are urgently needed.

In recent years, *in situ* immobilization of Pb^{2+} in contaminated soils with phosphate-based amendments has elicited a great deal of attention (Ma et al., 1995; Zhang and Ryan, 1998; Cao et al., 2002; Sonmez and Pierzynski, 2005). This approach reduces the Pb^{2+} mobility, and thus toxicity, by transforming the labile form of Pb^{2+} in soils to the geochemically much more stable pyromorphites ($Pb_5(PO_4)_3X$, where X = F, Cl, Br, OH) by amending contaminated soils with soluble phosphate salts or solid phosphate minerals such as apatite. Pyromorphites are considered as the most stable forms of Pb^{2+} under a wide range of environmental conditions, and are over 44 orders of magnitude less soluble than other common Pb^{2+} minerals in contaminated soils such as galena (PbS), anglesite ($PbSO_4$), cerussite ($PbCO_3$), and litharge (PbO) (Ruby et al., 1994; Laperche et al., 1996). For *in situ* immobilization of Pb^{2+} in soils, phosphate has been commonly applied to soils in its soluble forms such as phosphoric acid (Eighmy et al., 1997; Yang et al., 2001), NaH_2PO_4 (Stanforth and Qiu, 2001) or KH_2PO_4 (Berti and Cunningham, 1997), or in solid forms such as synthetic apatite (Ma et al., 1993; Zhang and Ryan, 1998), natural phosphate rocks (Ma and Rao, 1999), and even fishbone (with apatite being the effective composition) (Admassu and Breese, 1999; USEPA, 2001a). Among those additives, phosphoric acid was regarded as the most effective amendment (USEPA, 2001a) for its easy delivery and superior ability to dissolve Pb^{2+} from existing minerals and transform it to pyromorphites. Amendment dosage of 3% PO_4^{3-} by weight for soils has been proposed and applied by USEPA and other government agencies (USEPA, 2001a).

However, adding large amounts (e.g. the 3% PO_4^{3-} dosage) of highly soluble phosphoric acid or phosphate salts into the subsurface is limited by not only the material cost but the secondary contamination problems. Due to the high solubility of phosphate, groundwater and surface waters in the affected area may be contaminated by excessive nutrient input (Cotter-Howells and Caporn, 1996). To avoid phosphate leaching, solid phosphate (e.g. rock phosphate) was also studied (Ma et al., 1993; 1995; Ma and Rao, 1999). However, effectiveness of solid phosphate is hindered by the large size of the particles. In fact, even fine-ground solid phosphate particles are not mobile in soils, which prevents solid phosphate from being delivered to the lead-affected zone and from reaching and reacting with Pb^{2+} sorbed in soils.

In recent years, environmental application of nanoscale zero-valent iron (ZVI) has attracted considerable interests. In addition to reductive dechlorination uses (e.g. Zhang, 2003; He and Zhao, 2005), ZVI nanoparticles have been studied for transformation of inorganic contaminants such as chromate (CrO_4^{2-}) (Astrup et al., 2000), arsenate (AsO_4^{3-}) (Su and Puls, 2001), perchlorate (ClO_4^-) (Moore et al., 2003), and nitrate (NO_3^-*)* (Ginner et al., 2004). Compared to conventional powder or granular iron particles, nanoscale ZVI particles offer improved reactivity. However, ZVI nanoparticles can form micron-scale agglomerates rapidly, resulting in loss in soil mobility and reduced reactivity. To prevent nanoparticle agglomeration, He et al. (2006) developed a strategy to stabilize ZVI-nanoparticles using low-cost and environmentally friendly cellulose (CMC) as a stabilizer. The stabilized ZVI nanoparticles displayed much improved reactivity as well as soil mobility compared to non-stabilized counterparts. In addition, iron-oxide and iron sulfide nanoparticles were also studied for environmental uses. For instance, Chang and Chen (2005) used chitosan-bound Fe_3O_4 nanoparticles to remove Cu^{2+} from water, showing that the nanoparticles had a maximum capacity of 21.5 mg/g when applied to removal of Cu^{2+} at $pH > 2$. Xiong et al (2006) have observed a 99% reduction of the Hg leachability in soil using stabilized FeS nanoparticles. However, there have been no studies reported on the *in situ* immobilization of Pb^{2+} using nanoparticles.

The overall goal of this study is to prepare and test a new class of CMC-stabilized iron phosphate nanoparticles for immobilizing Pb^{2+} in soils and/or solid and hazardous wastes. The specific objectives are to: 1) prepare a new class of iron phosphate nanoparticles with CMC as a stabilizer; 2) test effectiveness of the nanoparticles for reducing the leachability of Pb^{2+} in three representative soils; and 3) determine the effects of soil type, treatment contact time (aging), and chloride on Pb^{2+} immobilization efficiency; and 4) probe speciation of soil-bound Pb^{2+} before and after the nanoparticle amendment. 5) test the effects of iron sulfide and iron oxide nanoparticle on soil Pb leachability as a comparison.

2. Materials and Methods

2.1 MATERIALS

All chemicals used in this study were of analytical or higher grade. The sodium form of carboxymethyl cellulose (molecular weight = 90,000), iron sulfate heptahydrate ($FeSO_4 \cdot 8H_2O$) and sodium chloride (NaCl) were purchased from Acros Organics, (Morris Plains, NJ, USA), all other chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA). All solutions were prepared with deionized water (18 MΩ-cm) from a Milli-QTM Water System.

Three top soils (up to ~25 cm) designated as acidic, neutral and calcareous soils (or AS, NS, and CS, respectively) based on the soil pH were collected from a farm in western Alabama, USA. **Table 5.1** gives salient physical and chemical properties for the soil samples. Before use, the soils were air-dried and passed through a 2-mm sieve. Soil *pH* was measured in 0.01 M $CaCl_2$ in a 1:1 soil:solution (g:mL) suspension (Thomas, 1996). Elemental analysis of the soils was conducted following EPA method 3050B and using an inductively coupled plasma optical emission spectrometer (Vista-MPX, Varian

TABLE 5.1
Salient physical and chemical properties of soils used in study.

Items	Acidic soil (AS)	Neutral soil (NS)	Calcareous soil (CS)
pH	4.36	6.93	7.85
*CEC7 (meq/100g soil)	10.77	18.17	34.85
Pb (g/kg)	0.024	0.139	0.062
OM (%)	1.2	0.9	0.6
$CaCO_3$ equiv. (%)	0.15	1.15	48.5
Sand (%)	69	18	15
Silt (%)	4	28	29
Clay (%)	27	54	56
**Clay minerals (%)	K(2.8) M(39.0) Q(39.5) S(18.7)	K(33.9) M(19.1) Q(18.8) S(28.2)	K(22.4) M(38.8) Q(21.7) S(17.1)

* cation exchange capacity at pH 7.0
**K= kaolinite, M=micas, Q=quartz, S= smectite

Inc., Palo Alto, CA, USA). The content of sand, silt and clay was determined following the pipette method (Gee and Or, 2002). Total nitrogen and total carbon were analyzed following the Dumas method with a LECO CN-2000 combustion unit (LECO Corp., Joseph, MI, USA) at 1050 °C, and organic carbon was measured using the same method except that soils were treated with 4 N H_2SO_4 to dissolve free carbonates before the dry combustion. The content of carbonates was then calculated from the difference between total carbon and organic carbon. Soil sulfur content was determined using a LECO SC-432 sulfur analyzer (LECO Corp., Joseph, MI, USA). Soil clay minerals were analyzed with an X-ray diffraction unit (Siemens D5000 X-ray diffractometer) after the soil samples were treated following the procedure by Kunze and Dixon (1986). The content of each clay mineral was estimated by the respective intensity of diffracted X-ray. The cation exchange capacity (CEC) was determined with 1 N NH_4OAc buffered at pH 7.0 following the method described in the Soil Survey Laboratory Methods Manual (USDA et al., 1996).

2.2. SOIL SPIKING WITH PB^{2+}

The three soils were first spiked with known concentrations of Pb^{2+} following the procedure described by Yang et al. (2003). In brief, a known mass of an air-dried soil was mixed with a solution containing ~70 mg/L Pb^{2+} and 10^{-3} M $CaCl_2$ and at a solution-to-solid ratio of 10:1 (mL/g) and at an initial pH 5.50. After mixing for 96 h, the mixture was centrifuged and supernatant decanted. The Pb-loaded soil was then washed twice with deionized water to remove water-soluble Pb^{2+}. The decanted supernatant and rinse water were filtered through 0.45-μm membrane filter, and analyzed for Pb using an atomic absorption spectrophotometer. The final Pb^{2+} uptake by each soil was calculated by mass balance calculations. The resultant Pb^{2+} uptake was 599 mg/kg for CS, 696 mg/kg for NS, and 640 mg/kg for AS, all of which were above the EPA action level of 400 mg/kg. These numbers were further verified with USEPA Method 3050B (1996), and the difference was < 5% in all cases. The Pb-loaded soils were then air-dried for subsequent experimental uses.

2.3. PREPARATION OF CMC-STABILIZED IRON PHOSPHATE NANOPARTICLES

A 1% (w/w) CMC solution, a 1.05 g/L PO_4^{3-} solution, and a 1.19 g/ L Fe^{2+} solution were prepared separately and then purged with nitrogen gas for ~4 hours to remove dissolved oxygen (DO). The nanoparticle preparation was then carried out in an anaerobic chamber (Model 10, Sheldon Manufacturing Inc., Oregon, USA), where 25 mL of the Fe^{2+} solution was dropwise added to 50 mL of the 1% CMC solution under mixing. After the mixture was stirred for 30 min, 25 mL of the phosphate solution was added to the mixture dropwise and under mixing. The molar ratio of Fe^{2+}-to-PO_4^{3-} was 3:2 in accord with the stoichiometry given in **Eqn.5.1**, through which the $Fe_3(PO_4)_2{\cdot}8H_2O$ (vivianite) nanoparticles are formed,

$$3Fe^{2+} + 2PO_4^{3-} + 8H_2O \Leftrightarrow Fe_3(PO_4)_2 \bullet 8H_2O \qquad \log K = 36.0 \qquad (5.1)$$

The final pH of the resultant nanoparticle suspension was measured to be 6.56, and the resultant nanoparticle suspension included: 0.5% CMC, 4.7 mM or 263 mg/L Fe and 3.1 mM or 298 mg/L PO_4^{3-}.

For TEM imaging, a drop of the vivianite nanoparticle suspension was placed on a carbon coated copper grid and then dried for 12 hours in the anaerobic chamber. The dried grid was then placed under a JEOL JEM-200CX transmission electron microscope. The images were taken at an acceleration voltage of 120 kV. The size of the stabilized nanoparticles was determined by measuring the size of a total of 450 nanoparticles from five representative TEM images.

2.4. SOIL TREATMENT WITH $FE_3(PO_4)_2$ NANOPARTICLES

Each Pb-spiked soil sample was amended by mixing 0.5 g (dry weight) of a soil with 1 mL of the nanoparticle suspension in 15 mL centrifuging tubes with screw caps, which resulted in a suspension-to-soil ratio of 2:1 (mL/g). The mixtures were sealed and shaken for 5 min, and then placed still in the hood at room temperature (21±2 ^{o}C) for aging. For comparison, the same amendment procedure was also conducted at a higher suspension-to-soil ratio of 10:1 (mL/g). For typographical convenience, the treatment at a suspension-to-soil ratio of 2:1 is denoted as Case 1, and that at 10:1 as Case 2. For the three soils, the Phosphate-to-Pb molar ratio ranged from 1.8 to 2.2 for Case 1, and from 9 to 11 for Case 2. To test the effect of contact/reaction time on Pb-immobilization, the soil-nanoparticle contact time was varied from 1 to 56 days. To ensure data quality, all soil treatment tests were performed in duplicates.

To determine the effect of chloride on Pb^{2+} immobilization, additional tests were carried out following the same experimental protocol but in the presence of 100 mg/L and 300 mg/L Cl^- (added as NaCl), respectively. The reaction time for these tests was set for 1 week.

Additionally, parallel experiments were conducted to compare the phosphate leachability when the soils were amended with the nanoparticle suspension or a soluble phosphate salt (300 mg/L PO_4^{3-} solution as NaH_2PO_4 at pH 6.7) following the same treatment procedures. The reaction time was set for 1 week for these tests. The phosphate concentrations in the supernatants were measured using a DX-120 Ion Chromatograph (Dionex Corporation, CA, USA) after the soil slurries were centrifuged at 3000×g for 10 min.

2.5. TCLP TESTS

TCLP tests were performed to quantify the Pb^{2+} leachability of the untreated and the nanoparticle-amended soils following EPA Method 1311 (USEPA, 1992). The TCLP fluid consisted of 0.1 M glacial acetic acid and 0.0643 M NaOH, which resulted in a pH of 4.93. At predetermined aging times, the soil-nanoparticle mixtures were centrifuged at 3000×g for 10 min. Upon decanting the supernatant, 10 mL of the TCLP fluid was added to each tube containing the treated soils. The mixture was then agitated on a shaker for 18 h at 21±2 ^{o}C and then centrifuged at 3000×g for 10 min. The supernatant was then passed through a 0.45 μm Ampore-size polycarbonate filter. The filtrate was then acidified using 1% HNO_3, and analyzed for Pb. For comparison, the same TCLP tests were also carried out on the untreated Pb-spiked soils. All tests were duplicated. The TCLP leachability of Pb was quantified via **Eqn. 5.2.**

$$Leachability\ of\ \ Pb\ in\ soil = \frac{(\ C_{TCLP,Pb}\,, mg\,/\,L)(0.01L)}{(Q_{Pb}\,, mg\,/\,g)(0.5g)} \times 100\% \qquad (5.2)$$

where $C_{TCLP,Pb}$ is the Pb concentration in the TCLP extract and Q_{Pb} is the Pb content in the soil sample before the TCLP treatment. To assure mass balance, the TCLP-extracted soil residues were further extracted and analyzed for residual Pb following EPA Method 3050B (USEPA, 1996).

2.6. PBET TESTS

In addition to the TCLP tests, a biochemically oriented method, PBET, was employed to evaluate the in vitro bioaccessibility of soil-sorbed Pb (Ruby et al., 1996; 1999). PBET employs an extraction solution of 30 g/L glycine (0.4 M) with a pH adjusted to ~2.3 with HNO_3 to mimic the conditions in the gastric system and small intestines. PBET tests were conducted following the procedures described by Kelley et al. (2002). Typically, 0.1 g of an air-dried soil sample (untreated or nanoparticle treated) was mixed with 10 mL of the extracting liquid in 15 mL high-density polyethylene vials. The mixtures were then mixed on an end-to-end rotator placed in an incubator at 37 °C. After 1 h, the vials were centrifuged at 3000×g for 10 min, supernatant filtered with 0.45-μm membrane filters, and then filtrate analyzed for Pb^{2+} with an atomic absorption spectrophotometer. The bioaccessibility of Pb^{2+} was quantified by

$$Bioaccessibility\ of\ \ Pb\,(\%) = \frac{(\,C_{PBET\,,Pb}\,, mg\,/\,L\,)(\,0.01L\,)}{(\,Q_{Pb}\,, mg\,/\,g\,)(\,0.1g\,)} \times 100 \tag{5.3}$$

where $C_{PBET,\,Pb}$ is the Pb concentration in the PBET solution and Q_{Pb} is the Pb content in the soil sample before the PBET extraction. All tests were performed in triplicate to ensure data quality.

2.7. SEQUENTIAL EXTRACTION OF SOIL-SORBED PB^{2+}

To probe the change in Pb speciation in the soils before and after the nanoparticle treatment, a sequential extraction procedure developed by Tessier et al. (1979) and then modified by others (Shuman, 1985; Han et. al 2001; Peltier, 2005) was employed to quantify the relative availability of Pb^{2+} based on fractions of various operationally defined Pb^{2+} species. **Table 5.2** shows the reagents and procedures used for each of the five sequential extraction steps. For each step, a given volume of a prescribed extractant was mixed with a soil sample (equivalent to 0.5 g dry weight) in a 15 mL Teflon centrifuge tube. At predetermined times (**Table 5.2**), the mixtures were centrifuged at 3000×g for 10 min. The supernatant was decanted, filtered through a 0.45-µm membrane filter, and then filtrate analyzed for Pb. All tests were carried out in an anaerobic atmosphere to mimic the subsurface redox condition. All extractions were duplicated to assure data quality.

2.8. PREPARATION AND TEST OF IRON SULFIDE AND IRON OXIDE NANOPARTICLES

Similar to the procedures for iron phosphate nanoparticles preparation, solutions used for iron sulfide nanoparticle synthesis were prepared in the anaerobic chamber using degassed DI water and all procedures for the preparation were also conducted in the chamber where 20 mL 7.20 mM $FeSO_4 \cdot 7H_2O$ was mixed with 50 mL 1% CMC solution for 30 min. Then 20 mL 7.20 mM $Na_2S \cdot 9H_2O$ was added to the mixture dropwise. 0.1 M NaOH was used to keep final pH of the solution at 7.3 (Osseo-Asare and Wei, 1993) and the final volume of the prepared solution was about 100 mL.

TABLE 5.2
Experimental conditions for sequential extraction of Pb from soils.

Step	Extractant	Target
1	12.5 mL, 1 M NH_4NO_3 , pH 7.0, 0.5 h shaking.	Water soluble / exchangeable fraction (EX)
2	12.5 mL, 1 M Na-acetate, pH 5.0, 6 h shaking.	Carbonate-bound fraction (CB)
3	12.5 mL, 0.04 M hydroxylamine hydrochloride in 25% acetic acid, 6 h at 95 ^oC in water bath.	Fe/Mn oxides-bound fraction (OX)
4	1.5mL of 0.01 *M* HNO_3 and 2.5 mL of 30% H_2O_2, 5 h at 85 °C in water bath, followed by an additional 1 mL of H_2O_2, 1h at 85 °C in water bath, then 7.5 ml of 1 *M* NH_4NO_3 , 10 min shaking at room temperature.	Organically Bound fraction(OM)
5	12.5 mL, 4 M HNO_3 16 h at 80 °C in water bath.	Residual fraction (RS)

The magnetite (Fe_3O_4) nanoparticles were prepared following a method described by Si et al, 2004. The major procedures were introduced as follows: An aqueous solution of $FeSO_4 \cdot 7H_2O$ (50 mL, 0.1 M) was added dropwise to a 50 mL aqueous solution of 1% (w/v) NaCMC with continuous stirring. The final concentration of the polymer in the medium was 0.5% (w/v). The mixture was stirred for 30 min to complete the formation of the iron-polymer complex. The pH of the solution was then increased slowly to higher than 10 by adding 0.1 M NaOH solution. The reaction mixture (150 mL in volume) was subsequently aged for 1 h with constant stirring. The final concentration of Fe in the solution was about 0.03 M and the final pH was 12.0.

Soil samples tested, soil amendment procedures, TCLP procedure used in researching the effects of FeS and Fe_3O_4 nanopartilces on soil Pb leachability were respectively identical to those introduced in Section 2.2, section 2.4 and section 2.5 except that an anaerobic hood was not used for iron oxide nanoparticle study.

3. Results and Discussions

3.1. CHARACTERIZATION OF IRON PHOSPHATE NANOPARTICLES

Figure 5.1a shows the suspension of the vivianite nanoparticle suspension in the presence of 0.5% (w/w) CMC. For comparison, **Figure 5.1b** shows the vivianite prepared following the same procure except that CMC was absent. While the CMC-stabilized vivianite nanoparticles remain fully suspended and the suspension appeared as a transparent solution for weeks, the non-stabilized counterparts agglomerated and precipitated in minutes. **Figure 5.1c** shows a representative TEM image of the CMC-stabilized vivianite nanoparticles (nitrogen-dried), which reveals that the particles were present as discrete nanoparticles. Based on 450 particles from five similar TEM images, the mean size of the iron phosphate nanoparticles was estimated to be 8.4±2.9 nm (standard deviation). Evidently, the presence of CMC effectively prevented the vivianite nanoparticles from agglomerating. Preliminary column tests with the acidic soil showed that the stabilized nanoparticles can easily pass the soil bed under gravity, while the agglomerated vivianite flocs were completely caught on top of the soil bed. The greater soil mobility enables the nanoparticles to be delivered to targeted contaminant source zones in the subsurface (i.e. applied in situ) and to access to and react with soil-sorbed Pb^{2+} ions.

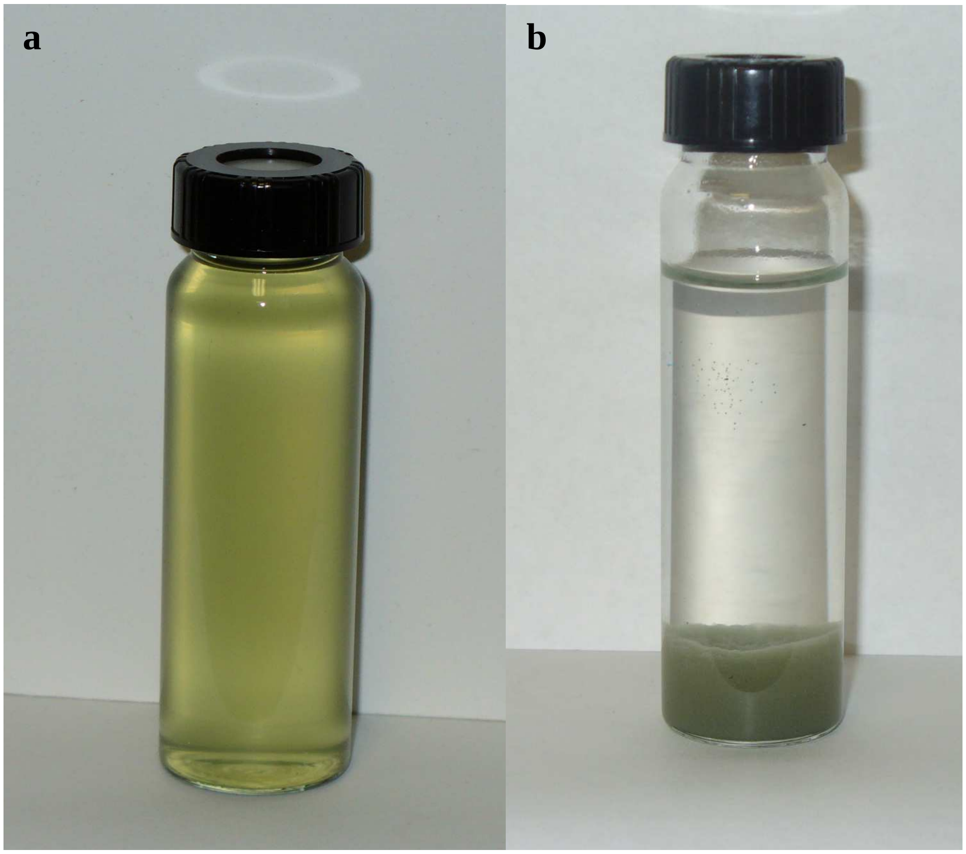


Figure 5.1a. Freshly prepared vivianite ($Fe_3(PO_4)_2\cdot 8H_2O$) nanoparticle suspension (1.56 mM) in the presence of 0.5% (w/w) NaCMC as a stabilizer; **b.** Vivianite precipitates (1.56 mM) in the absence of a stabilizer.

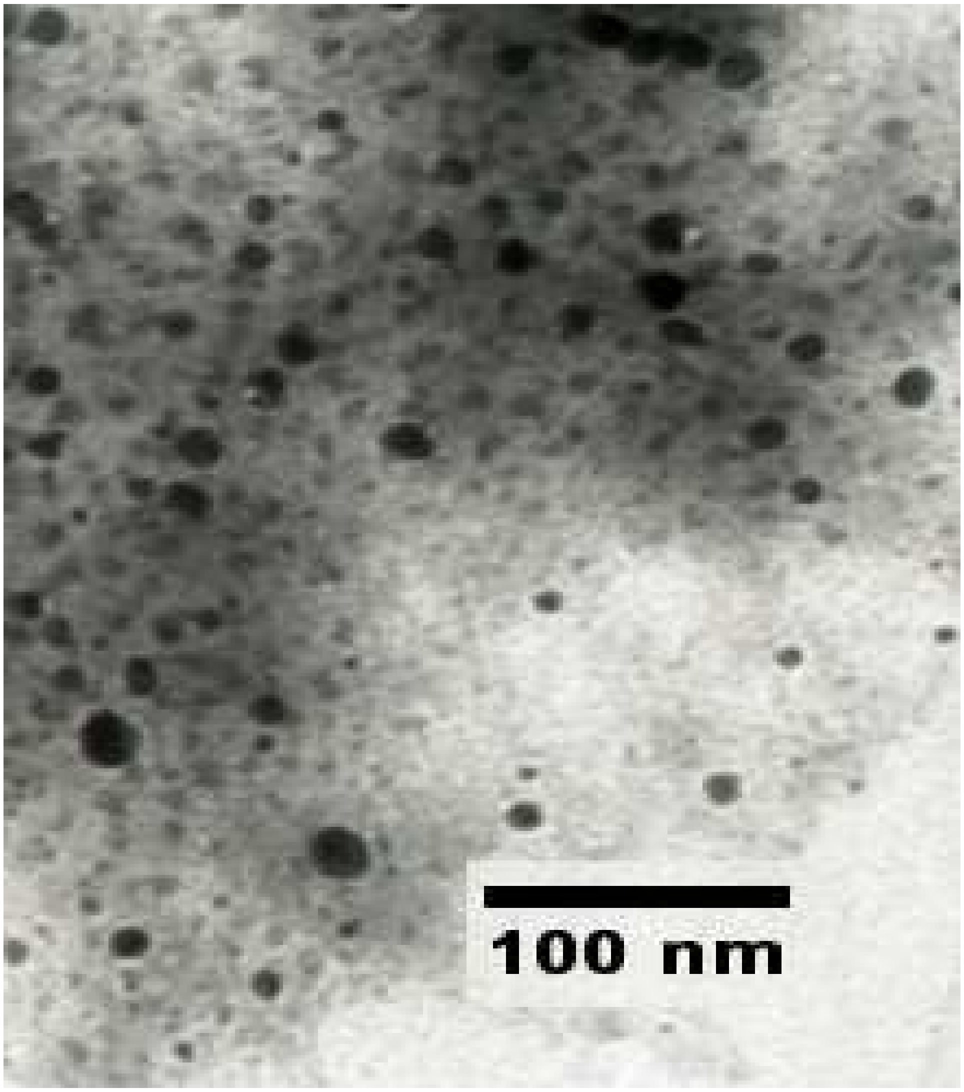


Figure 5.1c. TEM images of CMC-stabilized vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) nanoparticles.

Earlier, Si et al. (2004) and He et al. (2006) attributed the nanoparticle stabilization by poly-electrolytes to encapsulation of the nanoparticles with a thin layer of negative charges from the stabilizer such as CMC. They also reported that the size of the nanoparticles can be controlled by varying the concentration of a polyelectrolyte. In addition, Kim et al. (2003) claimed that the formation of a polymer layer on the surface of magnetite nanoparticles may prevent oxidation of the nanoparticles.

The solubility product value for vivianite was reported to be $\sim 10^{-36}$ (Nriagu, 1972). Therefore, dissolution of vivianite is thermodynamically rather unfavorable. Ignoring the influence of CMC in the nanoparticle suspension on the dissolution, Visual MINTEQ (Version 2.32) calculations showed that the equilibrium aqueous concentrations of Fe^{2+} (the predominant aqueous form of Fe) and total P (predominantly in $H_2PO_4^-$ at pH 6.56) in the nanoparticle suspension were 0.027 mM and 0.015 mM, respectively, i.e. >99.4% of Fe^{2+} and phosphate added to the solution were converted to vivianite precipitates during the nanoparticle synthesis.

3.2. TCLP LEACHABILITY OF SOIL-BOUND PB^{2+}

To test the effectiveness of the CMC-stabilized vivianite nanoparticles for enhancing Pb^{2+} immobilization, the TCLP leachability of Pb^{2+} in untreated and nanoparticle-amended soils was measured and compared. **Figure 5.2** shows the TCLP leachability of soil-sorbed Pb^{2+} for the three soils before and after the soils were treated with the nanoparticles under Case 1 (**a**) and Case 2 (**b**) conditions and at various treatment times. As usually is the case, the acidic soil displayed the greatest Pb^{2+} leachability (~44% for the untreated soil) than the other two soils (~6.6% for the neutral soil and ~1.5% for the

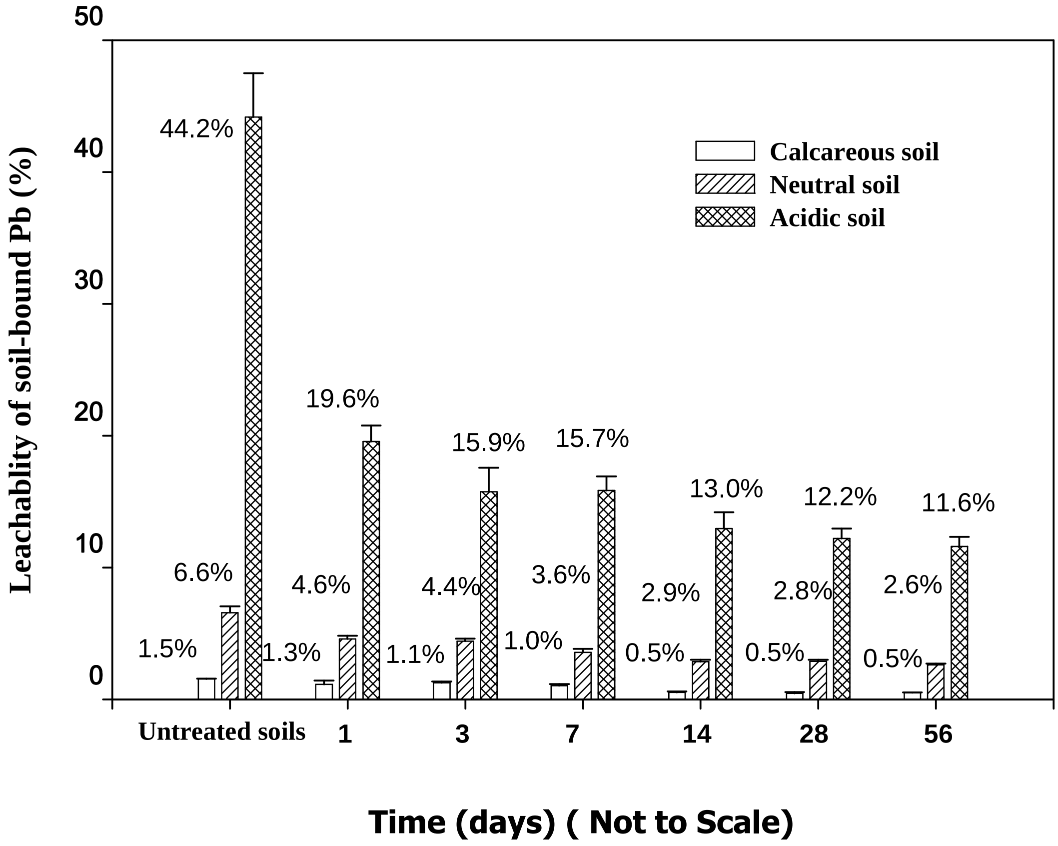


Figure 5.2a Reduction of TCLP-based leachability of soil-bound Pb with treatment time when soils were amended with 1.56 mM of CMC-stabilized vivianite nanoparticle suspension in Case 1 (suspension-to-soil ratio = 2 : 1 mL/ g)

calcareous soil). When the soils were amended with the vivianite nanoparticle suspension for 1 day in Case 1 (**Figure 5.2a**), the Pb^{2+} leachability for the acidic soil was decreased from its original 44% to 20%, i.e. a 56% reduction. The Pb^{2+} leachability was further reduced as the contact time was increased for all cases. For example, the leachability in the acidic soil was lowered to ~15% after 14 days of aging in Case 1. However, after 14 days, the aging effect became less conspicuous. For the acidic soil, the leachability from the 14th to 56th day was reduced by only 1%, resulting in a 74% of overall reduction in Pb^{2+} leachability in Case 1. From the standpoint of nanoparticle mass transfer and reaction kinetics, the observation revealed that the Pb^{2+} immobilization was kinetically most effective within one day and equilibrium was nearly reached in 14 days.

For the neutral soil in Case 1 (**Figure 5.2a**), the 1 day-treatment lowered the leachability from 6.6% to 4.6% (i.e. a 30% reduction). The leachability was further reduced to 2.8% after 14 days of treatment. The leachability for the untreated calcareous soil was quite low (~1.5%), which nonetheless was lowered to 0.5% after 14 days of treatment (a 65% reduction) although the Pb content in this soil was comparable to that for the other two soils. The calcareous soil contained a much higher level (48.5%) of carbonates than the other two soils (**Table 5.1**), which granted the calcareous soil a much greater alkalinity. Consequently, the final pH of the TCLP extracts was 6.0~6.5 for CS, 5.1~5.5 for NS and 4.8~5.0 for AS although all the TCLP tests were carried out under identical conditions and the initial TCLP fluid pH was 4.9. As a result, the extracting power of the TCLP fluid was diminished by the elevated pH so that the TCLP leachability of Pb^{2+} in the calcareous soil was much lower than that in the neutral or acidic soils although the three

TABLE 5.3

Changes of Pb concentrations in TCLP extracts with treatment time after soils were amended with vivianite nanoparticles in Case 1 (nanoparticle suspension-to-soil ratio= 2:1 mL/g).

Soil	Pb concentration in TCLP extracts (mean ± standard deviation, mg/L)						
	Untreated soil	Treatment time (days)					
		1	3	7	14	28	56
Calcareous soil (CS)	0.46 ±0.01	0.34 ±0.09	0.38 ±0.03	0.31 ±0.03	0.16 ±0.02	0.14 ±0.03	0.15 ±0.01
Neutral soil (NS)	2.29 ±0.17	1.60 ±0.08	1.54 ±0.06	1.24 ±0.09	0.99 ±0.06	1.00 ±0.04	0.91 ±0.03
Acidic soil (AS)	14.13 ±1.06	6.26 ±0.39	5.04 ±0.59	5.07 ±0.34	4.14 ±0.40	3.90 ±0.24	3.71 ±0.24

soils contained comparable amounts of Pb. Accordingly, after those soils were amended with the nanoparticles, the reduction of the Pb^{2+} leachability was much more distinctive for the acidic soil (56% reduction in 1 day amendment) than for the neutral (30% reduction in 1 day) and the calcareous soil (26% reduction in 1 day). This observation also suggested that the nanoparticle treatment is likely more suitable for immobilizing Pb^{2+} sorbed in acidic soils.

Table 5.3 gives the Pb concentrations in the TCLP extracts corresponding to those in **Figure 5.2a** (Case 1). The Pb concentration for the untreated acidic soil was nearly three times the EPA TCLP limit of 5 mg/L for identifying hazardous wastes (USEPA, 2006). Upon the nanoparticle treatment, the Pb concentration in the TCLP extracts was reduced to 3.7 mg/L.

Figure 5.2b shows that much greater reduction in Pb leachability was achieved when the soils were treated in Case 2, where a five times greater volume of the nanoparticle

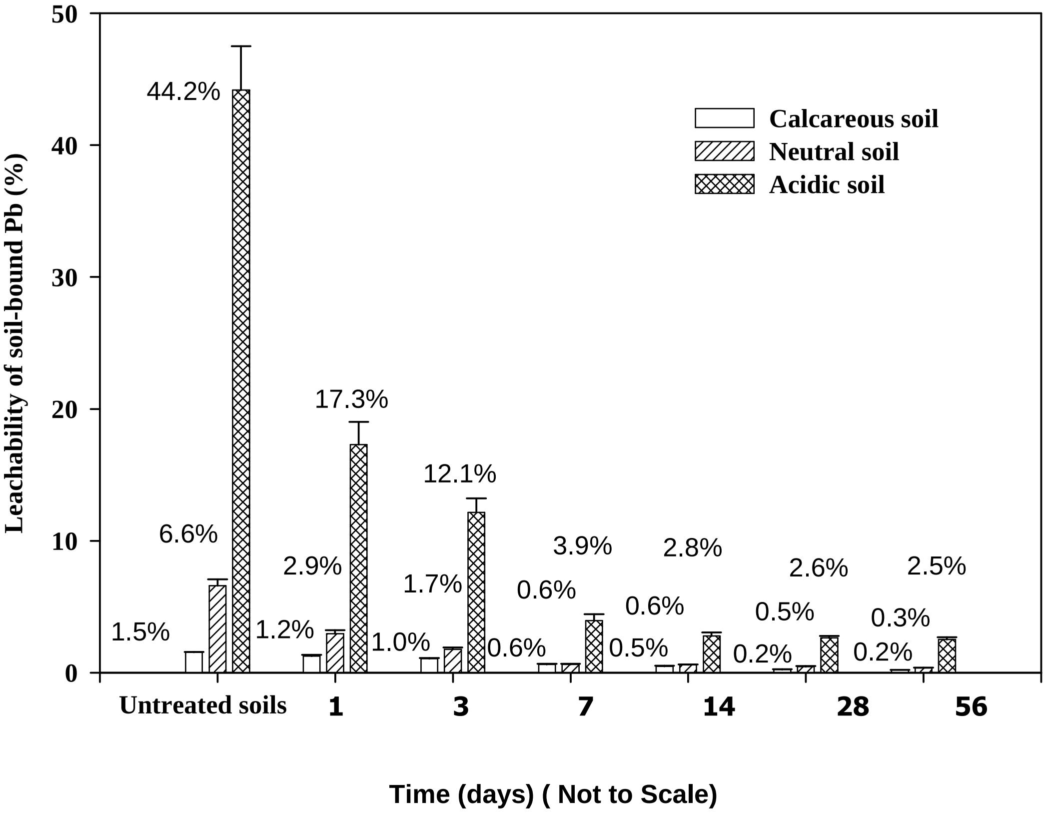


Figure 5.2b Reduction of TCLP-based leachability of soil-bound Pb with treatment time when soils were amended with 1.56 mM of CMC-stabilized vivianite nanoparticle suspension: in Case 2 (suspension-to-soil ratio = 10:1 mL/g).

suspension was used to amend the soils (i.e. vivianite:Pb molar ratio = 8.6:1). Again, after two weeks of the treatment, the leaching equilibrium was reached and Pb leachability was decreased by 94%, 95% and 70%, for the AS, NS and CS, respectively.

Table 5.4 indicates that in Case 2 Pb in the TCLP extracts was reduced to ~0.12 mg/L for NS and 0.06 mg/L for CS after 56-day reaction time. For AS, the TCLP leachable Pb concentration was about 0.89 mg/L after 14 days of treatment, and displayed a slow decreasing trend as the reaction time further increased. At day 56, the Pb concentration reached 0.80±0.05 mg/L.

TABLE 5.4

Changes of Pb concentration in TCLP extracts with treatment time after soils were amended with vivianite nanoparticles in Case 2 (nanoparticle suspension-to-soil ratio = 10:1 mL/g).

Soil type	*Pb* concentration in TCLP extracts (mean ± standard deviation, *mg/L*)						
	Untreated soil	Treatment time (days)					
		1	3	7	14	28	56
Calcareous soil (CS)	0.46 ±0.01	0.37 ±0.03	0.32 ±0.02	0.19 ±0.01	0.14 ±0.02	0.07 ±0.01	0.06 ±0.01
Neutral soil (NS)	2.29 ±0.17	1.02 ±0.09	0.61 ±0.05	0.22 ±0.02	0.20 ±0.02	0.16 ±0.01	0.12 ±0.01
Acidic soil (AS)	14.13 ±1.06	5.53 ±0.50	3.88 ±0.29	1.26 ±0.15	0.89 ±0.08	0.84 ±0.05	0.80 ±0.06

Prior findings by others who applied various phosphate-based agents to immobilize the soil-bound lead provided some important insights into the mechanistic processes. Laperche et al. (1996) reacted hydroxyapatite with litharge/massicot (PbO) and cerussite ($PbCO_3$) in the pH range 5-8 for 2 days and found that the formation of hydroxypyromorphite was the fastest at the lower end pH of 5. The researchers also claimed that at pH <8, hydroxypyromorphite formation appeared to be limited by kinetic rather than thermodynamic constraints. Ma and Rao (1999) found that phosphate rocks were less effective at higher soil pH for Pb^{2+} immobilization. Ryan et al. (2001) and Yang et al. (2001) asserted that dissolution of soil-sorbed Pb^{2+} is the rate-limiting step for formation of pyromorphite-like minerals when sufficient soluble phosphate is provided. Apparently, when solid phosphate (e.g. rock phosphate) is used, dissolution of the phosphate source is also important. Since lower pH favors dissolution of both Pb^{2+} and phosphate, improved Pb^{2+} immobilization is expected at a lower pH. Thus, the observed rapid and substantial reduction in the TCLP leachability for the acidic soil (**Figure 5.2**) is attributed to the relatively easy availability of soil-sorbed Pb^{2+} and the lower soil pH. In contrast, the immobilization rate for NS (pH =6.93) and especially CS (pH=7.85) was a relatively slower process, and thus, was not as effective as for AS.

The fact that the equilibrium was almost reached in ~14 days also revealed that the vivianite nanoparticles provide a relatively easily available source of phosphate for Pb^{2+} immobilization. Compared to macron-scale or granular phosphate sources, the nanoparticles offer much greater soil mobility, greater surface area, and reactivity toward soil-sorbed Pb^{2+}. Chen et al. (2005) studied the effect of grain size of rock phosphate on Pb^{2+} immobilization in various contaminated soils, and concluded that the rock phosphate with small grain size (<35 μm) performed much better than with larger grain size. Stanforth and Qiu (2001) reported that high dosages of solid phosphate such as hydroxyapatite ($Ca_5(PO_4)_3(OH)$) or rock phosphate were not effective for reducing the TCLP leachable Pb to <0.75 mg/L, which is the targeted level of Pb in treating Pb-laden hazardous waste, due to the slow dissolution of the phosphate minerals.

To enhance phosphate availability, various soluble phosphate sources were tested for immobilization of Pb in soils. Theodoratos et al. (2002) evaluated the efficiency of calcium monobasic phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) for heavy metals immobilization in contaminated soils, and observed that TCLP-leachable Pb^{2+} was reduced by 87% by applying >7% (w/w) PO_4^{3-} to soil samples for 2 month. Wang et al. (2001) reported that a combination of 6.25% $Ca(H_2PO_4)_2 \cdot H_2O$ (4.7% as PO_4^{3-}) and 6.25% $CaCO_3$ was able to decrease TCLP-leachable Pb^{2+} by 99% in a contaminated surface soil. Evidently, these soluble phosphate sources were able to offer comparable reduction of Pb^{2+} leachability to that with the nanoparticles. However, the reported treatment dosage of these soluble phosphate sources was typically more than one order of magnitude greater than that of the CMC-stabilized nanoparticles. In addition, application of large quantities of soluble phosphate is critically confined by the subsequent release of excessive amounts of phosphate in the affected environment.

Evidently, to achieve effective stabilization of Pb^{2+} in soils, an amending agent must be kinetically easily available, and yet, any release of excessive phosphate should be minimized. Compared to macro-scale phosphate source materials, the stabilized nanoparticles offer not only much improved soil mobility (i.e. they can be much more easily delivered to the targeted locations), but also much faster reaction kinetics, and thus, much improved effectiveness. Compared to soluble phosphate sources, the stabilized nanoparticles offer better controlled kinetics to sustain a sound formation rate of pyromorphite, yet result in much reduced phosphate leaching from the process as to be demonstrated in Section 3.6.

3.3. BIOACCESSIBILITY OF SOIL-BOUND PB

In vitro bioaccessibility of soil-bound Pb^{2+} has been evaluated by several authors using the PBET method, in which a much harsher solution of pH 2.3 and at a much higher liquid-to-soil ratio (100:1) are employed to simulate the gastrointestinal conditions. **Figure 5.3** shows the PBET-based bioaccessibility of Pb^{2+} in the three soils that were either untreated or treated for 56 days at the two doses of the nanoparticles. First of all, it was interesting that under the harsh PBET conditions, the Pb bioaccessibility for all three untreated soils ranged from 72% for NS to 98% for CS and followed a sequence of CS>AS>NS, which differed markedly from that for the TCLP leachability (**Figure 5.2**). This observation was consistent with the study by Ruby et al. (1999), who stated that Pb-laden mineral phases formed under acidic conditions tend to be more stable in the acidic conditions of the stomach and hence less bioaccessible than mineral phases formed under alkaline conditions. In fact, Pb^{2+} in calcareous soils is predominantly present in the form of $PbCO_3$ (Doner and Grossl, 2002), which will resist leaching by moderately acidic solutions such as the TCLP liquid due to the greater acid buffering capacity. However, under PBET conditions, the soil buffer capacity is exhausted, and as carbonate is dissolved, Pb^{2+} is released. In contrast, for acidic soils, Pb^{2+} is typically sorbed by iron/manganese oxides, organic matter, and clay minerals or even in pyromorphite form (Buatier et al., 2001), all of which are less available than the carbonate-bound form. **Figure 5.3** shows that ~14% of pre-loaded Pb in original AS was not bioaccessible. However, the bioaccessibility of Pb was effectively lowered when the soils were treated with the nanoparticles for 56 days. In Case 1, the bioaccessibility was reduced by 25% for CS, 33% for NS, and 21% for AS. When the dose was increased 5 times (Case 2), the

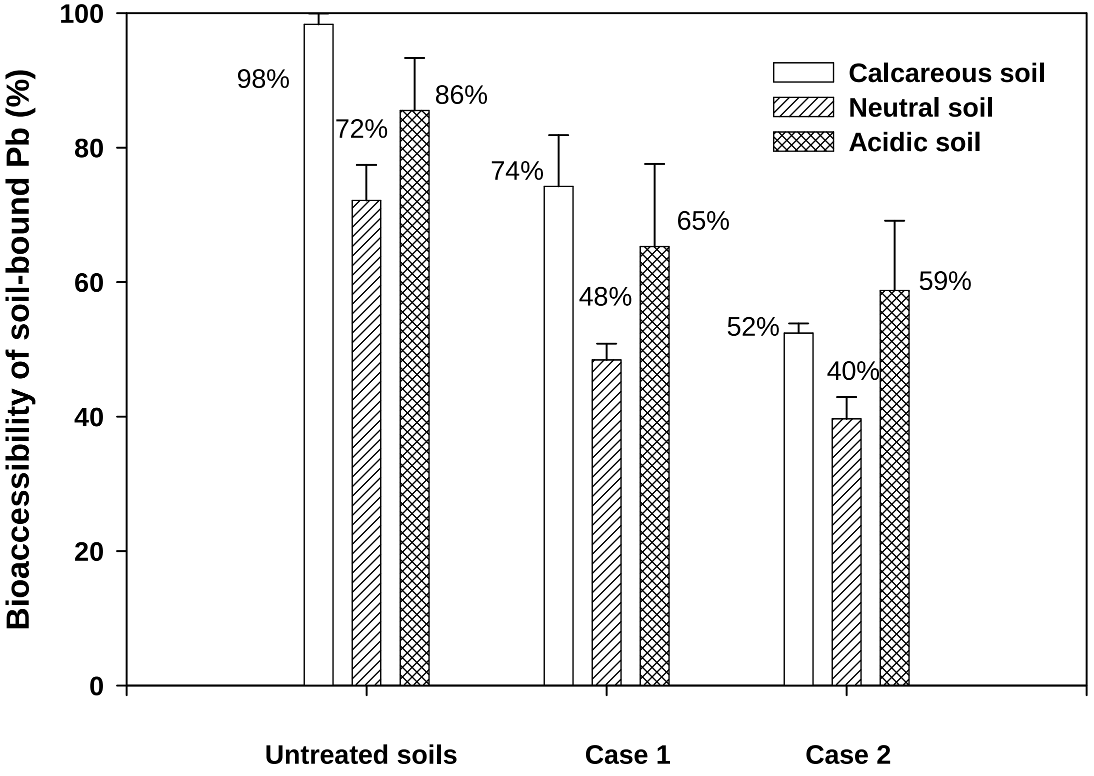


Figure 5.3. Reduction of PBET-based bioaccessibility of soil-bound Pb when soils were amended with 1.56 mM of vivianite nanoparticles for 56 days.

bioaccessibility was reduced by 47% for CS, 45% for NS, and 31% for AS compared to the untreated soils. Yang et al. (2001) reported that bioaccessible Pb in a smelter-contaminated soil (soil pH=7.1) was reduced by 60% after the soil was amended for 70 days with H_3PO_4 at 10 mg-P/g-soil, which was over 3 times greater than the highest dose used in our case. The Pb concentration in the PBET extracts is given in **Table 5.5**.

TABLE 5.5

Pb concentration (mean ± standard deviation, mg/L) in PBET extracts for three soils before and after nanoparticle treatments.

Soil type	Untreated soils	Amended with iron phosphate nanoparticles for 56 days	
		Case 1 (suspension to soil ratio =2:1 mL/g)	Case 2 (suspension to soil ratio =10:1 mL/g)
Calcareous soil	5.50 ± 0.09	4.15±0.42	2.93±0.08
Neutral soil	5.02±0.37	3.37±0.17	2.76±0.22
Acidic soil	5.47±0.50	4.18±0.79	3.76±0.66

3.4. EFFECT OF CHLORIDE ON PB IMMOBILIZATION

A number of researchers have reported direct evidence for the formation of pyromorphites in phosphate-amended Pb-contaminated soils through X-ray diffraction (XRD), scanning electron microscopy (SEM), atomic force microscopy (AFM), and extended X-ray absorption fine structure spectroscopy (EXAFS) measurements (Laperche et al., 1996; Lower et al., 1998; Traina and Laperche, 1999; Ryan et al., 2001; Cao et al, 2002). Based on work by others (Lindsay, 1979; Laperche et al., 1996; Yang et al., 2001), the following reaction stoichiometry is considered to be responsible for the enhanced Pb^{2+} immobilization when the soils are treated with vivianite nanoparticles under acidic conditions:

$$Fe_3(PO_4)_2 \cdot 8H_2O + 4H^+ \Leftrightarrow 3Fe^{2+} + 2H_2PO_4^- + 8H_2O \tag{5.4}$$

$$5Pb^{2+}(aq) + 3H_2PO_4^-(aq) + X^- \Leftrightarrow Pb_5(PO_4)_3X(s) + 6H^+(aq) \tag{5.5}$$

where $X^- = F^-, Cl^-, Br^-$ or OH^-.

At pH >7.20, the following reactions become predominant pathways:

$$Fe_3(PO_4)_2 \cdot 8H_2O + 2H^+ \Leftrightarrow 3Fe^{2+} + 2HPO_4^{2-} + 8H_2O \tag{5.6}$$

$$5Pb^{2+}(aq) + 3HPO_4^{2-}(aq) + X^- \Leftrightarrow Pb_5(PO_4)_3X(s) + 3H^+(aq) \tag{5.7}$$

The solubility product value (K_{sp}) for vivianite is 10^{-36}. However, the K_{sp} value for chloro-, bromo-, hydroxy- and fluoro-pyromorphites is $10^{-84.4}$, $10^{-78.1}$, $10^{-76.8}$, and $10^{-71.6}$, respectively (Ruby et al., 1994). In the absence of halogen or sufficient hydroxyl ions, it is possible that $Pb_3(PO_4)_2$ ($K_{sp} = 8.0\times10^{-43}$) will be formed. Although $Pb_3(PO_4)_2$ is also a fairly stable compound, the presence of halogen ions (such as chloride as commonly found in natural waters) can result in the much more stable halo-pyromorphites, thereby greatly enhancing Pb^{2+} immobilization. In addition, **Eqns 5.4-5.7** also reveal that the treatment pH can also strongly affect the equilibrium and kinetics of pyromorphite formation process, and thus, the Pb immobilization effectiveness.

The effect of chloride ions on immobilization of lead was investigated. **Table 5.6** presents the concentration of TCLP-leachable Pb^{2+} in the three soils amended with the two different doses (Case 1 and Case 2) of nanoparticles for one week and in the presence of 0, 100, and 300 mg/L of Cl^-, respectively.

For AS, the presence of 100 mg/L Cl^- decreased the TCLP leachability of Pb from 5.07 to 3.55 mg/L in Case 1 and 1.26 to 0.89 mg/L in Case 2, i.e. a reduction of ~30% for both cases.

TABLE 5.6

Effects of chloride on TCLP leachable Pb in soils amended with stabilized vivianite nanoparticles (data given as mean ± standard deviation).

Chloride in Nanoparticle Suspension (mg/L)	Acidic soil		Neutral Soil		Calcareous Soil	
	Nanoparticle suspension-to-soil ratio (mL/g)					
	2:1	10:1	2:1	10:1	2:1	10:1
	Pb concentration in TCLP extracts (mg/L)					
0	5.07±0.34	1.26±0.15	1.24±0.09	0.22±0.02	0.31±0.03	0.19±0.01
100	3.55±0.20	2.39±0.15	0.75±0.02	0.34±0.01	0.24±0.01	0.22±0.01
300	4.46±0.32	2.27±0.10	0.77±0.02	0.32±0.01	0.23±0.02	0.19±0.01

For NS, the presence of 100 mg/L Cl^- reduced the leachability of Pb by 40% in Case 1, but a modest 14% in Case 2. For CS, the presence of 100 mg/L Cl^- reduced the TCLP leachability of Pb by ~23% in both cases. In all cases, the effect of Cl^- appears more profound at the lower nanoparticle dose (Case 1), and it did not enhance the immobilization any further as the concentration of Cl^- was tripled from 100 to 300 mg/L. These observations suggest that promoting the formation of the highly stable chloro-pyromorphite can further enhance immobilization of Pb^{2+}; however, this positive effect can be limited by the relative availability of phosphate and Pb^{2+} in soils.

Earlier, Stanforth and Qiu (2001) studied effects of various anions including chloride on immobilization of soil-bound lead using sodium phosphate and concluded that presence of chloride can reduce the TCLP leachability of soil-bound Pb by up to 57~60%, whereas the effects of other anions such as F^- and SO_4^{2-} were less profound (37% reduction with F^-, 19% with SO_4^{2-}).

The fact that Cl^- addition showed more impact on AS and NS than CS also indicates that OH^- (or pH) also plays an important role as revealed by **Eqns. 5.4-5.7**. At alkaline pH, OH^- may compete with Cl^- and result in competitive formation of hydroxyl-pyromorphite and chloro-pyromorphite. Stanforth and Qiu (2001) also observed that the effect of chloride on lead leachability was more profound at pH < 7.0.

3.5. EFFECT OF NANOPARTICLE TREATMENT OF PB SPECIATION IN SOILS

Sequential extraction procedures have been often applied to identifying the relative availability/leachability of soil-sorbed metals by revealing the operationally defined speciation of a metal in the solid phase (Tessier et al., 1979; Shuman, 1985; Han et al., 2001; Scheckel et al., 2003; Peltier et al., 2005). Earlier, Ryan et al. (2001) employed the procedure to studying effects of phosphate amendments on Pb speciation in soils. They found that phosphate treatments resulted in significant shift in soil-bound Pb species from more easily extractable Pb to the least available form (the residual Pb). They also confirmed the formation of chloro-pyromorphite ($Pb_5(PO_4)_3Cl$) via Extended X-ray Absorption Fine Structure (EXAFS) spectroscopic studies. Similar results were also observed by Cao et al. (2002), who studied the soil Pb speciation in various types of soils treated with phosphate. **Figure 5.4** shows the changes in fractions of five operationally defined Pb species in the three soils untreated or treated with two different doses of the vivianite nanoparticles. The five Pb species are defined as Exchangeable (EX), Carbonate-Bound (CB), Fe/Mn Oxides-Bound (OX), Organic Matter-Bound (OM), and Residual (RS) Pb. The relative availability follows the sequence of

$$EX > CB > OX > OM > RS \tag{5.8}$$

Figure 5.4a shows that Pb in the untreated calcareous soil was bound to the soil predominantly in the forms of CB (63%) and OX (33%). The least available RS form accounted for only 2.1%, whereas the EX and OM fractions were negligible in this untreated soil. The observation is expected given the known high carbonate and Fe/Mn, but low organic contents of the soil (**Table 5.1**). It also agrees with the observed low TCLP leachability but high PBET bioaccessibility of Pb in this soil. When the soil was mixed with the nanoparticles in Case 1 and Case 2, Pb leached into the aqueous phase was less than 1% Pb initially in the soil. However, the treatments resulted in a substantial shift in Pb speciation from the more easily available CB species to much less available RS and OM species. After the soils were treated in Case 1 and 2, the CB fraction was reduced from 63% to 40% (a 36 % reduction) and to 22% (a 65% reduction), respectively. Accordingly, the RS fraction was increased from 2.1% to 22% (>10 times) and 32% (>15 times), respectively. Meanwhile, the OX fraction was also increased from 33% to 36% (Case 1) and 42% (Case 2), and OM fraction from 0.7% to 1.9% (Case 1) and 3.1% (Case 2). The distinctive alteration in Pb speciation, especially the substantial increase in the RS fraction, accounts for the reduced leachability and bioaccessibility of Pb by the vivianite nanoparticles. Based on eqns (5.4)-(5.5) and work by others (Cao et al., 2002; Chen et al., 2003; Melamed et al., 2003; Chen et al., 2005), the elevated RS Pb fraction is largely attributed to the formation of the highly stable pyromorphite. The transformation of more easily available Pb species to the less available pyromorphite was also revealed when the

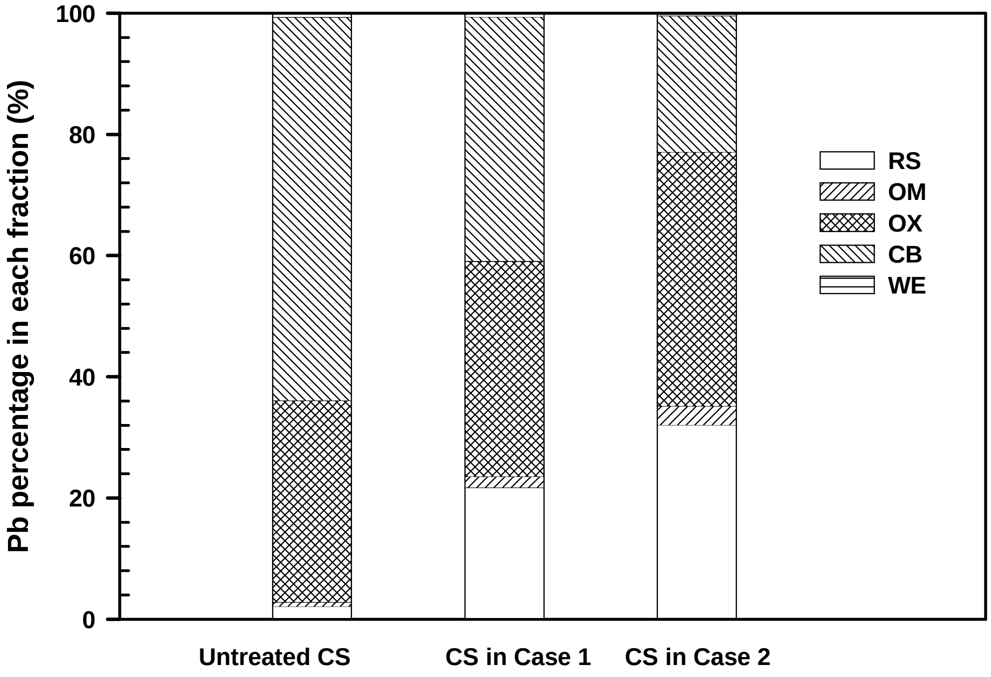


Figure 5.4a. Changes in Pb speciation after the calcareous soil was amended with 1.56 mM vivianite nanoparticle for 56 days (CS: the calcareous soil; WE: water soluble/ exchangeable fraction; CB: carbonate-bound fraction; OX: Fe/Mn oxides-bound fraction; OM-organically bound fraction; RS: residual fraction; Case 1: solution to soil ratio=2:1; Case 2: solution to soil ratio=10:1).

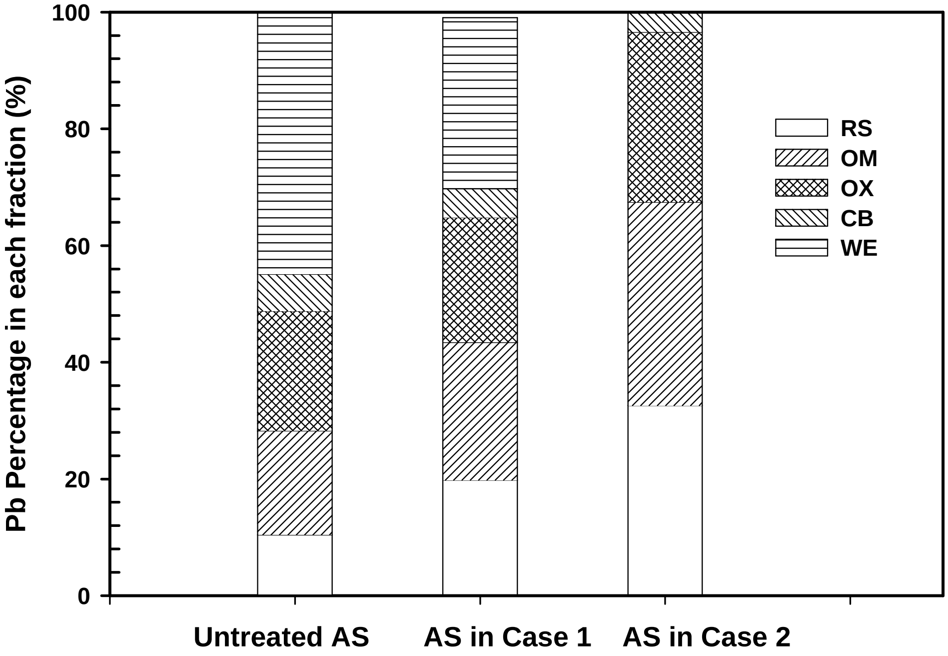


Figure 5.4b. Changes in Pb speciation after the acidic soil was amended with 1.56 mM vivianite nanoparticle for 56 days (AS: the acidic soil; WE: water soluble/ exchangeable fraction; CB: carbonate-bound fraction; OX: Fe/Mn oxides-bound fraction; OM-organically bound fraction; RS: residual fraction; Case 1: solution to soil ratio=2:1; Case 2: solution to soil ratio=10:1).

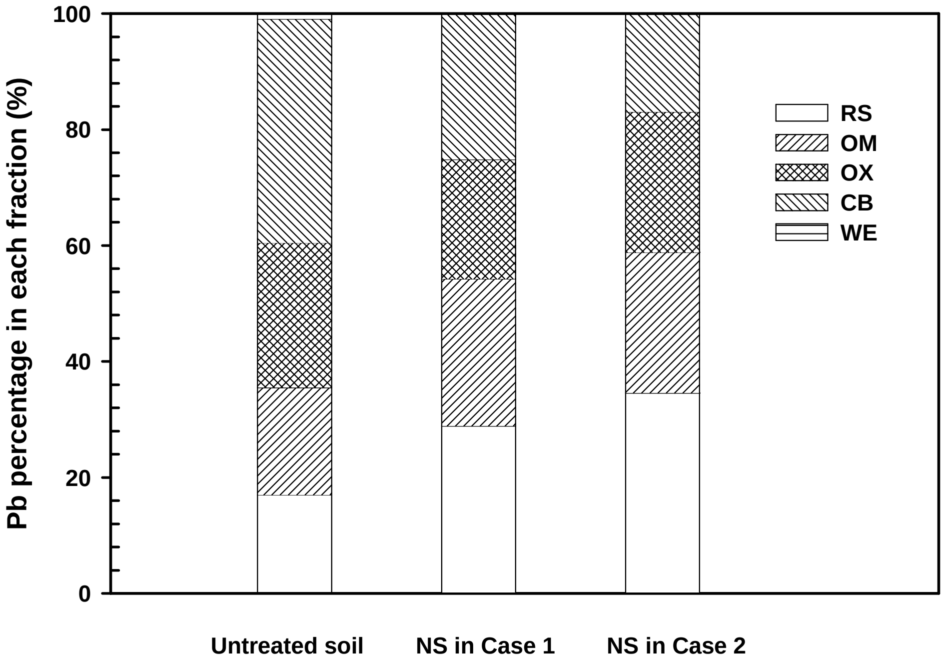


Figure 5.4c. Changes in Pb speciation before and after the neutral soil was amended with 1.56 mM vivianite nanoparticles for 56 days (NS: the neutral soil; WE: water soluble/ exchangeable fraction; CB: carbonate-bound fraction; OX: Fe/Mn oxides-bound fraction; OM-organically bound fraction; RS: residual fraction; Case 1: solution to soil ratio=2:1; Case 2: solution to soil ratio=10:1).

AS and NS soils were treated. **Figure 5.4b** shows that unlike the calcareous soil, the primary Pb species for the untreated acidic soil were split among EX (45%), OX (21%), OM (18%), and RS (10%). Because of the rather great EX fraction, ~3.5% and 5.2% of *Pb* initially in the soil were released into the aqueous phase when the soil was treated in Case 1 and Case 2, respectively. However, the EX *Pb* fraction was reduced by 35% in Case 1 and nearly 100% when the dose was pentupled (Case 2). It is evident from **Figure 5.4b** that the EX fraction was primarily converted to RS (increased by 90%, 210% in Case 1 and Case 2, respectively), OM (33% and 96%, respectively), and OX (4.2% and 42.2%, respectively). **Figure 5.4 c** shows that the distribution of various *Pb* species for the neutral soil lies between that for the calcareous and acidic soils. The nanoparticle treatments leached <1% *Pb* initially in the neutral soil. The conversion of the more easily available *Pb* (EX or CB) to the less available RS and OM species was consistently evident, and thus, is held responsible for the enhanced stability of *Pb* upon the nanoparticle treatment for all the soils.

3.6. POTENTIAL ENVIRONMENTAL IMPACTS OF IRON PHOSPHATE NANOPATILCE APPLICATION

Iron phosphate exists in natural soils, peat deposits and lake sediments often in the form of $Fe_3(PO_4)_2*8H_2O$ (vivianite) (Harris, 2002). There have been no data available on any toxic impacts on the environmental and human health for this mineral (Ralph and Chau, 2006). However, our knowledge on the environmental consequences of synthetic nanoparticles is rather rudimentary, and thus, the long-term environmental fate of the vivianite nanoparticles is yet to be explored. It is also well known that phosphate is a

TABLE 5.7

Phosphate leached from soils after being amended with vivianite nanoparticle suspension or sodium phosphate (NaH_2PO_4) solution for 7 days (data given as mean ± standard deviation)

Soil type	Phosphate (PO_4^{3-}) concentration (C) in solution or mass % leached							
	Vivianite nanoparticle suspension (300 mg/L as PO_4^{3-})				Sodium phosphate solution (300 mg/L as PO_4^{3-})			
	Suspension-to-soil ratio = 2:1 (mL/g)		Suspension-to-soil ratio = 10:1 (mL/g)		Solution-to-soil ratio = 2:1 (mL/g)		Solution-to-soil ratio = 10:1 (mL/g)	
	C (mg/L)	%	C (mg/L)	%	C (mg/L)	%	C (mg/L)	%
Calcareous soil (CS)	6.19 ±0.65	2.08 ±0.22	1.01 ±0.24	0.34 ± 0.08	14.64 ±1.68	4.88 ±2.56	193.23 ±12.62	64.41 ± 5.54
Neutral soil (NS)	9.08 ±1.22	3.05 ±0.23	1.55 ±0.30	0.52 ± 0.10	1.98 ±0.24	0.66 ±0.08	149.01 ±10.62	49.67 ±3.45
Acidic soil (AS)	8.42 ±0.98	2.83 ± 0.33	21.02 ±3.63	7.06 ±0.58	4.68 ±0.33	1.56 ± 0.11	146.01 ±15.63	48.67 ± 5.21

nutrient that often causes eutrophication problem in surface waters; and soluble phosphate is known to be more bioavailable than in its solid forms (Reynolds and Davies, 2001). To address the phosphate leachability, parallel experiments were conducted whenthe equivalent amount (based on PO_4^{3-} concentration) of the CMC-stabilized nanoparticles or a soluble phosphate (NaH_2PO_4) was applied to the treat the three soils in the batch reactors. **Table 5.7** gives the amount of phosphate remained in the supernatant solution after the soils were treated with the two different forms of phosphate for 7 days. Evidently, when the phosphate dosage was low (Case 1), the aqueous phosphate concentration was low and accounted for less than 5% of total phosphate applied for both forms of phosphate and for all the three soils. However, when the phosphate dosage was pentupled (Case 2), nearly 50% of the soluble phosphate applied remained in the aqueous phase compared to less than 1% for CS and NS and ~7% for AS when the vivianite nanoparticles were applied. This observation indicates that the stabilized nanoparticles not only offer great Pb immobilization, but may potentially produce much less leachable phosphate than soluble forms of phosphate. Earlier, Yang et al (2001) also studied Pb immobilization in a smelter-contained urban soil using phosphoric acid, and observed that about 150 mg/L PO_4 (or 38% of phosphate added) remained in the solution after the soil samples were treated with 4 mg/g-soil phosphoric acid for 16 days, and the aqueous phosphate was increased to 460 mg/L PO_4 (i.e. 58% of phosphate added) when the amendment dosage was increased to 8 mg/g-soil.

In order to compare the soil Pb immobilization effectiveness of vivianite nanoparticles and that of soluble phosphate, experiments were also conducted using the same three soil samples to test the effectiveness of soil Pb leachability reductions by NaH_2PO_4 amendment at suspension to soil ratio of 2:1 and 10:1 for 7 days. **Figure 4.5** shows the temporal changes of TCLP leachable Pb in three soils after those samples were amended with 300 mg/L soluble NaH_2PO_4, which contained the same amount of PO_4 as in the nanoparticles. For comparison purpose, the Pb immobilization through nanoparticles amendment results (**Tables 5.3** and **5.4**) are also plotted in the same figure. Experimental results showed that TCLP leachable Pb in the calcareous soil decreased from an initial 0.26 mg/L to 0.0 mg/L after 7-day amendment using NaH_2PO_4 for both suspension to soil ratios, resulting in a 100% Pb immobilization in 1 week (**Figure 5.5a**). However, using the nanoparticle amendment, only 32.6% and 58.7% of Pb were immobilized in a week, although higher reductions were observed for nanoparticles in 2 month (**Tables 5.3** and **5.4**). For the neutral soil, the reduction of TCLP leachable Pb by 10:1 nanoparticles was observed (90.3%) much close to those using 2:1 (72.6%) and 10:1

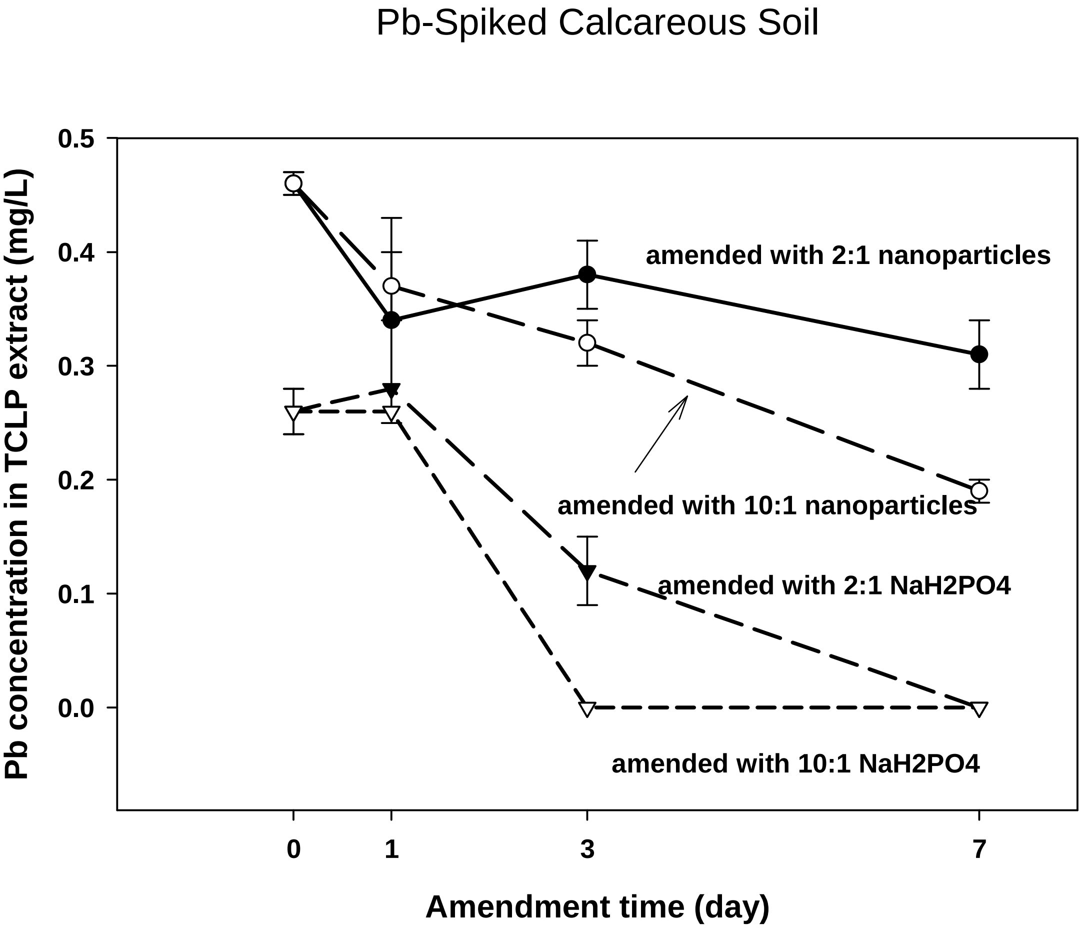


Figure 5.5a. A comparison of nanoparticles and NaH_2PO_4 amendment on Pb immobilization in calcareous soil.

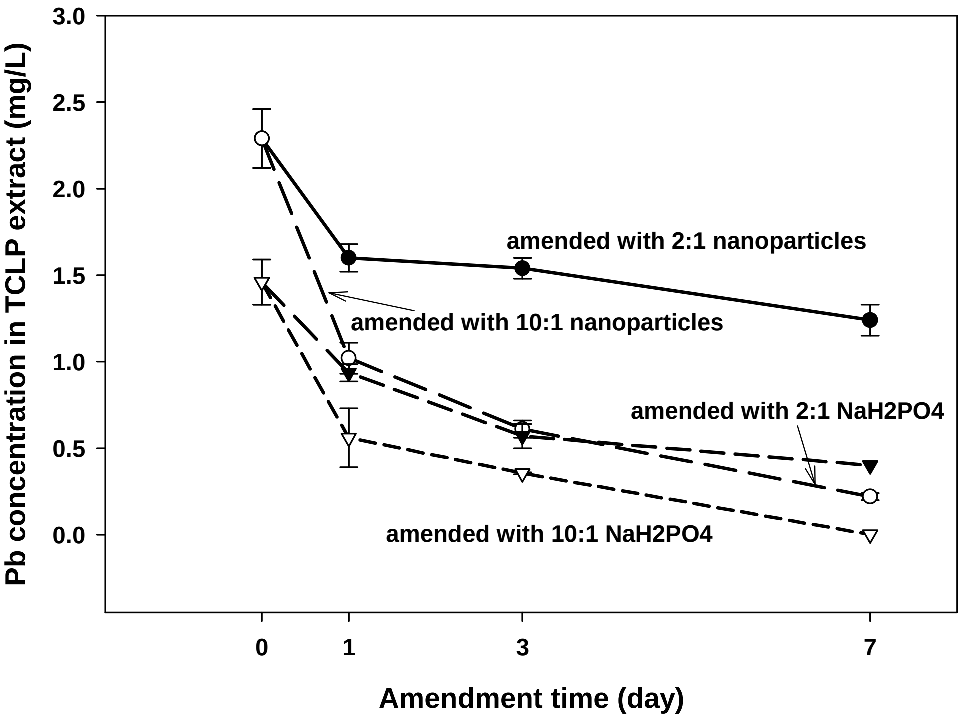


Figure 5.5b A comparison of nanoparticles and NaH_2PO_4 amendment on Pb immobilization in neutral soil.

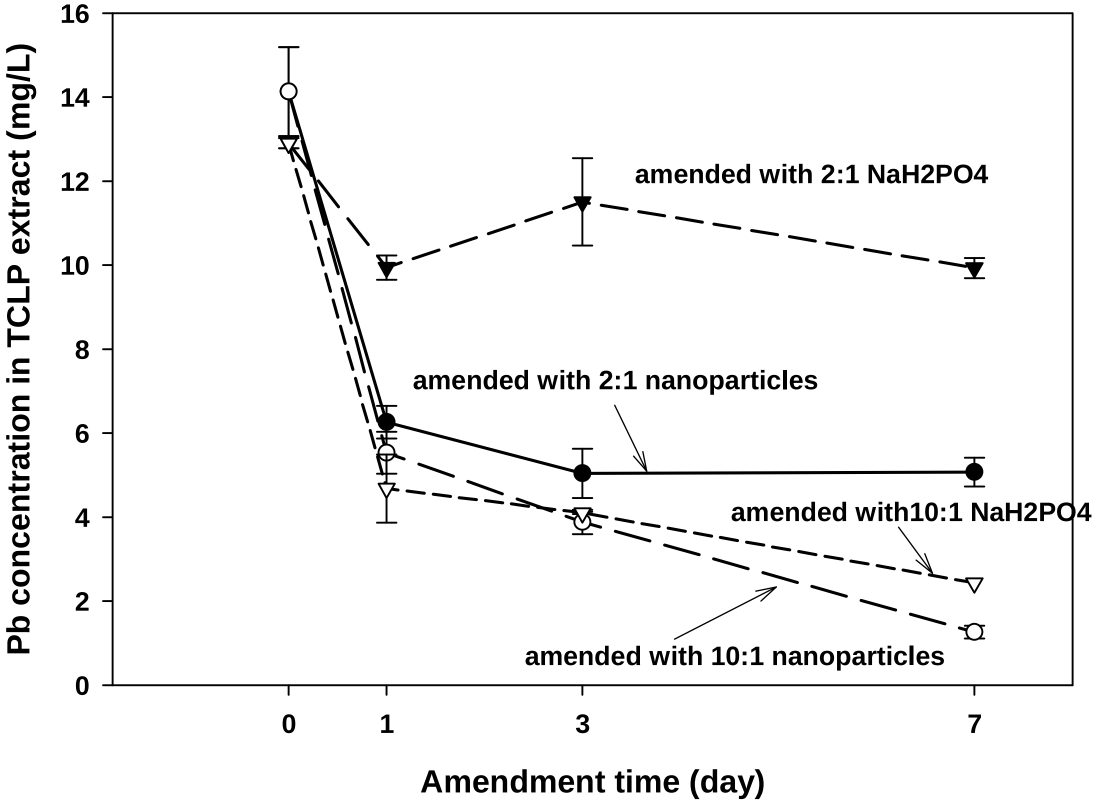


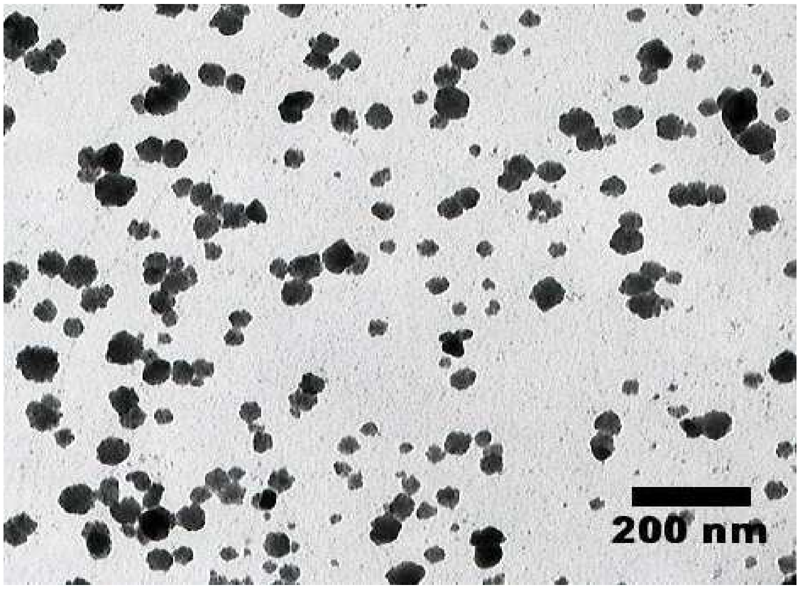
Figure 5.5c. A comparison of nanoparticles and NaH_2PO_4 amendment on Pb immobilization in acidic soil.

NaH_2PO_4 (100 %) while 2:1 nanoparticles achieved only 45.9% of Pb leachability reduction (**Figure 5.5b**). Not much difference was observed in Pb immobilization rate in the acidic soil between nanoparticle and NaH_2PO_4 amendment with 64.7% and 91.1% for the former and 23.4% and 81.5% for the latter. The nanoparticles even exhibited a better immobilization effect than the soluble phosphate. Those results show that the vivianite nanoparticle exhibits a similar Pb immobilization effectiveness with the soluble phosphate for the acidic soil, and higher soil pH inclined to inhibit nanoparticles immobilization process, resulting in lower Pb immobilization effectiveness of nanoparticles than that of the soluble phosphate for the neutral soil and calcareous soil. These results further confirmed that dissolution of the nanoparticle is the controlling step for Pb immobilization as shown in equations 5.4 and 5.6, and higher pH hinders the process and thus the immobilization effectiveness. Accordingly NaH_2PO_4 immobilization results were not affected much by soil pH.

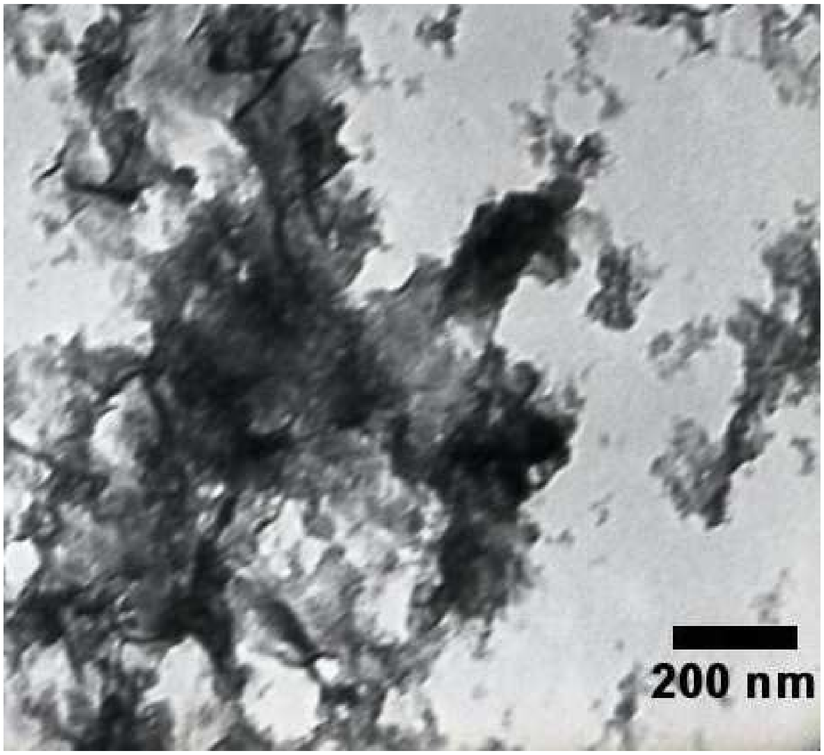
3.7. EFFECTS OF IRON SULFIDE NANOPARTICLES ON THE LEACHABILITY OF SOIL-BOUND PB

3.7.1 Characterization of the iron sulfide nanoparticles

Figure 5.6 shows that CMC stabilized iron sulfide precipitant was present as discrete particles with average size about 40±28 nm (**a**) while the non-stabilized counterpart showed as agglomerated flocs in morphology (**b**). Therefore stabilized iron sulfide should have higher transportability than the non-stabilized one, suggesting the promising



(a)



(b)

Figure 5.6 TEM images of iron sulfide (FeS) **a**. with CMC stabilizer; **b** without CMC stabilizer (by courtesy of Mr. Z. Xiong).

application of the iron sulfide nanoparticles in remediating the heavy metal contaminants in subsurface.

Minteq calculation indicated that the 99.3 % of added Fe^{2+} and S^{2+} in the solution precipitated with 0.01 mM the iron and sulfide remained in the solution and 1.43 mM FeS solid (Mackinawite) was produced as nanosized particles. The following reaction (**Eqn 5.9**) reveals the process of FeS formation:

$$Fe^{2+} + \quad S^{2-} \Leftrightarrow \quad FeS \downarrow \tag{5.9}$$

3.7.2 Effects of FeS nanoparticles on Pb leachability

Figure 5.7a shows the changes of Pb leachability in three soils with time after the soil samples were amended with FeS nanoparticles in case 1 where the ratio of the nanoparticle to soil was 2:1 and the molar ratio of FeS to Pb in soils was about 1:1. Results indicated that the nanoparticle showed a prominent impact by decreasing the Pb leachability for acidic soil. Pb leachbility deceased from 44% to 28% after 1 M with a reduction of 36.4%. The Pb concentrations in the TCLP extract reduced from 14 mg/L to 9.00 mg/L (**Table 5.8**). For the neural soil and calcareous soil, the Pb leachability in the exhibited a slower but also obvious tendency of reduction: one-month reduction was 21% and 40% for the NS and CS, respectively.

Increased reduction rate of Pb leachability was observed (**Figure 5.7b**) when the amendment ratio increased for 2:1 to 10:1 where the molar ratio of FeS to soil Pb was 5:1. In this case, the 1-month reduction rates were 52.3% for acidic soil, 28.8% for the neutral soil and 53.3% for the calcareous soil. The changes of Pb concentration in the TCLP

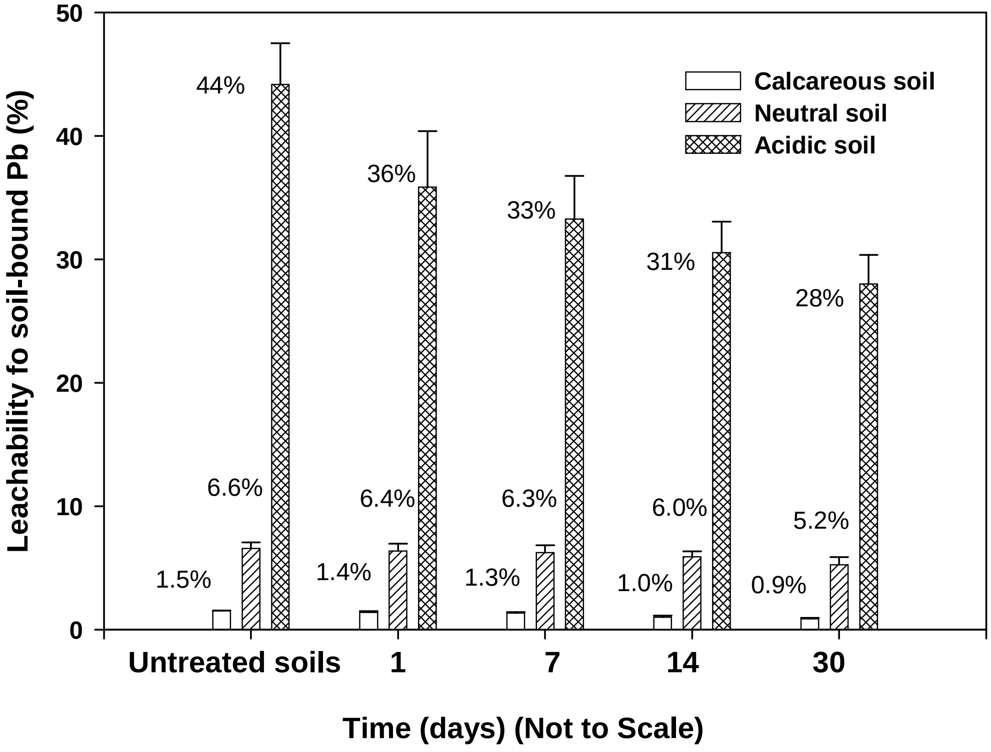


Figure 5.7a. Reduction of Pb leachability in soils with treatment time when the soils were amended with 1.43 mM FeS nanoparticle suspension at a ratio of 2:1(mL/g)

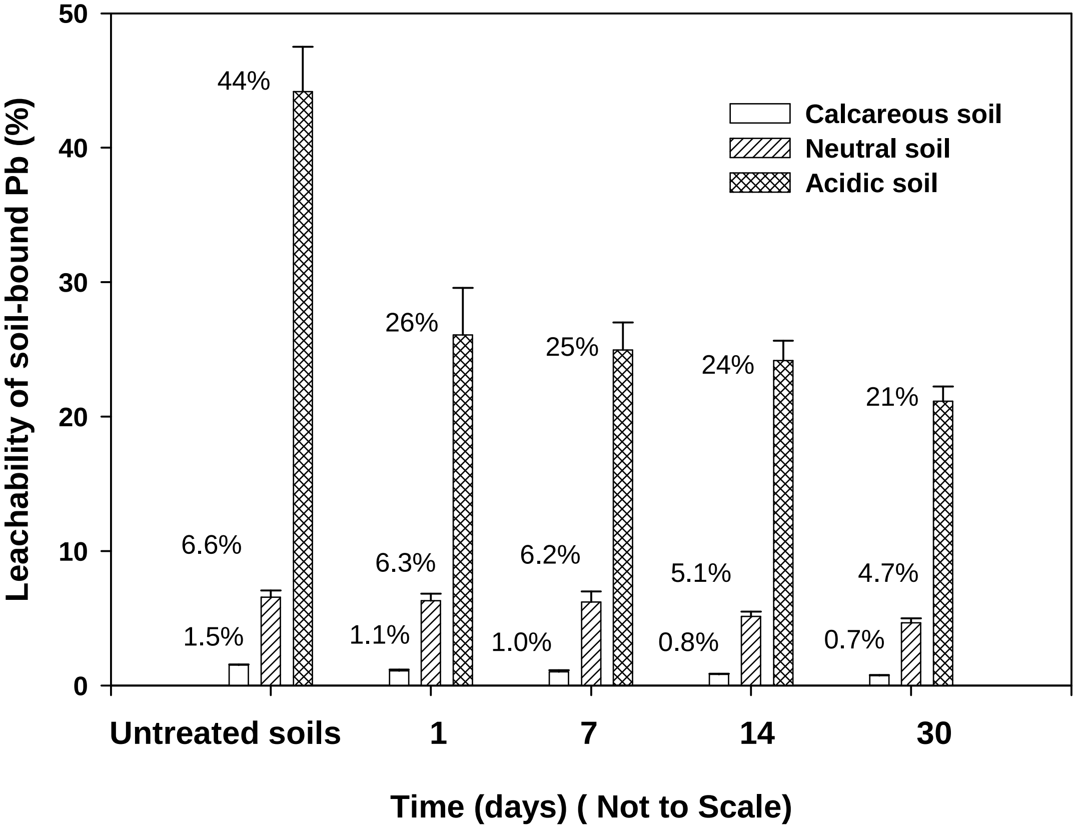


Figure 5.7b. Reduction of Pb leachability in soils with treatment time when the soils were amended with 1.43 mM FeS nanoparticle suspension at a ratio of 10:1(mL/g)

TABLE 5.8

Changes of Pb concentrations in the TCLP extracts with treatment time after soils were amended with 1.43 mM FeS nanoparticle suspension

Aging time (day)	Solution to soil ratio 2:1			Solution to soil ratio 10:1		
	CS	NS	AS	CS	NS	AS
Untreated	0.46±0.01	2.29±0.17	14.13±1.06	0.46±0.01	2.29±0.17	14.13±1.06
1	0.42±0.04	2.21±0.22	11.47±1.44	0.33±0.04	2.20±0.18	8.34±1.12
7	0.40±0.04	2.17±0.21	10.64±1.12	0.30±0.05	2.17±0.27	7.99±0.65
14	0.30±0.05	2.05±0.16	9.77±0.80	0.25±0.02	1.79±0.12	7.73±0.46
30	0.26±0.04	1.83±0.22	8.96±0.75	0.21±0.02	1.63±0.11	6.76±0.35

extract could be found in **Table 5.8**. The following equation (**Eqn 5.10**) may be responsible for the Pb leachability changes with FeS amendment.

$$FeS \quad + \quad Pb^{2+} \Leftrightarrow PbS \quad + \quad Fe^{2+} \tag{5.10}$$

Where Ksp for FeS and PbS are 8×10^{-19} and 3×10^{-28}

3.8. EFFECTS OF IRON OXIDE (MAGNETITE, FE_3O_4) ON THE LEACHABILITY OF SOIL-BOUND PB

3.8.1. Characterization of the iron oxide nanoparticles

Si et al (2004) indicated that the TEM particle size of the magnetite nanoparticle prepared with 0.5% NaCMC was 14.4±0.92 nm (standard deviation). The authors suggested the following reaction (**Eqn 5.11**) may be responsible for the formation of magnetite in this study although the detailed mechanism is still unclear (Si et al, 2004).

$$Fe^{2+} \quad + \quad O_2 \quad + \quad OH^- \Leftrightarrow Fe_3O_4 \quad + \quad H^+ \tag{5.11}$$

3.8.2. Effects of magnetite (Fe_3O_4) nanoparticles on Pb leachability

Changes of soil Pb leachability with magnetite nanoparticle amendments were shown in **Figures 5.8a** and **5.8b**. As lower amendment ratio (solution to soil=2:1, Fe:Pb molar ratio = 20~23) was applied, Pb leachability exhibited a moderate decreases with aging time (**Figure 5.8a**). For example, the acidic soil reduced by 54.5% in Pb leachability in a month while the neutral soil and calcareous soil showed 43.5% and 60% reductions, respectively. Significant Pb immobilization was observed for those three soils when the amendment rate increased to 10:1 where Fe:Pb molar ratio=98~114 (**Figure 5.8b**): TCLP leachability of Pb in the acidic soil decreased by 93.4 in a month from the initial 44% to the final 20% and 86.4% reduction was observed for the neutral soil after 1 month aging. For the calcareous soil, the reduction was 73.3%. **Table 5.9** shows the changes of Pb concentrations in the TCLP extracts of the three soils with time.

Magnetite nanoparticles operate in reducing the TCLP leachability of soil-bound Pb. The mechanisms might include the adsorption of Pb on the magnetite surface or in the lattice, or just due to the increased soil pH caused by the nanoparticle solution with a pH of 12. Soil pH changes due to nanoparticle amendment was shown in **Figure 5.9**.

Overall, experimental data showed that iron phosphate nanoparticles exhibited better results in reducing the leachability of soil-bound Pb than iron sulfide or iron oxide nanoparticles. But the latter may pose less risk in surface water eutrophication and iron oxide nanoparticles were rather stable in the oxidizing environment. Detailed study is

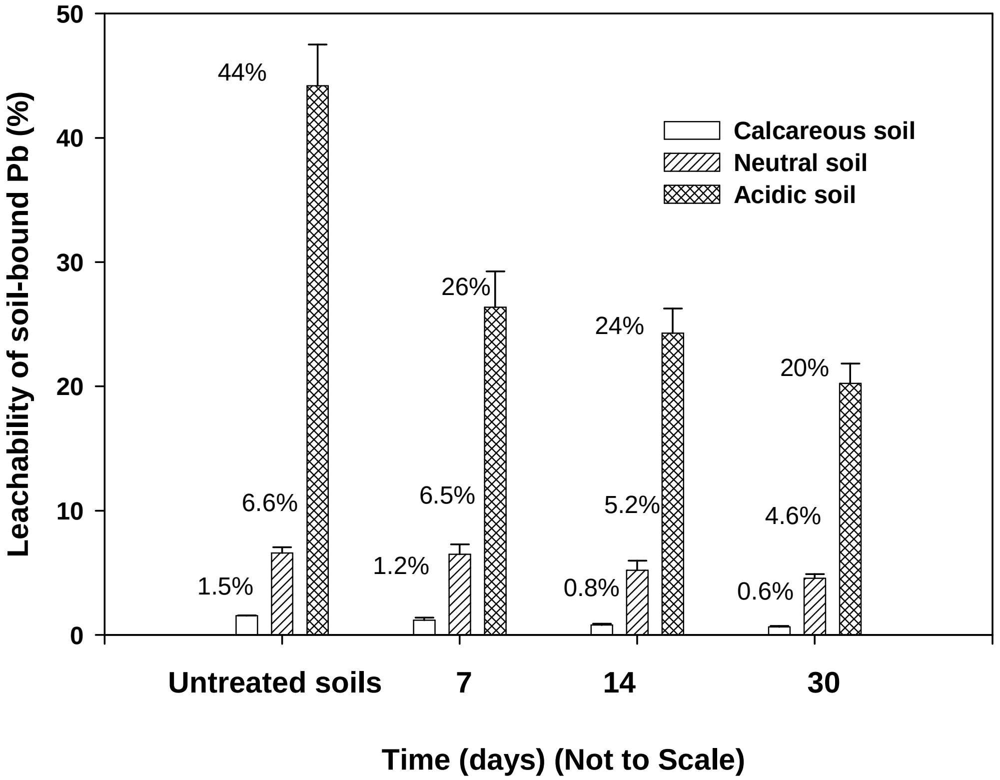


Figure 5.8a. Reduction of Pb leachability in soils with treatment time when the soils were amended with 30 mM magnetite nanoparticles suspension at a ratio of 2:1(mL/g)

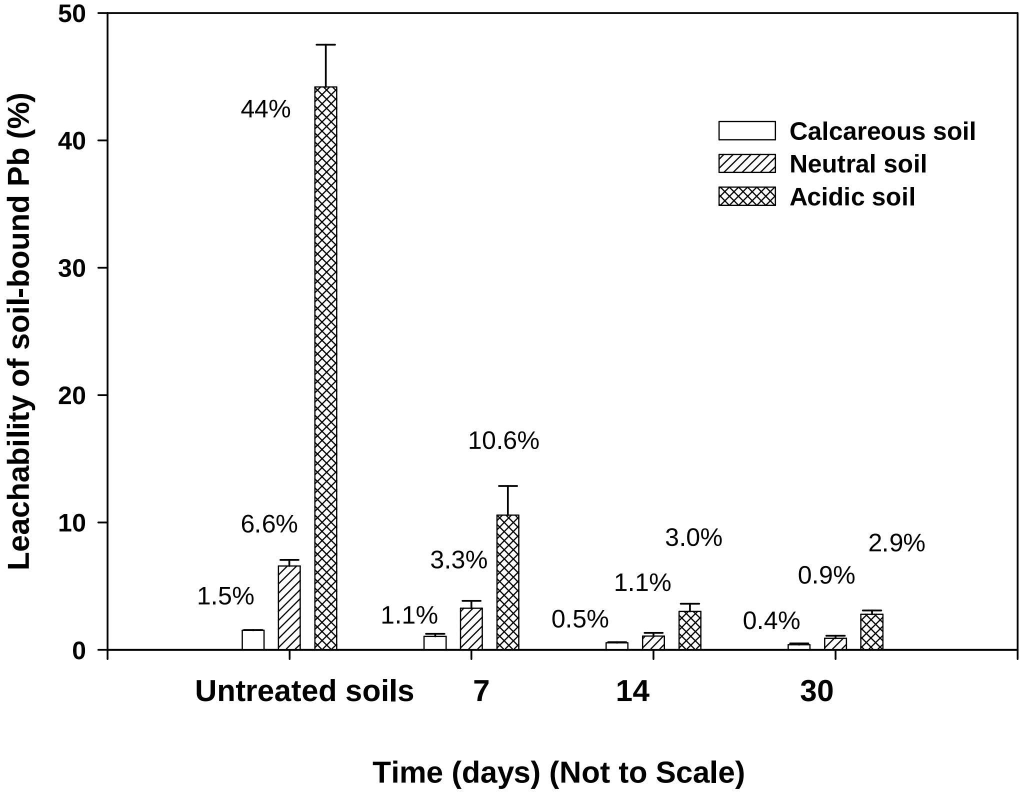


Figure 5.8b. Reduction of Pb leachability in soils with treatment time after the soils were amended with 30 mM magnetite nanoparticle suspension at a ratio of 10:1(mL:g)

needed to probe the mechanics and feasibility of application of iron sulfide and iron oxide nanoparticles to immobilize soil-bound heavy metals

TABLE 5.9

Changes of Pb concentrations in the TCLP extracts with treatment time after soils were amended with 30 mM magnetite nanoparticle suspension

Aging time (day)	Solution to soil ratio 2:1			Solution to soil ratio 2:1		
	CS	NS	AS	CS	NS	AS
Untreated	0.46±0.01	2.29±0.17	14.13±1.06	0.46±0.01	2.29±0.17	14.13±1.06
7	0.36±0.09	2.26±0.28	8.43±0.93	0.32±0.09	1.13±0.21	3.38±0.73
14	0.24±0.04	1.81±0.26	7.76±0.64	0.16±0.02	0.37±0.09	0.97±0.19
30	0.18±0.03	1.59±0.11	6.47±0.51	0.12±0.04	0.31±0.07	0.89±0.10

Conclusions

This work reports a new class of iron phosphate nanoparticles prepared using low concentrations of a modified food-grade cellulose (CMC) as a stabilizer. Compared to soluble phosphate or granular solid phosphate sources, the stabilized nanoparticles displayed some unique properties such as improved control of soil mobility, deliverability and reactivity. When used for in situ immobilization of Pb in soils, the nanoparticles offered both fast reaction kinetics and minimal phosphate leachability.

Batch experimental data showed that relatively low dosages (P:Pb molar ratio = 1.8-11) of the stabilized vivianite nanoparticles can effectively decrease the TCLP leachability and PBET bioaccessibility of Pb in three representative soils (calcareous, neutral and acidic). The significant decrease of Pb availability in the soils is attributed to

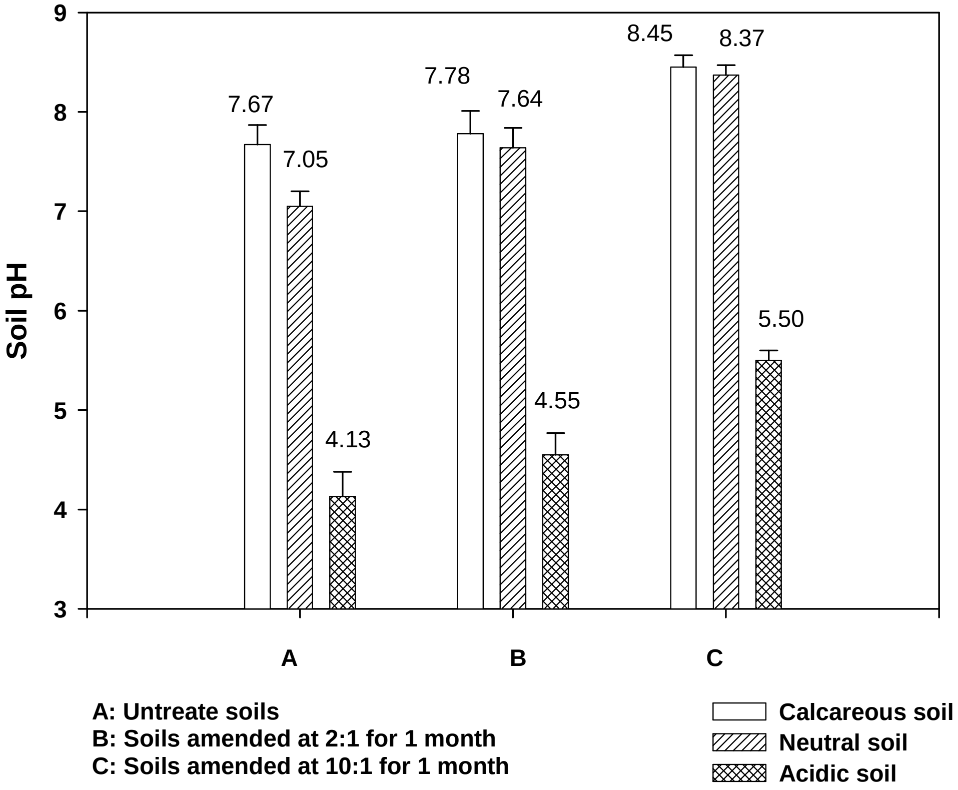


Figure 5.9 Changes of soil pH due to magnetite nanoparticle amendment

the formation of pyromorphite minerals through the iron phosphate nanoparticle amendment.

Lead leachability in the acidic soil was reduced by 56% after the soil was treated with the nanoparticles at a P:Pb molar ratio of 1.8-2.2 for 1 day compared to a 30% and 26% reduction for the neutral soil and the calcareous soil, respectively. At a higher P:Pb molar ratio of 9.0 -11, the 1-day treatment reduced Pb leachability by 61% for the acidic soil, 55% for the neutral soil and 19% for the calcareous soil. Faster kinetics of Pb immobilization was observed in the acidic soil samples, confirming that the lead immobilization is facilitated by the formation of pyromorphites and is affected by the availability of both Pb^{2+} and PO_4^{3-} in soils.

Addition of chloride (Cl^-) further decreased the TCLP leachability of Pb^{2+} in the soils, which supports the assertion that formation of the least soluble chloro-pyromorphite is responsible for enhanced immobilization of soil-bound Pb. The presence of 100 mg/L of chloride was able to reduce the TCLP leachability of Pb by 30% for the acidic soils, 40% for the neutral soil, and 23% for the calcareous soil.

Results from a sequential extraction procedure showed that the nanoparticle treatment of the soils converted large fractions of water soluble/exchangeable and carbonate-bound Pb^{2+} to the most stable form of the residual Pb^{2+}, i.e. the highly stable pyromorphite minerals, resulting in the enhanced Pb^{2+} immobilization. The stabilized iron phosphate nanoparticles produce much less phosphate leachate compared to soluble phosphate salts, especially when greater dosage of phosphate is needed, which substantially reduces the risk of secondary contamination (i.e. eutrophication). The newly prepared nanoparticles and the proposed technology may offer a useful alternative for in situ remediation of Pb-contaminated soils or solid wastes.

Iron sulfide and iron oxide nanoparticles also exhibited apparent effects in immobilizing the soil-bound Pb, but less significant than iron phosphate nanoparticles.

CHPATER VI

OVERALL CONCLUSIONS AND FUTURE WORK

1. Summary

The general conclusions of this dissertation are as follows,

1. All Cu added to the catfish pond transferred to the pond bottom in 2 days after each application and the remaining Cu concentration in the water was less than 30 μg/L.
2. Copper became associated with particles as soon as it was applied to the pond water through absorption and precipitation processes, which suggested that transport of Cu in the catfish pond was primarily in particulate form (particle-facilitated transport).
3. Application of Cu in the catfish pond did not result in significant Cu accumulation in the catfish meat. Contamination of the ground water and surface water by the application of Cu in the catfish pond aquaculture also seemed insignificant
4. Care should be taken in disposal of the Cu -laden pond sediment and the pond effluent because the bioaccessibility of the sediment-bound Cu is still very high. Hazardous effects may occur when the Cu-sensitive species keep feeding on the sediments.

5. The residual phase is an important Cu phase in the commercial catfish pond sediments, suggesting Cu was bound strongly with the sediment particles. However, AVS (acid volatile sulfide) was not observed as a significant binding phase for Cu in the sediments.
6. The synthetic precipitation leaching procedure (SPLP), which simulates the acid rain, released less than 1% of the sediment-bound Cu, and the toxic characteristic leaching procedure (TCLP), which resembles the landfill environment, leached about 1~8% of the Cu while the physiological based extraction test (PBET), mimicking the gastrointestinal conditions, could dissolve up to 100% of the sediment-bound Cu.
7. Availability of Cu in the sediment was highly correlated to the speciation of the metal in the solid phase.
8. Aging and higher content of certain soil iron oxides, organic matter and clay minerals could decrease the Cu bioaccessibility in the soils but soil carbonates have little effect.
9. Vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) nanoparticles were successfully synthesized using carboxymethylcellulose as a stabilizer. The average particle size was 8.4 nm
10. Immobilization of soil-bound Pb using the iron phosphate nanoparticles showed a promising technology in heavy metal remediation with easy delivery and low phosphate leaching.

2. Recommendations for Future Work

Overall the dissertation researched and discussed the interactions of heavy metals (Cu and Pb) with soil composition through various leaching procedures. The results and conclusions of this dissertation might be useful to environmental scientists and engineers, soil scientists, limnologist, and those concerned in legal and management aspects of surface and groundwater. More research is needed to study the heavy metal interaction with soil using spectroscopic methods. Study and modeling on the particle-facilitated transport of heavy metals seems also necessary. Moreover, practical application of the nanotechnology of heavy metal immobilization requires additional research on the nanoparticle transport in the subsurface and effect of iron phosphate nanoparticle on stabilization of other heavy metals. The specific recommendations were made as follows:

1. Investigate Cu speciation in the sediment using spectroscopic methods such as X-ray diffraction spectroscopy (XRD) and X-ray absorption spectroscopy (XAS) and find the correlation between Cu speciation based on the spectroscopic methods and that based on sequential extraction method.
2. Model the Cu transport in the subsurface environment in particulate form and validate the models using experimental research and field investigations. The model (s) can facilitate in understanding the behaviors of some contaminants which are tightly absorbed by soil particles such as Cu, Pb, and nuclides.
3. Provide the spectroscopic evidence of formation of pyromorphite after the Pb-contaminated soil (or pure Pb minerals) reacts with the vivianite nanoparticles to further support the conclusions on Pb immobilization.

4. Study and model the transport of the vivianite nanoparticles in the subsurface and field test (e.g. in a shooting range) the efficiency of Pb immobilization using the nanoparticles.
5. Investigate the effects of oxygen on Pb immobilization using the nanoparticles.
6. Research the immobilization of other heavy metals in soils such as Cu, Cd, Zn and Ni using the vivianite nanoparticles.
7. Vivianite nanoparticles contain not only the ligand to sequester heavy metals (PO_4^{3-}) but also a reductive component (Fe(II)). The optimum detoxifying result may be achieved if the nanoparticles are applied to treat some contaminants whose toxicity could be lessened by reduction reaction and whose solubility could be greatly deceased by phosphate sequestration such as uranium.

REFERENCES

Abdel-Rahman, M. S., Skowronski, G. A., & Turkall, R. M. (2005). *In vitro* penetration of pig skin by heavy metals in soil. *Soil and Sediment Contamination*, 14, 123-134

Abou-Arab, A. A. K., Ayesh, A. M., Amra, H. A., & Naguib, K. (1996). Characteristic levels of some pesticides and heavy metals in imported fish. *Food Chemistry*, 57, 487-492

Admassu, W. & Breese, T. (1999). Feasibility of using natural fishbone apatite as a substitute for hydroxyapatite in remediating aqueous heavy metals. *Journal of Hazardous Materials*, 69, 187-196

Akcay, H., Oguz, A., & Karapire, C. (2003). Study of heavy metal pollution and speciation in Buyak Menderes and Gediz river sediments. *Water Research,* 37, 813-822

Allen, H. E., Fu, G., & Deng, B. (1993). Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. *Environmental Toxicology and Chemistry,* 12, 1441-1453

Allen, H. E. (Ed) (1995). *Metal Contaminated Aquatic Sediments*. (Chelsea, Michigan: Ann Arbor Press, Inc.)

Anderson, J. U. (1963). An improved pretreatment for mineralogical analysis of samples containing organic matter. *Clays and Clay Minerals*, 10, 380-38

Ankley, G. T., Mattson, V. R., Leonard, E. N., West, C. W., & Bennett, J. L. (1993). Predicting the acute toxicity of copper in freshwater sediments: evaluation of the role of acid-volatile sulfide. *Environmental Toxicology and Chemistry*, 12, 315-320

Ankley, G. T., Di Toro, D. M., Hansen, D. J. & Berry, W. J. (1996). Technical basis and proposal for deriving sediment quality criteria for metals. *Environmental Toxicology and Chemistry*, 15, 2056-2066

Astrup, T., Stipp, S. L. S., & Christensen, T. H. (2000). Immobilization of chromate from coal fly ash leachate using an attenuating barrier containing zero-valent iron. *Environmental Science and Technology*, 34, 4163-4168

ATSDR: Agency for Toxic Substances and Disease Registry. (2004, September). ToxFAQs^{TM} for Copper. Retrieved July 18, 2005, from http://www.atsdr.cdc.gov /tfacts132.html

ATSDR: Agency for Toxic Substances and Disease Registry. (2005, February). Top 20 hazardous substances from the 2005 CERCLA priority list of hazardous substances. Retrieved February 20, 2005, from http://www.atsdr.cdc.gov/cxcx3.html

Avnimelech, Y. & Zohar, G. (1986). The effect of local anaerobic conditions on growth retardation in aquaculture systems. *Aquaculture*, 58, 167-174

Barrow, N. J. (1987). *Reaction with Variable-Charge Soils.* (Boston, Massachusetts: Martinus Nijhoff Publishers). pp. 73-74.

Basta, N. T., Gradwohl, R., Snethen, K. L. & Schroder, J. L. (2001). Chemical immobilization of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate. *Journal of Environmental Quality*, 30, 1222-1230

Benjamin, M. M. & Leckie, J. O. (1980). Adsorption of metals at oxide interfaces: Effects of the concentrations of adsorbate and competing metals. (In R. A. Baker (Ed.), *Contaminants and Sediments (Volume 2): Analysis, Chemistry, Biology* (pp.305-332). Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.)

Berry, W. J., Hansen, D. J., Mahony, J. D., Robson, D. L., Di Toro, D. M., Shipley, B. P., Rogers, B., Corbin, J. M., & Boothman, W. S. (1996). Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. *Environmental Toxicology and Chemistry*, 15, 2080-2094

Berti, W. R. & Cunningham, S. D. (1997). In place inactivation of Pb in Pb contaminated soils. *Environmental Science and Technology*, 31, 1359-1364

Boyd, C. E. (1982). Hydrology of small experimental fish ponds at Auburn, Alabama. *Transactions of the American Fisheries Society*, 111, 638-644

Boyd, C. E. (1990). *Water Quality in Ponds for Aquaculture*. (Birmingham, Alabama: Birmingham Publishing Co.). p. 340

Boyd, C. E. (1995). *Bottom Soils, Sediment, and Pond Aquaculture*. (New York: Chapman &Hall). pp. 194, 214, 257-258, 261, 262, 287, 297

Boyd, C. E. & Tucker, C. S. (1998). *Pond Aquaculture Water Quality Management*. (Norwell, Massachusetts: Kluwer Academic Publishers). pp. 413-437

Boyd, C. E., Queiroz, J., Lee, J., Rowan, M., Whitis, G., & Gross, A. (2000). Environmental assessment of channel catfish *Ictalurus punctatus* farming in Alabama. *Journal of the World Aquaculture Society*, 31, 511-544

Boyd, C. E. (2000). EPA rule-making for aquaculture effluents in the United States. *The Advocate (December)*, 81-82

Boyer, L. F., McCall, P. L., Soster, F. M., & Whitlatch, R. B. (1990). Deep sediment mixing by burbot (*Lota lota*), Caribou Island Basin, Lake Superior, USA. *Ichnos*, 1, 91-95

Bremner, I. (1979). Copper toxicity studies using domestic and laboratory animals. (In J. O. Nriagu (Ed.), *Copper in the Environment (part II: Health Effects)* (p.285-306). New York: John Wiley & Sons)

Buatier, M. D., Sobabska, S., & Elsaas, F. (2001). TEM and EDX investigation on Zn and Pb contaminated soils. *Applied Geochemistry*, 16, 1165-1177

Bull, D. C. & Williamson, R. B. (2001). Prediction of principal metal-binding solid phases in estuarine sediments from color image analysis. *Environmental Science and Technology*, 35, 1658-1662

Cao, X., Ma, L. Q., Chen, M., Singh, S. P. & Harris, W. G. (2002). Impacts of phosphate amendments on lead biogeochemistry at a contaminated site, *Environmental Science and Technology*, 36, 5296-5304

Cao, X., Dermatas, D. & Christodoulatos, C. (2005, October). A challenge for the applicability of regulatory leaching tests for assessing lead leachability in shooting range soils: comparison of TCLP and SPLP. (Paper presented at the 21st annual international conference of soil, sediment and water, University of Massachusetts, Amherst, Massachusetts)

Chang, C. & Chen, D. (2005). Preparation and adsorption properties of monodisperse chitosan-bound Fe_3O_4 magnetic nanoparticles for removal of Cu(II) ions. *Journal of Colloid and Interface Science*, 283, 446-451

Chen, M., Ma, Q. L., Singh, P. S., Cao, R. X., & Melamed, R. (2003). Field demonstration of in situ immobilization of soil Pb using P amendments. *Advances in Environmental Research*, 8, 93-102

Chen, S. B., Zhu, Y. G., & Ma, Y. B. (2005). The effect of grain size of rock phosphate amendment on metal immobilization in contaminated soils. *Journal of Hazardous Materials*, 134, 74-79

Clesceri, L. S., Greenberg, A. E., & Eaton, A. D. (Eds.) (1998). *Standard Methods for the Examination of Water and Wastewater (20th edition)*. (Washington, D.C.: American Public Health Association). P.1.33 and p.2.27

Cotter-Howells, J., & Caporn, S. (1996). Remediation of contaminated land by formation of heavy metal phosphates. *Applied Geochemistry*, 11, 335-342

Deng, Y. J. & Dixon, J. B. (2002). Soil organic matter and organic –mineral interactions. (In J. B. Dixon & D. G. Schulze (Eds), *Soil mineralogy with environmental applications* (pp.69-107). Madison, Wisconsin: Soil Science Society of American, Inc.)

Di Toro, D. M. (2001). *Sediment Flux Modeling*. (New York: John Wiley & Sons). p.18

Di Toro, D. M., Mahony, J. D., Hansen, D. J., Scott, K. J., Carlson, A. R., & Ankley, G. T. (1992). Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. *Environmental Science and Technology*, 26, 96-101

Dollar, N. L., Souch, C. J., Filippelli, G. M., & Mastalerz, M. (2001). Chemical fractionation of metals in wetland sediments: Indiana Dunes National Lakeshore. *Environmental Science and Technology*, 35, 3608-3615

Doner, H. E., & Grossl, P. R. (2002). Carbonates and evaporates. (In J. B. Dixon & D. G. Schulze (Eds.), *Soil Mineralogy with Environmental Applications* (pp.217-218). (Madison, Wisconsin: Soil Science Society of American, Inc.)

Eighmy, T .T., Crannell, B. S., Butler, L. G., Cartledge, F. K., Emery, E. F., Oblas, D., Krzanowski, J. E., Eusden, J. D., Jr., Shaw, E. L., & Francis, C. A. (1997). Heavy metal stabilization in municipal solid waste combustion dry scrubber residue using phosphate. *Environmental Science and Technology*, 31, 3330-3338

Ewers, U. & Schlipköter, H.-W. (1991). Lead (In E. Merian (Ed.), *Metals and Their Compounds in the Environment* (pp.971-1014). New York: VCH Publishers, Inc.)

Fan, W., Wang, W., Chen, J., Li, X., & Yen, Y. (2002). Cu, Ni, and Pb speciation in surface sediments from a contaminated bay of northern China. *Marine Pollution Bulletin*, 44, 816-832

Fan, W. & Wang, W. (2003). Extraction of spiked metals from contaminated coastal sediments: a comparison of different methods. *Environmental Toxicology and Chemistry*, 22, 2659-2666

Fang, T., Li, X., & Zhang, G. (2005). Acid volatile sulfide and simultaneously extracted metals in the sediment cores of the Pearl River Estuary, South China. *Ecotoxicology and Environmental Safety*, 61, 420-431

Farkas, A., Salánki, J., & Specziár, A. (2003). Age- and size-specific patterns of heavy metals in the organs of freshwater fish Abramis brama L. populating a low-contaminated site. *Water Research*, 37, 959-964

Fendorf, S., La Force, M. J., & Li., G. (2004). Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium, and lead. *Journal of Environmental Quality*, 33, 2049-2055

Fernandes, H. M. (1997). Heavy metal distribution in sediments and ecological risk assessment: the role of digenetic processes in reducing metal toxicity on bottom sediments. *Environmental Pollution*, 97, 317-325

Filgueiras, A. V., Lavilla, I., & Bendicho, C. (2002). Chemical sequential extraction for metal partitioning in environmental solid samples. *Journal of Environmental Monitoring*, 4, 823-857

Förstner, U. (1981). Metal transfer between soil and aqueous phases. (In U. Förstner & F. T. W. Wittmann (Eds), *Metal Pollution in the Aquatic Environment (2nd edition)* (pp.197-269). Berlin, Germany: Springer-Verlag)

Förstner, U. & Wittmann, G. T. W. (1981). *Metal Pollution in the Aquatic Environment (2nd edition)*. (New York: Springer-Verlag)

Frampton, J. A. & Reisenauer, H. M. (1978). The association of Co, Ni, Cu and Zn with Fe and Mn oxides in soils and soil concretions. (In: *1978 Agronomy Abstracts* (p.24). Madison, Wisconsin: American Society of Agronomy, Inc., Crop Science Society of America, Inc. and Soil Science Society of American, Inc.)

Furman, O., Strawn, D. G., Heinz, G. H., & Williams, B. (2006). Risk assessment test for lead bioaccessibility to waterfowl in mine-impacted soils. *Journal of Environmental Quality*, 35, 450-458

Gaetke, L. M. & Chow, C. K. (2003). Cu toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, 189, 147-163

Gee, G. W. & Or, D. (2002). Particle-size analysis: Pipette method. (In J. H. Dane & G. C. Topp (Eds.), *Methods of Soil Analysis (Part 4): Physical Methods* (pp.272-278). Madison, Wisconsin: Soil Science Society of American, Inc.)

Ghabru, S. K., Arnaud, R. J., & Mermut, A. R. (1990). Association of DCB-extractable iron with minerals in coarse soil clays. *Soil Science*, 149, 112-120

Ghosh, A., Mukiibi, M., & Ela, W. (2004). TCLP underestimates leaching of arsenic from solid residuals under landfill conditions. *Environmental Science and Technology*, 38, 4677-4682

Ginner, J. L. Alvarez, P. J. J., Smith, S. L., & Scherer, M. M. (2004). Nitrate and nitrite reduction by Fe-0: Influence of mass transport, temperature, and denitrifying microbes. *Environmental Engineering Science*, 21, 219-229

Gleyzes, C., Tellier, S., & Astruc, M. (2002). Fractionation studies of trace elements in contaminated soils and sediments: a review. *Trends in Analytical Chemistry*, 21, 451-467

Halim, M., Conte, P., & Piccolo, A. (2003). Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere*, 52, 265-275

Hamel, S. C., Buckley, B., & Lioy, P. J. (1998). Bioaccessibility of metals in soils for different liquid to solid ratios in synthetic gastric fluid. *Environmental Science and Technology*, 32, 358-362

Han, F. X, Hargreaves, J. A., Huggett, D. B., Kingery, W. L., & Schlenk, D. K. (2001a). Accumulation, distribution, and toxicity of copper in sediments of catfish ponds receiving periodic copper sulfate applications. *Journal of Environmental Quality*, 30, 912-919

Han, F. X., Banin, A, & Triplett, G. B. (2001b). Redistribution of heavy metals in arid-zone soils under a wetting-drying cycle soil moisture regime. *Soil Science*, 166, 18-28

Hansen, D. J., Berry, W. J., Mahony, J. D., Boothman, W. S., Di Toro, D. M., Robson, D. L., Ankley ,G. T., Ma, D., Yan, Q., & Pesch, C. E. (1996). Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations. *Environmental Toxicology and Chemistry*, 15, 2080-2094

Harris, W. G. (2002). Phosphate minerals. (In J. B. Dixon & D. G. Schulze (Eds.), *Soil Mineralogy with Environmental Applications* (pp.637-662). Madison, Wisconsin: Soil Science Society of American, Inc.)

Haughey, M. A., Anderson, M. A., Whitney, R. D., Taylor, W. D. & Losee, R. F. (2000). Forms and fate of Cu in a source drinking water reservoir following $CuSO_4$ treatment. *Water Research*, 34, 3440-3452

He, F. & Zhao, D. (2005). Preparation and characterization of a new class of starch-stabilized bimetallic nanoparticles for degradation of chlorinated hydrocarbons in water. *Environmental Science and Technology*, 39, 3314-3320

He, F., Zhao, D., Liu, J., & Roberts, C. B. (2006). Stabilization of Fe-Pd nanoparticles with sodium carboxymethyl cellulose for enhanced transport and dechlorination of trichloroethylene in soil and groundwater. *Industry Engineering Chemistry Research* (in press)

Hettiarachchi, G. M., Ryan, J. A., Chaney, R. L., & Fleur, C. M. L. (2003a). Sorption and desorption of cadmium by different fractions of biosolids-amended soils. *Journal of Environmental Quality,* 32, 1684-1693

Hettiarachchi, G. M., Pierzynski, G. M., Oehme, F. W., Sonmez, O. & Ryan, J. A. (2003b). Treatment of contaminated soil with phosphorus and manganese oxide reduces lead absorption by Sprague–Dawley rats. *Journal of Environmental Quality,* 32, 1167-1172

Hinz, C. & Selim, H. M. (1999). Kinetics of Zn sorption-desorption using a thin disk flow method. *Soil Science,* 164, 92-100

Hogg, D. S., Mclaren, R. G., & Swift, R. S. (1993). Desorption of copper from some New Zealand soils. *Soil Science Society of America Journal,* 57, 361-366

Itami, K. & Yanai, J. (2006). Sorption and desorption properties of cadmium and copper on soil clays in relation to charge characteristics. *Soil Science and Plant Nutrition*, 52, 5-12

Kabata-Pendias, A. & Pendias, H. (1984). *Trace Elements in Soils and Plants*. (Florida: CRC Press, Inc.)

Kanrathansis, A. D. (1999). Subsurface migration of Cu and zinc mediated by soil colloids. *Soil Science Society America Journal*, 6, 830-838

Kelley, M. E., Brauning, S. E., Schoof, R. A., & Ruby, M. V. (2002). *Assessing Oral Bioavailability of Metals in Soil*. (Columbus, Ohio: Battelle Press)

Kenchtenhofer, L. A., Xifra, I. O., Scheinost, A. C., Fluhler, H., & Kretzschmar, R. (2003). Fate of heavy metals in a strongly acidic shooting-range soil: small-scale metal distribution and its relation to preferential water flow. *Journal of Plant Nutrition and Soil Science*, 166, 84-92

Kersting, A. B., Efurd, D. W., Finnegan, D. L., Rokop, D. J., Smith, D. K., & Thomason, J. L. (1999). Migration of plutonium in groundwater at the Nevada Test Site. *Nature* 397, 56-59

Kim, D. K., Mikhsylova, M., Zhang, Y. & Muhammed, M. (2003). Protective coating of superparamagnetic iron oxide nanoparticles. *Chemistry of Material*, 15, 1617-1627

Kingham, N. & Semenak, B. (2000). SPLP gaining ground acceptable, even preferred, leachability test. *Georgia and Southeast Environmental News*, July/August 2000 Issue

Kraemer, L. D., Campbell, P. G. C., & Hare, L. (2005). Dynamics of Cd, Cu and Zn accumulation in organs and sub-cellular fractions in field transplanted juvenile yellow perch (*Perca flavescens*). *Environmental Pollution*, 138, 324-337

Kunze, G. W. & Dixon, J. B. (1986). Pretreatment for mineralogical analysis. (In A. Klute (Ed.), *.Methods of Soil Analysis (Part 1), Physical and Mineralogical Methods* (pp.91-100). Madison, Wisconsin: American Society of Agronomy)

Laperche, V., Traina, S. J., Gaddam, P. & Logan, T. J. (1996). Chemical and mineralogical characterizations of Pb in a contaminated soil: Reactions with synthetic apatite. *Environmental Science and Technology*, 30, 3321-3326

Lee, Y. J., Elzinga, E. J. & Reeder, R. J. (2005). Cu(II) adsorption at the calcite–water interface in the presence of natural organic matter: Kinetic studies and molecular-scale characterization. *Geochimica et Cosmochimica Acta*, 69, 49-61

Leonard, E. N., Mattson, V. R., Benoit, D. A., Hoke, R. A., & Ankley, G. T. (1993). Seasonal variation of acid volatile sulfide concentration in sediment cores from three northeastern Minnesota lakes. *Hydrobiologia*, 271, 87-95

Leonard, E. N., Mount, D. R., & Ankley, G. T. (1999). Modification of metal partitioning by supplementing acid volatile sulfide in freshwater sediments. *Environmental Toxicology and Chemistry*, 18, 858-864

Lindsay, W. L. (1979). *Chemical Equilibria in Soils*. (New York: John Wiley &Sons, Inc.). p.164

Liu, R., Zhao, D. & Barnett, M. O. (2006). Fate and transport of copper applied in channel catfish ponds. *Water, Air, and Soil Pollution*, 176, 136-162

Locat, J., R. Galvez-Cloutier, R. Chaney, & K. Demars (Eds) (2003). *Contaminated Sediments: Characterization, Evaluation, Mitigation, Restoration and Management Strategy Performance*. (West Conshohocken, Pennsylvania: ASTM International)

Lower, S. K., Maurice, P. A., & Traina, S. J. (1998). Simultaneous dissolution of hydroxylapatite and precipitation of hydroxypyromorphite: Direct evidence of homogeneous nucleation. *Geochimica et Cosmochimica Acta*, 62, 1773-1780

Lowery, G. V., Shaw, S., Kim, C. S., Rytuba, J. J., & Brown, G. E., Jr. (2004). Macroscopic and microscopic observations of particle-facilitated mercury transport from New Idria and Sulphur Bank mercury mine tailings. *Environmental Science and Technology*, 28, 5101-5110

Ma, Q. Y.; Traina, S. J., & Logan, T. J. (1993). In situ lead immobilization by apatite. *Environmental Science and Technology*, 27, 1803-1810

Ma, Q. Y., Logan, T. J., & Traina, S. J. (1995). Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks. *Environmental Science and Technology*, 29, 1118-1126

Ma, L. Q. & RAO, G. N. (1999). Aqueous Pb reduction in Pb-contaminated soils by phosphate rocks. *Water, Air, and Soil Pollution*, 110, 1-16

Madrid, L. & Diaz-Barrientos, E. (1992). Influence of carbonate on the reaction of heavy metals in soils. *Journal of Soil Science*, 43,709-721

Marschner, B., Welge, P., Hack, A., Wittsiepe, J. & Wilhelm, M. (2006). Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction. *Environmental Science and Technology*, 40, 2812-2818

Masuda, K. & Boyd, C. E. (1994). Chemistry of sediment pore water in aquaculture ponds built on clayey ultisols at Auburn, Alabama. *Journal of the World Aquaculture Society*, 25, 396-404

Matisoff, G. (1995). Effects of biotubration on solute and particle transport in sediment. (In H. E. Allen (Ed.), *Metal Contaminated Aquatic Sediments* (pp.201-253). Chelsea, Michigan: Ann Arbor Press, Inc.)

McBride, M. B., Martinez, C. E., Topp, E. & Evans, L. (2000). Trace metal solubility and speciation in a calcareous soil 18 years after no-till sludge application. *Soil Science*, 165, 646-656

McMurtry, G. M., Schneider, R. C., Colin, P. L., Buddemeier, R. W., & Suchanek, T. H. (1986). Vertical distribution of fallout radionuclides in Enewetak Lagoon sediments: effect of burial and bioturbation on the radionuclide inventory. *Bulletin of Marine Science*, 38, 35-55

Melamed, R., Cao, X., Chen, M., & Ma, L. Q. (2003). Field assessment of lead immobilization in a contaminated soil after phosphate application. *Science of the Total Environment*, 305, 117-127

Moore, A. M., De Leon, C. H. & Young, T. M. (2003). Rate and extent of aqueous perchlorate removal by iron surfaces. *Environmental Science and Technology*, 37, 3189-3198

Morse, J. W. & Rickard, D. (2004). Chemical dynamics of sedimentary acid volatile sulfide. *Environmental Science and Technology*, 131A-136A (April 1)

Munsiri, P., Boyd, C. E. & Hajek, B. F. (1995). Physical and chemical characteristics of bottom soil profiles in ponds at Auburn, Alabama, USA and a proposed system for describing pond soil horizons. *Journal of the World Aquaculture Society*, 26, 346-377

Nriagu, J. O. (1972). Stability of vivianite and ion-pair formation in the system $Fe_3(PO_4)_2$ $-H_3PO_4-H_2O$. *Geochimica et Cosmochimica Acta*, 36, 459-470

O'Day, P. A., Chisholm-Brause, C. J., Towle, S. N., Parks, G. A., & Brown, G. E. Jr. (1996). X-ray absorption spectroscopy of Co (II) sorption complexes on quartz (α-SiO2) and rutile (TiO2). *Geochimica et Cosmochimica Acta*, 60, 2515-2532

Oldewage, A. A. & Marx, H. M. (2000). Bioaccumulation of chromium, copper and iron in the organs and tissues of *Clarias gariepinu*s in the Olifants River, Kruger National Park. *Water South Africa*, 26, 569-582

Osseo, K. & Wei, D., 1993. Semiconductor electrochemistry of cola pyrite, Technical Progress Report, Jan.-March, 1993. http://www.osti.gov/bridge/servlets/purl/10134237-SvoClB/native/10134237.pdf

Oste, L. A., Dolfing, J., Ma, W., & Lexmond, T. M. (2001). Effect of beringite on cadmium and zinc uptake by plants and earthworms: more than a liming effect. *Environmental Toxicology and Chemistry*, 20, 1339-1345

Owen, C. A. (1981). *Copper Deficiency and Toxicity: Acquired and Inherent, in Plants, Animals, and Man*. (New Jersey: Noyes Publications)

Papadopoulos, P., & D. L. Rowell. (1989). The reaction of copper and zinc with calcium carbonate surfaces. *Journal of Soil Science*, 40, 39-48

Park, C. C. (1987). *Acid Rain: Rhetoric and Reality*. (London: Methuen & Co. Ltd)

Partrick, W. H., Gambrell, R. P., & Faulkner, S. P. (1996). Rodox measurements of soils. (In D. L. Sparks (Ed.), *Methods of Soil Analysis (Part 3): Chemical Methods* (pp. 1254-1273). Madison, Wisconsin: Science Society of American, Inc. and American Society of Agronomy, Inc.).

Patton, G. W. & Crecelius, E. A. (2001, January). Simultaneously extracted metals/acid-volatile sulfide and total metals in surface sediment from the Hanford reach of the Columbia River and the Lower Snake River (Prepared for the U.S. Department of Energy). Retrieved November, 20, 2005 from http://www.pnl.gov/main/publications/external/technical_reports/PNNL-13417.PDF

Peltier, E., Dahl, A. L., & Gaillard, J.-F. (2005). Metal speciation in anoxic sediments: when sulfides can be construed as oxides. *Environmental Science and Technology*, 39, 311-316

Peng, S., Wang, W., Li, X., & Yen, Y. (2004). Metal partitioning in river sediments measured by sequential extraction and biomimetic approaches. *Chemosphere*, 57, 839-851

Petterson, G. S., Ankley, G. T., & Leonard, E. N. (1996). Effect of bioturbation on metal-sulfide oxidation in the surficial freshwater sediments. *Environmental Toxicology and Chemistry*, 15, 2147-2155

Prosi, F. (1981). Heavy metals in aquatic organisms. (In U. Förstner & F. T. W. Wittmann (Eds), *Metal Pollution in the Aquatic Environment (2nd edition)* (pp.271-318). Berlin, Germany: Springer-Verlag)

Raicevic, S., Kaludjerovic-Radoicic, T., & Zouboulis, A. I. (2005). In situ stabilization of toxic metals in polluted soils using phosphates: theoretical prediction and experimental verification. *Journal of Hazardous Materials*, 117, 41-53

Ralph, J. & Chau, I. (2006). *Vivianite*. Retrieved June, 06, 2006, from http://www.mindat.org/copyrights.php

Ramos, L., Gonzalez, M. J., & Hernandez , L. M. (1999). Sequential extraction of copper, lead, cadmium, and zinc in sediments from Ebro River Spain: relationship with levels detected in earthworms. *Bulletin of Environmental Contamination and Toxicology*, 63, 301-308

Reynolds, C. S. & Davies, P. S. (2001). Sources and bioavailability of phosphorus fractions in freshwaters: a British perspective. *Biological Reviews*, 76, 27-64

Riemer, D. N. & Toth, S. J. (1970). Adsorption of Cu by clay minerals, humic acid and bottom muds. *Journal of the American Water Work Association*, 62, 195-197

Robinson, E. H., Li, M. H., & Manning, B. B. (2001). A practical guide to nutrition, feeds, and feeding of catfish. Retrieved June, 06, 2006, from http://www.uaex.edu/aquaculture/ verification/ catfish_ feeding. pdf

Rodriguez-Rubio, P., Morillo, E., Mardrid, L., Undabeytia, T., & Maqueda, C. (2003). Retention of copper by a calcareous soil and its textural fractions: influence of amendment with two agroindustrial residues. *European Journal of Soil Science*, 54, 401-409

Ruby, M. V., Davis, A., & Nicholson, A. (1994). In situ formation of lead phosphates in soils as a method to immobilize lead. *Environmental Science and Technology*, 28, 646-654

Ruby, M. V., Davis, A., Schoof, R., Eberle, S., & Sellstone, C. M. (1996). Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environmental Science and Technology*, 30, 422-430

Ruby, M. V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D. E., Castee, S. I, Berti, W. W., Carpenter, M., Edwards, D., Cragin, D., & Chappell, W. (1999). Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environmental Science and Technology*, 33, 3697-3705

Ryan, J. A., Zhang, P., Hesterberg, D., Chou, J., & Sayers, D. E. (2001). Formation of chloropyromorphite in a lead-contaminated soil amended with hydroxyapatite. *Environmental Science and Technology*, 35, 3798-3803

Sáenz, V., Blasco, J., & Gomez-Parra, A. (2003). Speciation of heavy metals in recent sediments of three coastal ecosystems in the gulf of Cadiz, southwest Iberian Peninsula. *Environmental Toxicology and Chemistry*, 22, 2833-2839

Scheckel, K., Impellitteri, C. A., Ryan, J. A., & McEvoy, T. (2003). Assessment of a sequential extraction procedure for perturbed lead-contaminated samples with and without phosphorus amendments. *Environmental Science and Technology,* 37, 1892-1898

Scheidegger, A. M. & Sparks, D. L. (1996). A critical assessment of sorption-desorption mechanisms at the soil mineral/water interface. *Soil Science,* 161, 813-831

Scheinberg, H. (1991). Copper. (In E. Merian (Ed.), *Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance* (pp.893-908). New York: VCH Publishers, Inc.)

Schmidt, N. W. & Kögel-Knabner, I. (2002). Organic matter in particle-size fractions from A and B horizons of a Haplic Alisol. *European Journal of Soil Science,* 53, 383-391

Shuman, L. M. (1982). Separating soil iron and manganese oxides fractions for microelement analysis. *Soil Science Society of America Journal,* 46, 1099-1102

Shuman, L. M. (1983). Sodium hypochlorite method for extracting microelements associated with soil organic matter. *Soil Science Society of America Journal*, 47, 656-660

Shuman, L. M. (1985). Fractionation method for soil microelements. *Soil Science*, 140, 11-22

Si, S., Kotal, A., Mandal, T. K., Giri, S., Nakamura, H., & Kohara, T. (2004). Size-controlled synthesis of magnetite nanoparticles in the presence of polyelectrolytes. *Chemistry of Material*, 16, 3489-3496

Singh, B. R. & Oste, L. (2001). In situ immobilization of metals in contaminated or naturally metal-rich soils. *Environmental Review*, 9, 81-97

Sonmez, O. & Pierzynski, M. (2005). Phosphorus and manganese oxides effects on soil lead bioaccessibility: PBET and TCLP. *Water, Air, and Soil Pollution*, 166, 3-16

Sparks, D. L. (1995). *Environmental Soil Chemistry*. (New York: Academic Press)

Sparks, D. L. & Schreurs, B. G. (2003). Trace amount of Cu in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 11065-11069. Retrieved October 13, 2005, from http: www.pnas.org/ cgi/doi/10.1073/ pnas. 1832769100

Sprague, L. A., Herman, J. S., Hornberger, G. M., & Mills, A. L. (2000). Atrazine adsorption and colloid-facilitated transport through the unsaturated zone. *Journal of Environmental Quality*, 29, 1632-1641

Stanforth, R. & Qiu, J. (2001). Effect of phosphate treatment on the solubility of lead in contaminated soil. *Environmental Geology*, 41, 1-10

Stewart, M. A., Jardine, P. M., Barnett, M. O., Mehlhorn, T. L., Hyder, L. K., & McKay, L. D. (2003a). Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium(III). *Journal of Environmental Quality*, 32, 129-137

Stewart, M. A., Jardine, P. M., Brandt, C. C., Barnett, M. O., Fendorf, S. E., McKay, L. D, Mehlhorn, T. L., & Paul, K. (2003b). Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in soil. *Soil and Sediment Contamination*, 12, 1-21

Straus, D. L. & Tucker, C. S. (1993). Acute toxicity of copper sulfate and chelated copper to channel catfish *Ictalarus punctatus. Journal of the World Aquaculture Society*, 24, 390-395

Su, C. & Puls, R. W. (2001). Arsenate and arsenite removal by zero-valent iron: Kinetics, redox transformation, and implications for in situ groundwater remediation. *Environmental Science and Technology*, 37, 1487-1492

Sun, Y., & Torgersen, T. (2001). Adsorption–desorption reactions and bioturbation transport of ^{224}Ra in marine sediments: A one-dimensional model with applications. *Marine Chemistry*, 74, 227-243

Tack, F. M. G., Singh, S. P., & Verloo, M. G. (1999). Leaching behavior of Cd, Cu, Pb and Zn in surface soils derived from dredged sediments. *Environmental Pollution*, 106, 107-114

Tessier, A., Campbell, P. G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 51, 844-851

Theodoratos, P., Papassiopi, N., & Xenidis, A. (2002). Evaluation of monobasic calcium phosphate for the immobilization of heavy metals in contaminated soils from Lavrion. *Journal of Hazardous Materials*, 94, 135-146

Thomann, R. V., Shkreli, F., & Harrison, S. (1997). A pharmacokinetic model of cadmium in rainbow trout. *Environmental Toxicology and Chemistry*, 16, 2268-2274

Thomas, G. W. (1996). Soil pH and soil acidity. (In D. L. Sparks (Ed.), *Method of Soil Analysis (Part 3): Chemical Methods* (pp.475-489). Madison, Wisconsin: Science Society of American, Inc. and American Society of Agronomy, Inc.)

Thornton, I. (1976). Copper in soils and sediment. (In J. O. Nriagu (Ed.), *Copper in the Environment (Part I: Ecological Cycling)* (pp.171-216). New York: John Wiley & Sons)

Tipping, E., Hetherington, N. B., Hilton, J., Thompson, D. W., Howles, E., & Hamilton-Taylor, J. (1985). Artifacts in the use of selective chemical-extraction to determine distributions of metals between oxides of manganese and iron. *Analytical Chemistry*, 57, 1944-1946

Townsend, T., Tolaymat, T., Solo-Gabriele, H., Brajesh, Dubey, B., Stook, K., & Wadanambi, L. (2004). Leaching of CCA-treated wood: Implications for waste disposal. *Journal of Hazardous Material*, B114, 75-91

Traina, S. J.; & Laperche, V. (1999). Contaminant bioavailability in soils, sediments and aquatic environments. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 3365-3371

USDA: United States Department of Agriculture, Natural Resources Conservation Service & National Soil Survey Center (1996). *Soil Survey Laboratory Methods Manual: (Soil Survey Investigation Report No.42, Version 3.0).* pp.203-206

USDA: United State Department of Agriculture (2004, October). *National Agricultural Statistics Service, Catfish Production*. Retrieved June 10, 2005, from http: // jan. mannlib. cornell.edu/ reports/ nassr/ other/pcf-bbc/

USEPA: United State Environmental Protection Agency, (1992, July). Toxicity Characteristic Leaching Procedures, SW-846, Method 1311. Retrieved May 31, 2003, from

http://www.epa.gov/sw-846/pdfs/1311.pdf

USEPA: United State Environmental Protection Agency, (1994, September). Synthetic Precipitation Leaching Procedures, SW-846, Method 1312. Retrieved May 31, 2003, from http://www.epa.gov/sw-846/pdfs/1312.pdf

USEPA: United State Environmental Protection Agency, (1996, December). Acid digestion of sediments, sludges, and soils, Method 3050B, Revision 2. Retrieved May 31, 2002, from http://www.epa.gov/SW-846/pdfs/3050b.pdf

USEPA: United States Environmental Protection Agency (1997). *The Incidence and Severity of Sediment Contamination in Surface Waters of the United States*: *Volume 1: National Sediment Quality Survey*. (Washington, D.C.)

USEPA: United State Environmental Protection Agency (1999). *National Priorities List (NPL), Superfund*. Retrieved June, 10, 2005, from http://www.epa.gov/superfund/ sites /query/advquery.htm

USEPA: United States Environmental Protection Agency (2000). *Appendix to Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment*. (Washington, D.C.)

USEPA: United State Environmental Protection Agency Region 10 (2001a, February). *Consensus Plan for Soil and Sediment Studies: Coeur d'Alene River Soils and Sediments Bioavailability Studies* (URS DCN: 4162500.06161.05.a. EPA:16.2).1-16. Retrieved June 10, 2006, from

http:// yosemite.epa.gov /.../ 503bcd6aa1bd60a288256cce00070286/$ F I L E / s o I l_amend_consensus_ final _ 0 2 2 801.PDF

USEPA: United State Environmental Protection Agency (2001b, March), *Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites*. Retrieved March 20, 2005, from

http://www.epa.gov/superfund/resources/soil/ssgmarch01.pdf

USEPA: United States Environmental Protection Agency (2002, July). *List of Contaminants & Their MCLs.* Retrieved February 3, 2006, from http://www.epa.gov/safewater/mcl.html#mcls.

USEPA: United States Environmental Protection Agency (2003). *Code of Federal Regulations (Title 40: Protection of the Environment, parts 150-189).* (Washington, D.C.). p.539

Van Den Berg, G. A., Loch, J. P. G., Van Der Heijdt, L. M., & Zwolsman, J. J. G. (1998). Vertical distribution of acid-volatile sulfide and simultaneously extracted metals in a recent sedimentation area of the river Meuse in the Netherlands. *Environmental Toxicology and Chemistry*, 17, 758-763

Vdovic, N., Billon, G., Gabelle, C., & Potdevin, J. (2006). Remobilization of metals from slag and polluted sediments (Case Study: The canal of the Deule River, northern France). *Environmental Pollution*, 141, 359-369

Wang, Y. M., Chen, T. C., Yeh, K. J., & Shue, M. F. (2001). Stabilization of an elevated heavy metal contaminated site. *Journal of Hazardous Materials*, 88, 63-74

Whittig, L. D. & Allardice, W. R. (1996). X-ray diffraction techniques. (In A. Klute (Ed.), *Methods of Soil Analysis (Part 1): Physical and Mineralogical Methods (2nd edition)* (pp.340-342). Madison, Wisconsin: Science Society of American, Inc. and American Society of Agronomy, Inc.)

Wijnhoven, S., Van Der Velde, G., Leuven, R. S. E. W., Eijsackers, H. J. P., & Smits, A. J. M. (2006). The effect of turbation on zinc relocation in a vertical floodplain soil profile. *Environmental Pollution*, 140, 444-452

Wittmann, G. (1981). Toxic metals. (In U. Förstner & G. T. W. Wittmann (Eds.), *Metal Pollution in the Aquatic Environment* (pp.3-68). New York: Springer-Verlag)

Xiong, Z., Zhao, D., Barnett, M. O., & Harper, W. F. (2006) Immobilization of mercury from water and soils using stabilized iron sulfide nanoparticles. 2006 Alabama Water Resources Conference, Orange beach, Al., September 7-8.

Yang, J., Mosby, D. E., Casteel, S. W., & Blanchar, R. W. (2001). Lead immobilization using phosphoric acid in a smelter-contaminated urban soil. *Environmental Science. and Technology*, 35, 3553-3559

Yang, J. K., Barnett, M. O., Jardine, P. M., Basta, N. T., & Casteel, S. W. (2002). Adsorption, sequestration, and bioaccessibility of As(V) in soils. *Environmental Science and Technology*, 36, 4562-4569

Yang, J. K., Barnett, M. O., Jardine, P. M., & Brooks, S. C. (2003). Factors controlling the bioaccessibility of arsenic(V) and lead(II) in soil. *Soil and Sediment Contamination*, 12, 165-179

Yang, J. K., Barnett, M. O., Zhuang, J. Fendore, S. E., & Jardine, P. M. (2005). Adsorption, oxidation, and bioaccessibility of As(III) in soils. *Environmental Science and Technology*, 39, 7102-7110

Yoo, K. H. & Boyd, C. E. (1994). *Hydrology and Water Supply for Pond Aquaculture*. (New York: Chapman & Hall)

Young, R. N. & MacDonald, E. M. (1998). Influence of pH, metal concentration, and soil component removal on retention of Pb and Cu by an illitic soil. (In E. A. Jenne (Ed.), *Adsorption of Metals by Geomedia: Variables, Mechanisms and Model Application* (pp.230-251). San Diego, California: Academic Press)

Yu, J. & Klarup, D. (1994). Extraction kinetics of copper, zinc, iron, and manganese from contaminated sediment using disodium ethylenediaminetetraacetate. *Water, Air, and Soil Pollution*, 75, 205-225

Yu, K. C., Tsai, L. J., Chen, S. H., & Ho, S. T. (2001). Chemical binding of heavy metals in anoxic river sediments. *Water Research*, 35, 4086-4094

Yu, S., He, Z. L., Huang, C. Y., Chen, G. C., & Calvert, D. V. (2004). Copper fractionation and extractability in two contaminated variable charge soils. *Geoderma*, 123, 163-175

Zhang, P., & Ryan, J. A. (1998). Formation of pyromorphite in anglesite-hydroxyapatite suspensions under varying pH conditions. *Environmental Science and Technology*, 32, 3318-3324

Zhang, W. (2003). Nanoscale iron particles for environmental remediation: An overview. *Journal of Nanoparticle Research*, 5, 323-332

Zhang, M. K., He, Z. L., Calvert, D. V., Stoffella, P. J., Yang, X. E., & Li, Y. C. (2003). Phosphorus and heavy metal attachment and release in sandy soil aggregate fractions. *Soil Science Society of America Journal*, 67, 1158–1167

Zietz, B., Vergara, J.D., & Dunkelberg, H. (2003). Cu concentrations in tap water and possible effects on infant’s health-results of a study in Lower Saxony, Germany. *Environmental Research*, 92, 129-138

APPENDIX

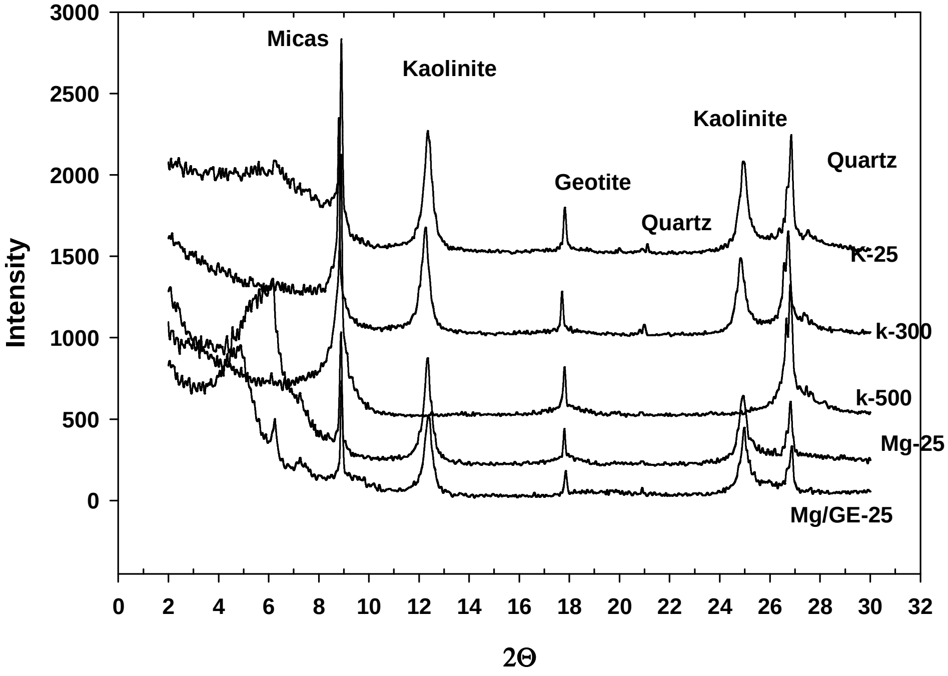


Figure A1 XRD pattern of clay fraction in the calcareous soil (CS)

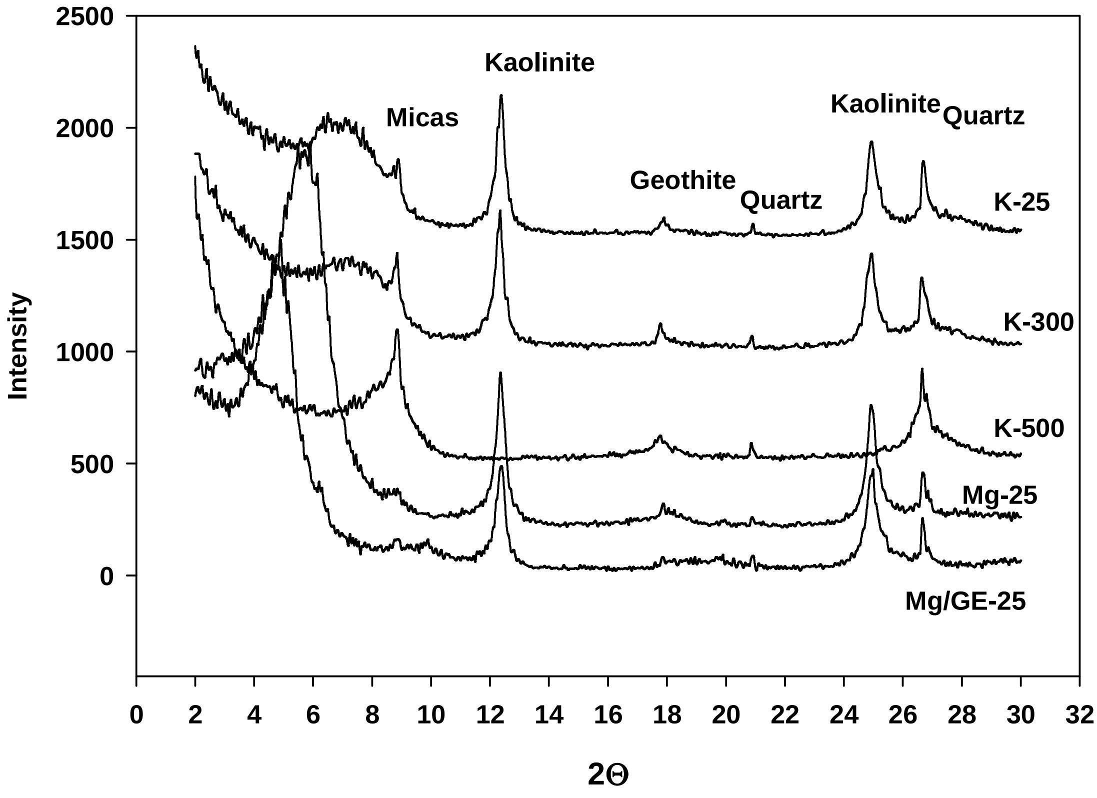


Figure A2 XRD pattern of clay fraction in of the neutral soil (NS)

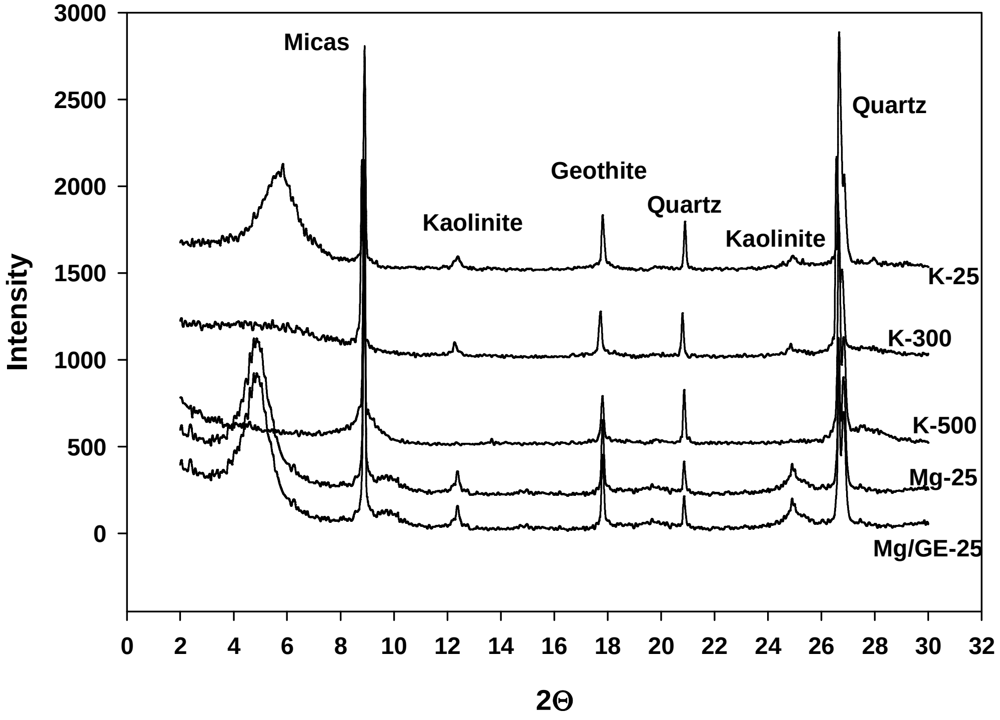


Figure A3 XRD pattern of the clay fraction in the acidic soil (AS)

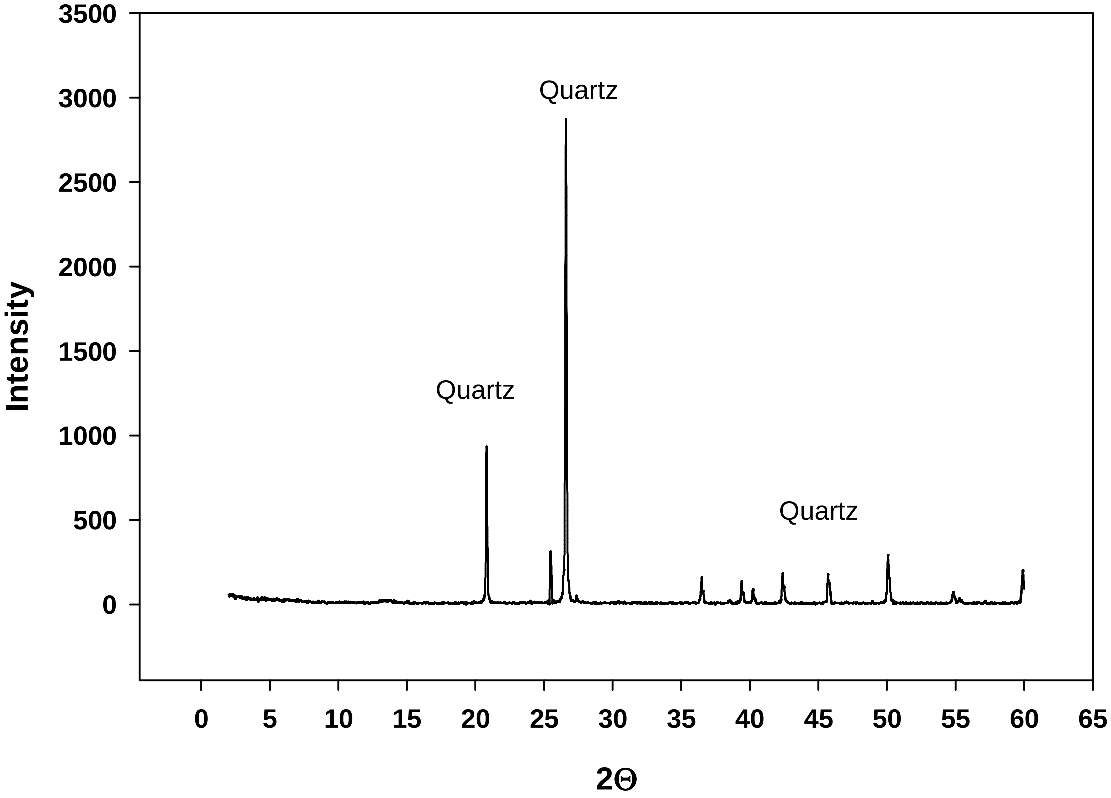
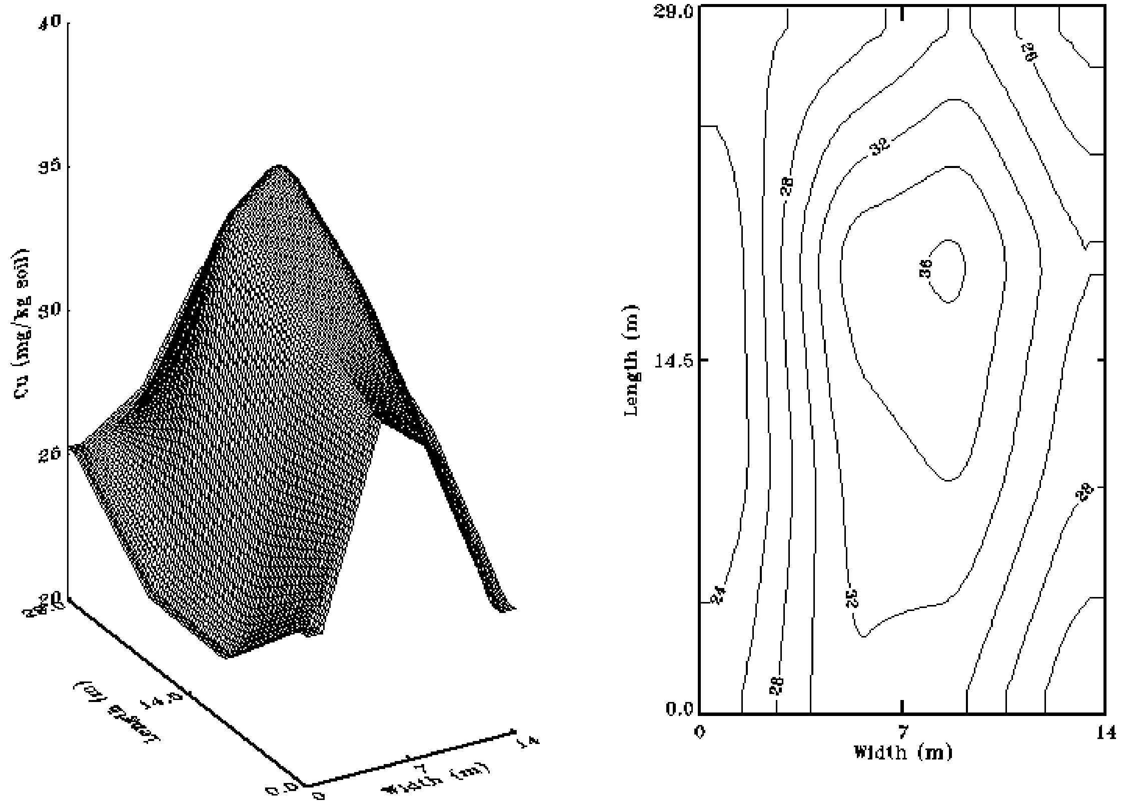
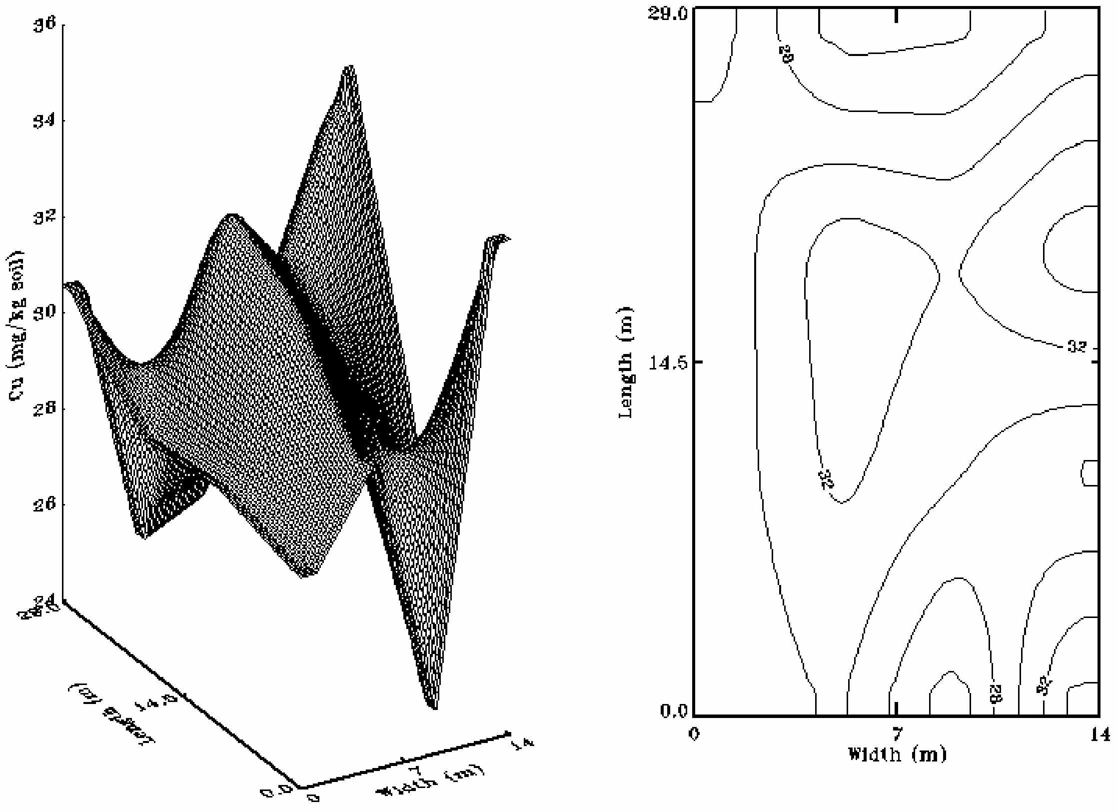


Figure A4 XRD pattern of sand fraction of the neutral soil (NS). Similar results were also observed for those in the calcareous and acidic soils



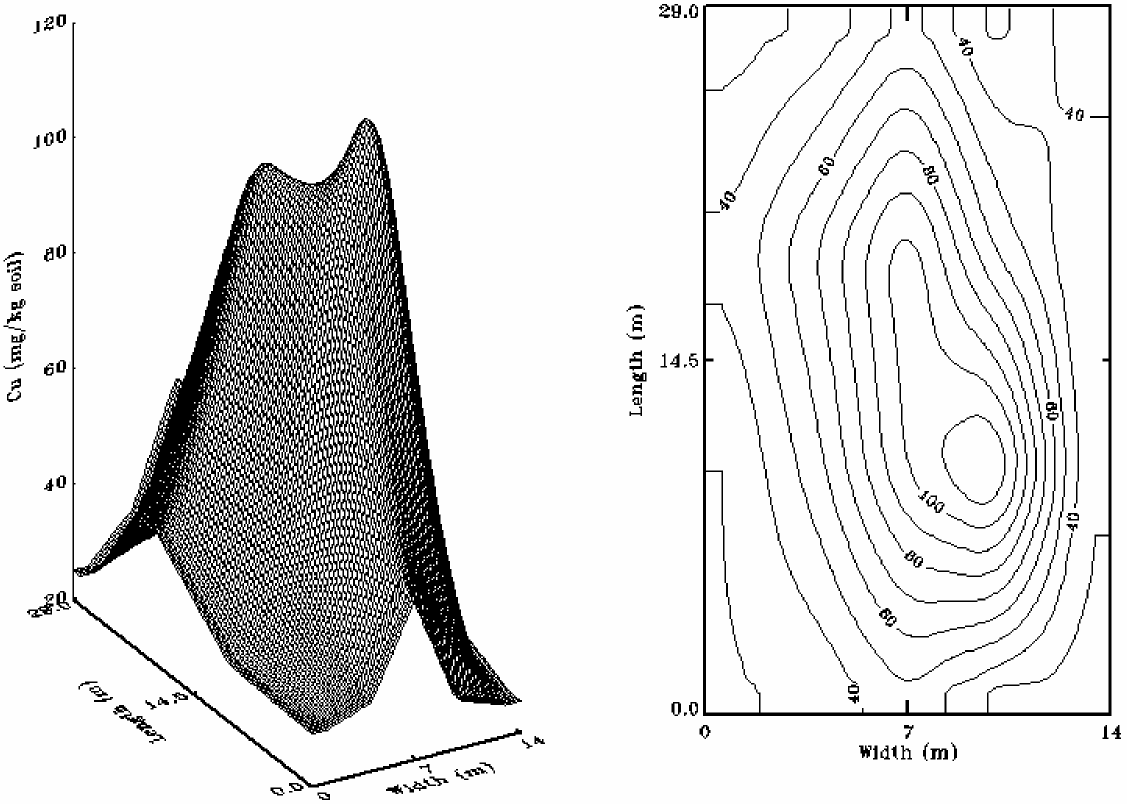
COPPER DISTRIBUTION IN POND SEDIMENT (0- 2cm)

Figure A5 copper distributions in selected layers of the experimental pond sediment before the copper application season (a)



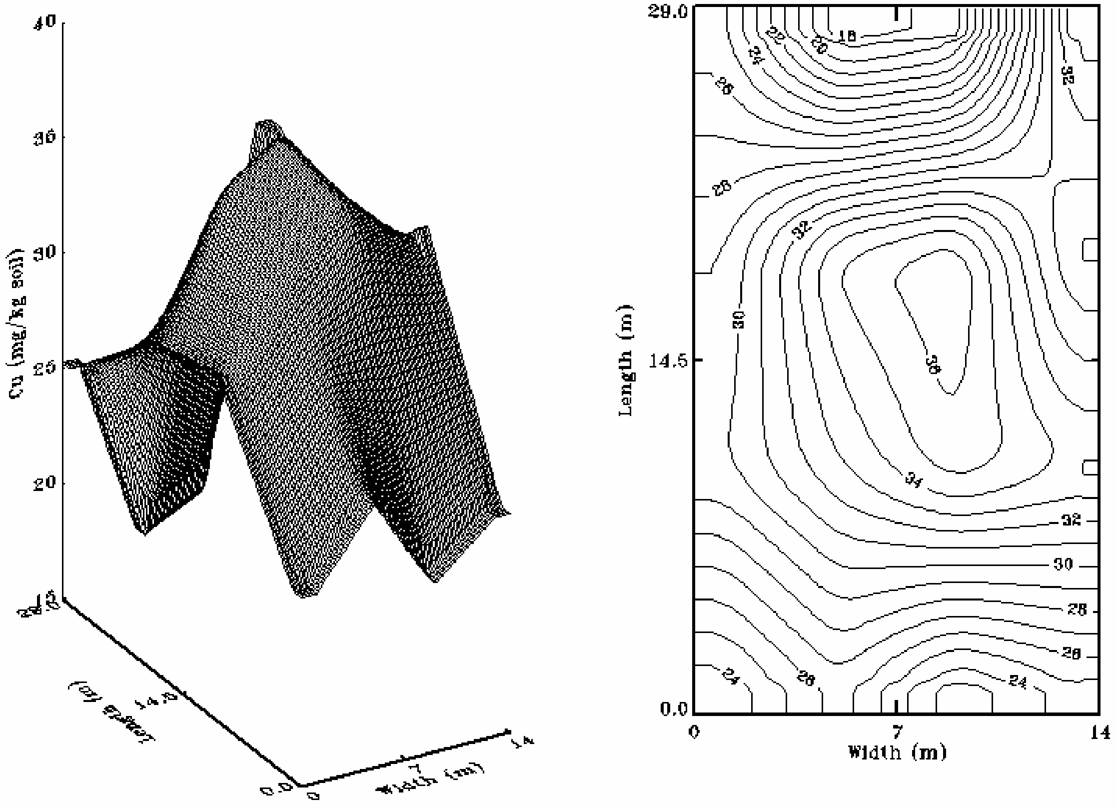
COPPER DISTRIBUTION IN POND SEDIMENT (4- 6cm)

Figure A5 copper distributions in selected layers of the experimental pond sediment before the copper application season (b)



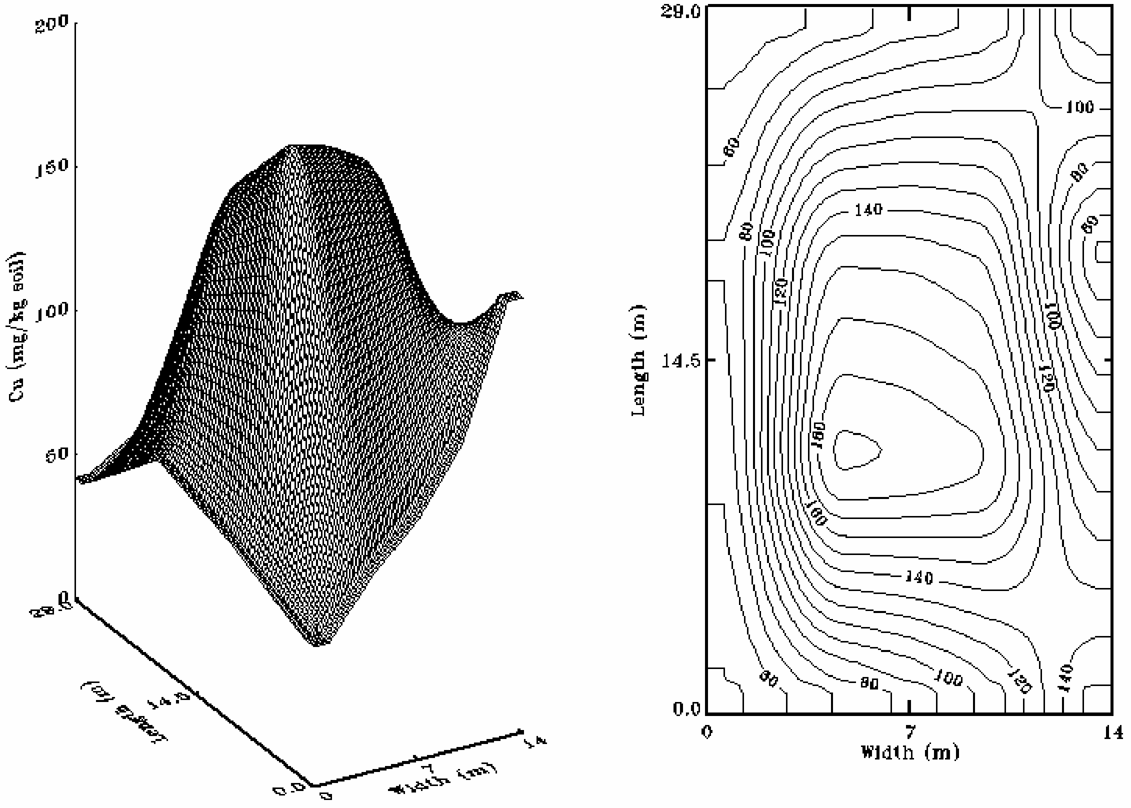
COPPER DISTRIBUTION IN POND SEDIMENT (8−10cm)

Figure A5 copper distributions in selected layers of the experimental pond sediment before the copper application season (c)



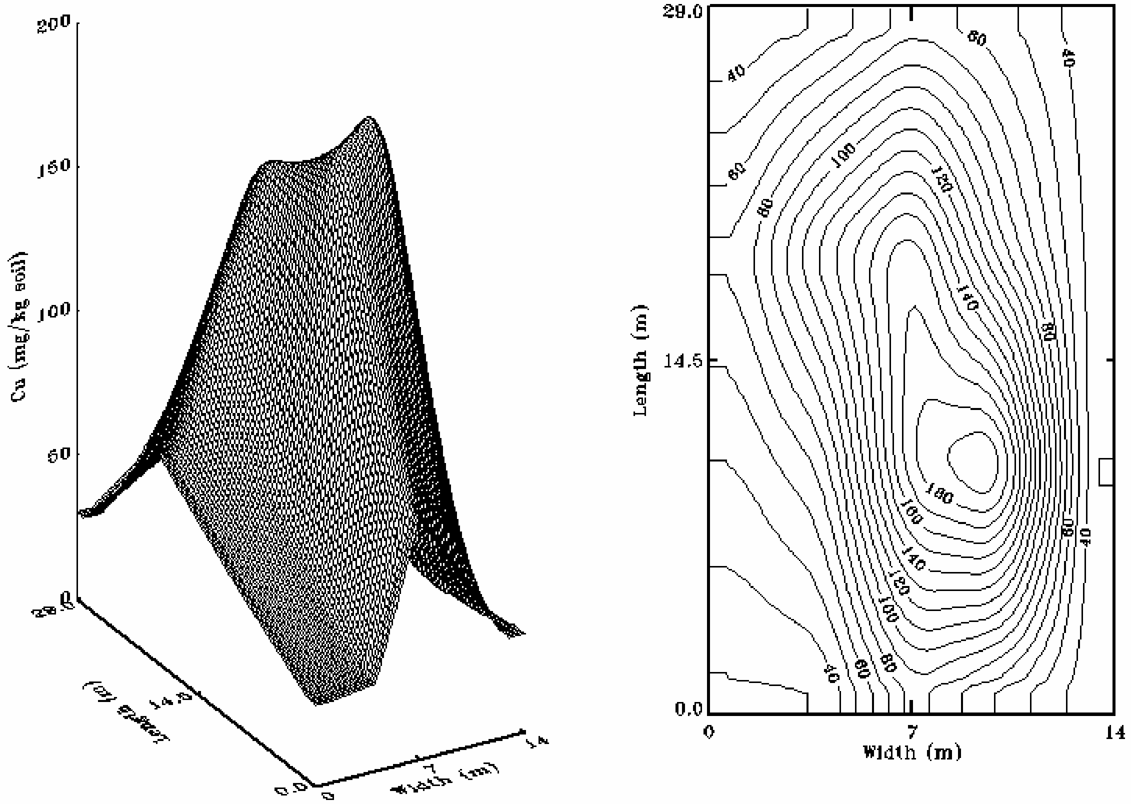
COPPER DISTRIBUTION IN POND SEDIMENT (14−16cm)

Figure A5 copper distributions in selected layers of the experimental pond sediment before the copper application season (d)



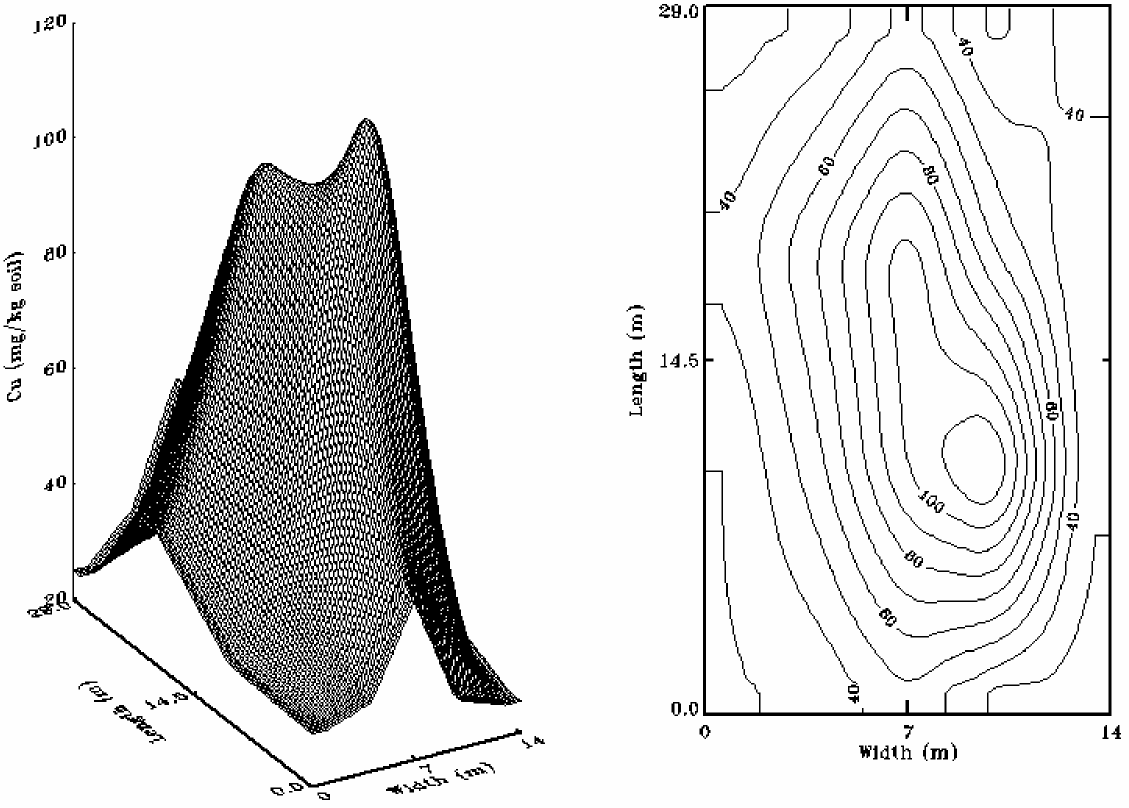
COPPER DISTRIBUTION IN POND SEDIMENT (0- 2cm)

Figure A6. Copper distribution in selected layers of the experimental pond sediment after the copper application season (a)



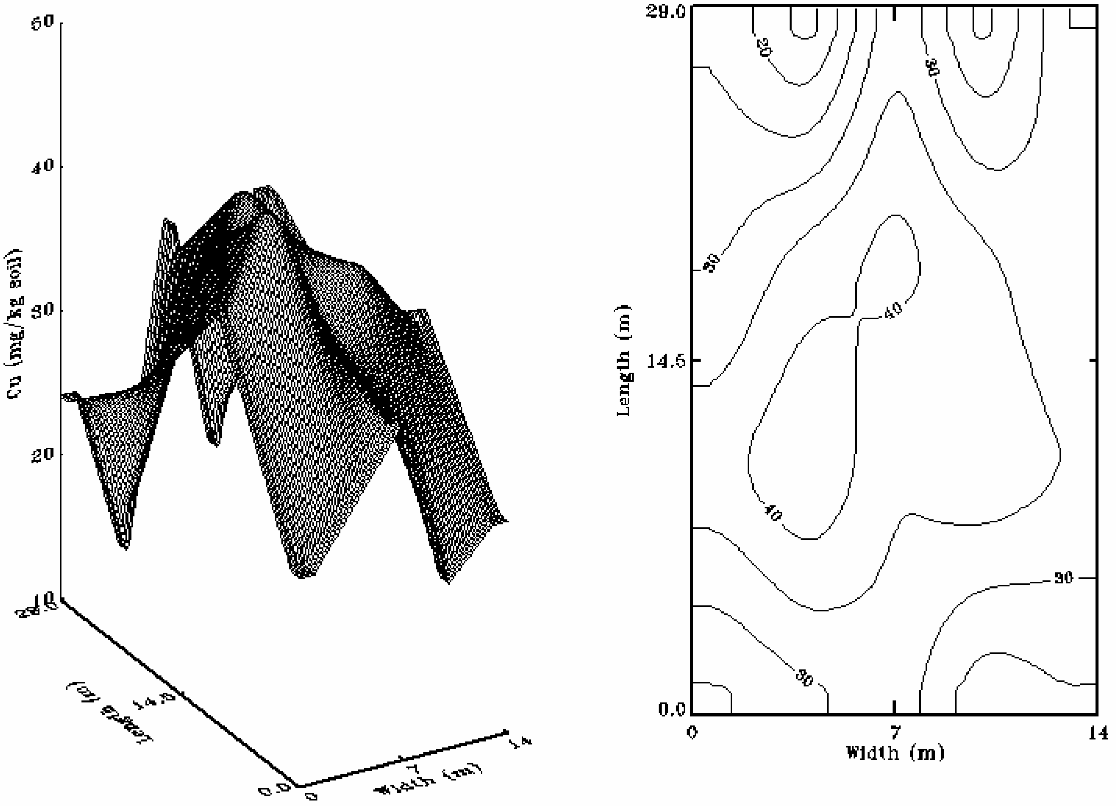
COPPER DISTRIBUTION IN POND SEDIMENT (4- 6cm)

Figure A6. Copper distribution in selected layers of the experimental pond sediment after the copper application season (b)



COPPER DISTRIBUTION IN POND SEDIMENT (8-10cm)

Figure A6. Copper distribution in selected layers of the experimental pond sediment after the copper application season (c)



COPPER DISTRIBUTION IN POND SEDIMENT (14-16cm)

Figure A6. Copper distribution in selected layers of the experimental pond sediment after the copper application season (d)

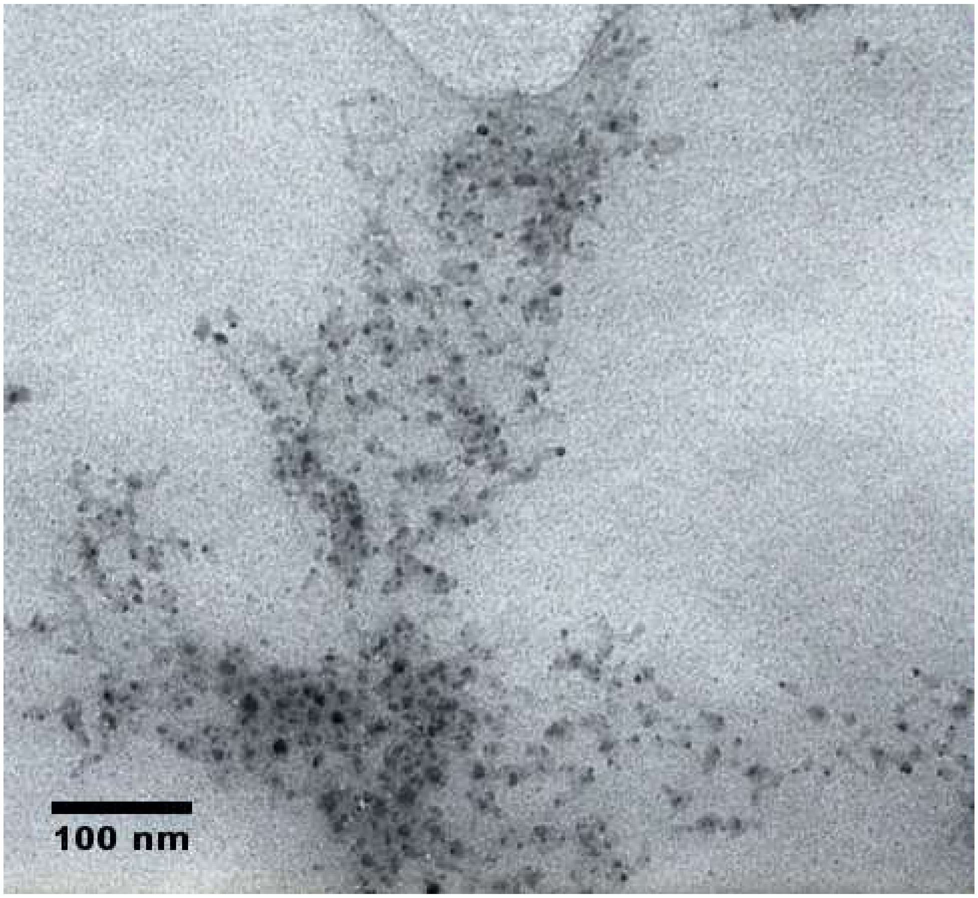


Figure A7. More TEM images of the iron phosphate nanoparticles (a)

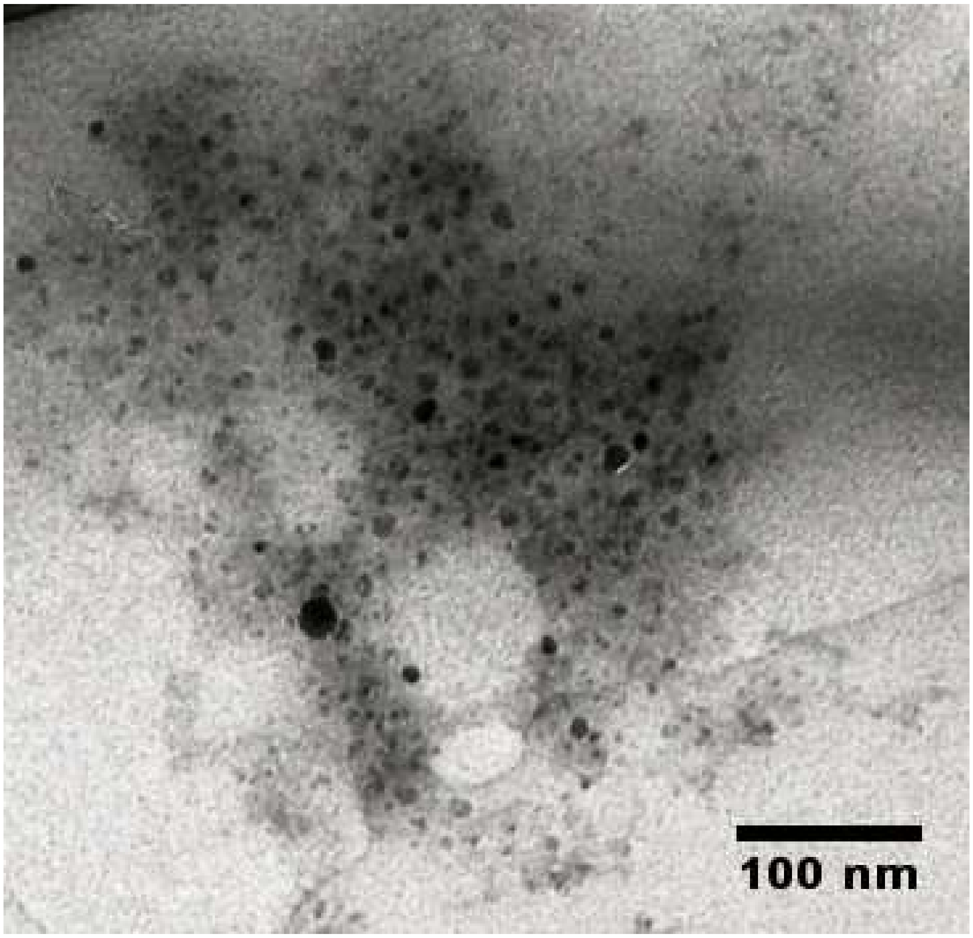


Figure A7. More TEM images of the iron phosphate nanoparticles (b)

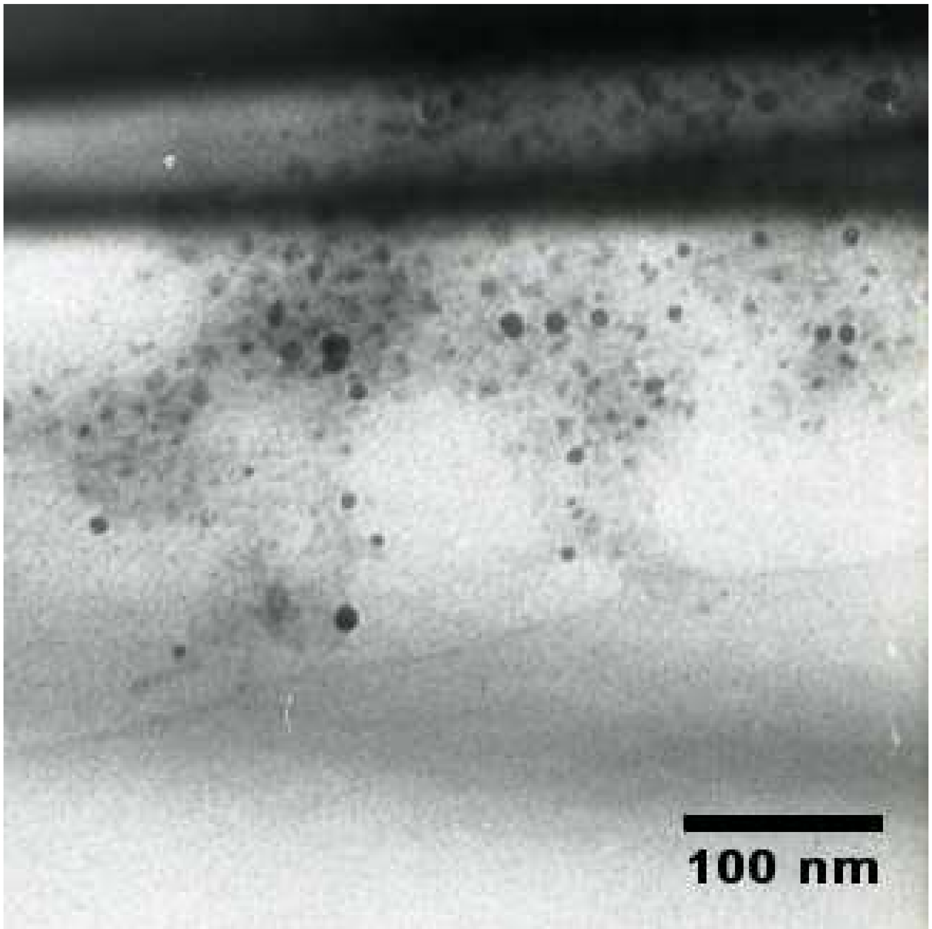


Figure A7. More TEM images of the iron phosphate nanoparticles (c)

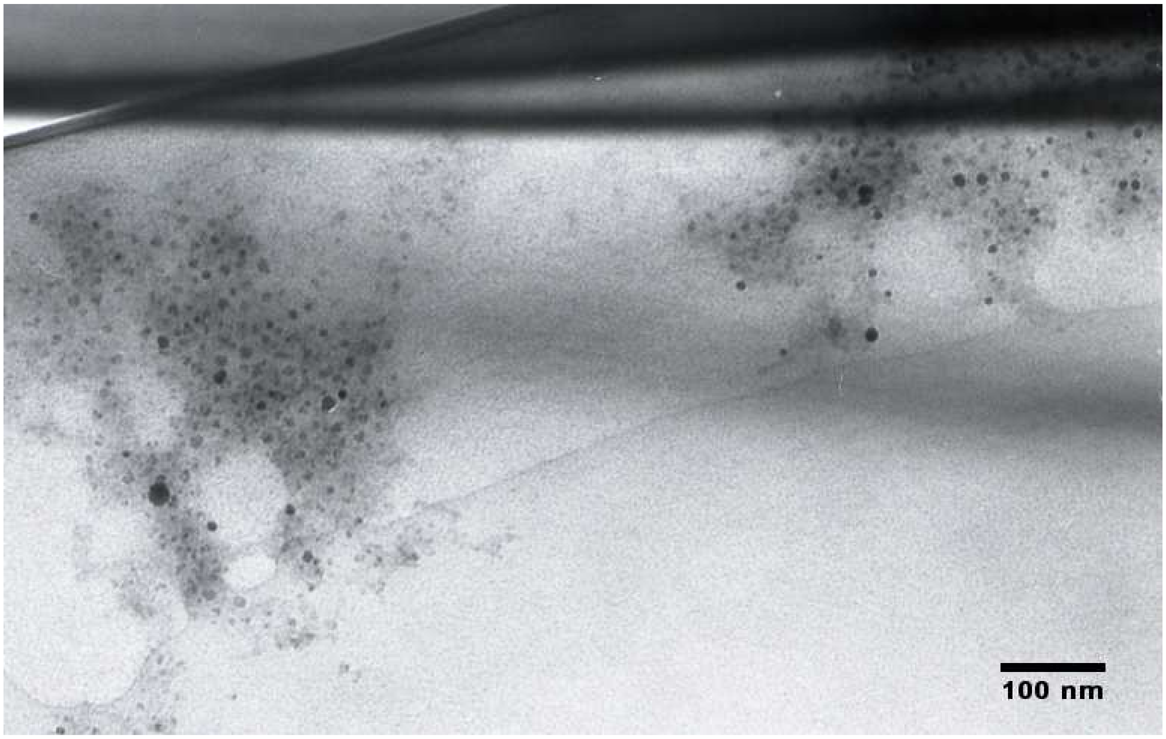


Figure A7. More TEM images of the iron phosphate nanoparticles (d)