SPECIATION AND RELEASE KINETICS OF CADMIUM AND ZINC IN PADDY SOILS

by

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ABSTRACT

Cadmium contamination in paddy soils of rice-producing countries has been reported at many locations near Zn mining and smelting plants. Cadmium in these soils can contaminate rice and subsequently enter the human food chain. Long-term Cd consumption causes chronic *Itai-itai* disease, which is characterized by renal proximal tubular dysfunction and osteomalacia. Understanding the chemical forms in which Cd and Zn are present in paddy soils is needed to develop efficient and cost-effective strategies to clean up the soils, and/or minimize Cd uptake by rice. Knowledge of metal speciation is essential because it directly controls metal mobility and bioavailability. The main goals of this study are to investigate Cd and Zn speciation and release kinetics in a paddy soil contaminated by Cd and Zn, obtained from the Mae Sot district, Tak province, Thailand. This study was conducted with a soil untreated (referred as the alkaline soil), or acidified to pH 6 and spiked with K_2SO_4 (referred as the acidified soil). These two soil samples were studied under various flooding periods and draining conditions, to mimic lowland rice culture. The secondary goal of this study is to identify the predominant low molecular weight organic acids (LMWOAs) excreted from two Thai rice cultivar under Cd stress, and to elucidate the effect of these LMWOAs on Cd and Zn desorption in the paddy soils. Synchrotron-based techniques, including bulk x-ray absorption fine structure (bulk-XAFS) spectroscopy, micro x-ray fluorescence (μ-XRF) spectroscopy, and micro x-ray absorption near-edge structure (μ-XANES) spectroscopy, and a stirred-flow kinetic method were employed to elucidate Cd and Zn speciation, metal distribution/association, as well as Cd and Zn release kinetics, respectively.

Results from the alkaline soil revealed that, under all flooding and draining conditions, less than 25% of Cd was released during 2 hours of desorption using the strong chelating agent, DTPA-TEA-Ca. Bulk-XAFS analysis revealed that Cd carbonates were the most important species in the alkaline soil. Cadmium associated with humic acid was found in the air-dried soil and after 1 day of flooding. However, this species was not detected after 30 days of flooding. Small amounts of CdS were present after 30 days of flooding, and persisted over 150 days of flooding. Other species, e.g. Cd-kaolinite and Cd-ferrihydrite, were found after short periods of flooding, but were no longer significant after 150 days of flooding.

In the acidified soil, Cd sorbed to kaolinite was the dominant species after flooding, and its amount increased with increasing flooding periods. Cadmium sorbed to humic acid was found in the acid air-dried soil, spiked with K_2SO_4, and its amount increased after flooding. However, this species was not detected after 150 days of flooding. Flooding conditions did not cause the acidified soil to become as highly reducing as expected, which more likely due to high soluble nitrate or salinity in the acidified soil. During 2 hours of desorption using the strong chelating agent, DTPA-TEA-Ca, cumulative Cd desorption reached 100% under air-dried soil conditions, and greater than 50%, at all flooding periods and draining conditions. The μ-XRF maps showed that there was no association between Cd and Zn.

The study on Zn speciation and release kinetics showed that almost no change occurred in Zn speciation and release kinetics in the soils due to flooding or acidification, although the alkaline and acidic soils were subjected to different flooding periods, and drained to field capacity or saturation. After 2 hours of stirred-flow experiment using the strong chelating agent, DTPA-TEA-Ca, the cumulative Zn desorption was less than 25% in all soil samples. Only two main phases were identified by LLSF, i.e. Zn-layered double hydroxides (Zn/Mg-hydrotalcite-like, and ZnAl-LDH) and Zn-phyllosilicates (Zn-kerolite). Micro-XRF maps showed that Zn was localized and associated with Cu in most samples.

The *in vitro* study on root exudates from two local Thai rice cultivars (jasmine and glutinous) under Cd stress revealed that the pH in the rhizosphere was not dependent on Cd concentration, but decreased when roots of each rice cultivar were introduced into the media. Additionally, the types and concentrations of LMWOAs secreted by the two rice cultivars were not dependent on Cd concentration. Both rice cultivars secreted three main LMWOAs, in the following order: oxalic > citric > maleic acids. The amounts of Cd and Zn released were dependent on the types and concentrations of the organic acids, and soil pH. Citric acid showed the greatest ability to desorb Cd and Zn from soil components.

Chapter 1

INTRODUCTION

1.1 Cadmium contamination in paddy soil

Cadmium and zinc have similar electronic configurations, valence states, and affinities for S, N, and O donor ligands (Kummerova *et al.*, 2010). Zinc ores usually contain between 0.1 and 5% of cadmium (Nan *et al.*, 2002). Although Cd and Zn share similar geochemical and environmental properties, Zn is an important nutrient to plants, animals and humans, while Cd has no biological function in plants, and it is toxic to humans. Because Cd and Zn are usually co-contaminants, have similar properties in soil and plants, and are readily absorbed and translocated to plant shoots, any study on Cd should be consider Zn as well (Chaney, 2010).

Rice (*Oryza sativa* L.) is the most produced cereal in the world after maize. The daily food of more than 3 billions of people is based on rice, and half of these rice consumers are very poor (Timmer, 2008). The number of rice consumers continues to increase worldwide, and might reach 5 billions in 2030 (Khush, 2005). The largest rice producing countries in the world are mainly located in Asia, including Thailand. These countries face more and more difficulties to satisfy the world demand. Firstly, the population growth results in smaller areas of lands that can be used for agriculture. Additionally, intensive agricultural land exploitations over centuries decrease the fertility of the soil. The increasing frequency and intensity of droughts limit the amount of water available for farming. The poorest rice producing countries also face the increasing cost of labor and prices of fertilizers and pesticides.

Moreover, Cd, Zn, and As contamination in soils can be also an issue for some of these countries. The amount of agricultural soils contaminated by Cd increased worldwide last century, due to modern industrial activities, including Zn mining and smelting operations, applications of sewage sludge and phosphate fertilizers (Alloway, 1995; Simmons *et al.*, 2003; Song *et al.*, 20 04).

"Paddy soil" refers to a soil mainly used, or potentially used to grow rice. The word "Paddy" has several meanings, and can refer to a rice plant, an unhusked rice grain, or a rice field. It can also specifically refer to the rice produced by the "lowland rice culture", which is the main rice culture system in Asia. Farmers practice this type of culture because of its high yields, low maintenance, and the resistivity of the rice plants to harsh climate conditions (Kyuma, 2004). The lowland rice culture employs a cyclical process of submerged and drained conditions. This process alters the chemical, physical, and biological properties of the paddy soil. Under flooding conditions, the rates of oxygen diffusion in water are very low. The gas exchanges between the soil and the atmosphere, and the decomposition of organic matter in the soil, are inhibited. Therefore, the soil is mostly anaerobic under flooding conditions (Kyuma, 2004). Conversely, the soil becomes aerobic during drained conditions. The changes in soil pH and Eh during the submergence and drainage cycles of lowland rice culture can greatly impact the chemical properties of soil components, e.g. organic matters, clays, or oxides present in the soil, where nutrients and contaminants can be retained.

Several studies have shown that some rice producing countries are faced with Cd contamination in their paddy soils, especially at locations near mining and

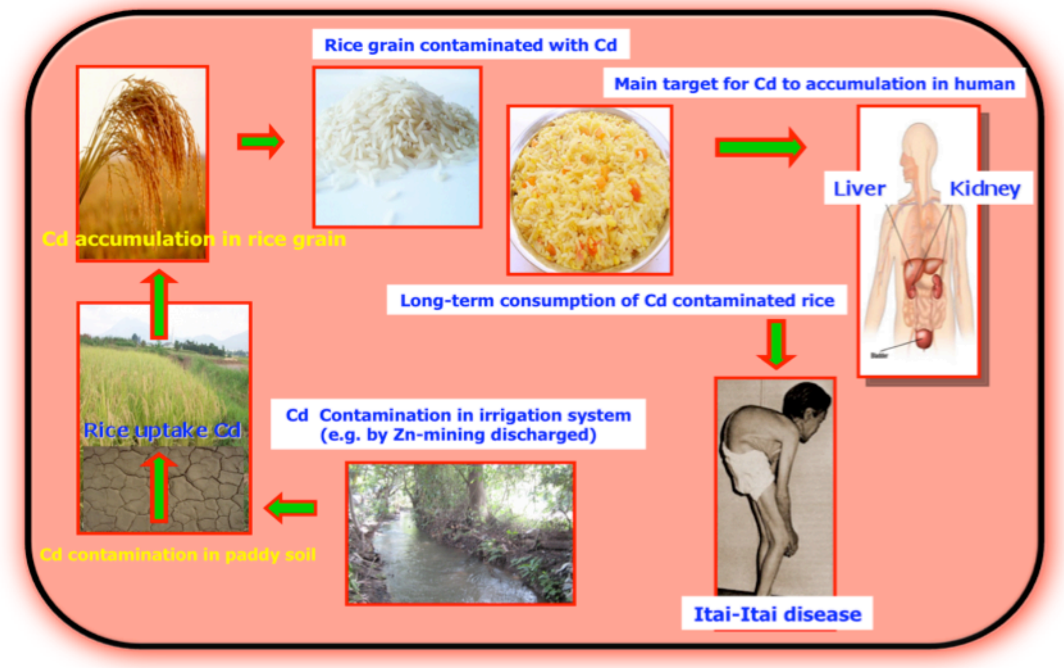

Figure 1.1 How Cd enters in the human food chain via rice grown under Cd contaminated paddy soil.

smelting industrial areas (Kawada and Suzuki, 1998; Simmons and Pongsakul, 2003; Simmons *et al.*, 2005a and b; Liao *et al.*, 2005). Rice plants, grown in contaminated paddy soils, can uptake Cd and accumulate this metal in their grains. The presence of Cd in paddy soils is problematic due to the toxicity of this metal to humans. Once in the human body, Cd can accumulate in the kidneys and liver. Long-term Cd consumption causes chronic *Itai-itai* disease, which is characterized by renal proximal tubular dysfunction and osteomalacia (Nogawa *et al.*, 1983). Figure 1.1 illustrates how

Cd enters the human food chain via rice grown under Cd contaminated paddy soil. The amount of Cd taken up by rice depends on the speciation of this metal in the soil. Previous studies suggested that rice takes up less Cd under flooded conditions than non-flooded condition, due to the formation of cadmium sulfide, based on Cd concentration measurements in the rice (Ito and Iimura, 1975; Bingham *et al.*, 1975). However, no microscopic evidence for cadmium sulfide phase formation was provided in these previous studies.

1.2 Cadmium contamination at Mae Sot districk, Tak province, Thailand

In 2003, the International Water Management Institute (IWMI) reported that mining activities in the Mae Sot district, which is located in the northern part of Thailand, have resulted in Cd contamination of about 2,201 hectares of agricultural paddy soils. Therefore, the ~6000 villagers living in the Mae Sot district have been potentially exposed to cadmium. The rice produced in the Cd-contaminated areas of this district was not exported, and only sold to the local population, according to Simmons *et al.* (2005 a and b). In some areas of Mae Sot, the total concentrations of Cd and Zn in paddy soils ranged from 0.5 to 284 ppm and 100 to 8,036 ppm, respectively, which were above the cadmium and Zn background levels in Thai soils, i.e. 0.15 and 70 ppm, respectively (Zarcinas *et al.*, 2003). The concentration of Cd in rice grains ranged from 0.05 to 7.7 ppm, most of which were above the Cd Maximum Permissible Level in rice grain set at 0.4 ppm fresh weight by the Codex Committee on Food Additives and Contaminants (CCFAC) (Simmons *et al.*, 2005 a and b).

In 2003, the Royal Thai Government suspended rice production in the contaminated areas of Mae Sot, due to public health concerns. Financial compensations were given to the farmers, based on the amount of rice that they had stored at their farms, and the total area of paddy fields that they owned. In 2006, the Thai Ministry of Agriculture advised the farmers to produce non-food crops, such as sugarcane, oil palm, and rubber plantation, instead of rice. However, rice is not only a source of income for the Thai farmers, but also their principal food, consumed on a daily basis. When the financial compensations were no longer given, many farmers started to use again the Cd contaminated paddy soils for rice production (Figure 1.2). Additionally, some farmers have let their cows pasture on wild grasses grown in some paddy soils that have high Cd contents, which are no longer used for cultivation (Figure 1.3). Consequently, the food consumed by the population living at Mae Sot can still be contaminated with Cd. Two studies reported that people regularly consuming rice, grown in contaminated paddy soils at Mae Sot, had high Cd concentrations in their urine, and high risks of developing renal dysfunction problems (Swaddiwudhipong *et al.*, 2007; Teeyakasem *et al.*, 2007).

Padaeng deposit is a Zn-mining site located near some rice paddy fields in Mae Sot, Thailand. It is the first supergene nonsulfide Zn deposit in the world that has been exploited for large modern mining operations. Ores found in this deposit are hemimorphite, smithsonite, hydrozincite, and sphalerite (Reynolds, 2003). This site is believed to be the major source of Cd and Zn contamination in the district of Mae Sot (Simmons *et al.*, 2005 a; Swaddiwudhipong *et al.*, 2007).

1.3 Root exudates and metal bioavailability

Plant roots exude many compounds, called “root exudates”, into the

Figure 1.2 Paddy soil at Mae Sot, Thailand, contaminated by Cd and Zn, where lowland rice culture is practiced (picture taken in 2007).

Figure 1.3 Paddy field uncultivated due to Cd contamination, where cows pasture on wild grasses (picture taken in 2007).

rhizosphere (Bais *et al.*, 2006). Low-Molecular Weight Organic Acids (LMWOAs) are commonly found in root exudates, whose compositions depend on plant species, plant age, and the nature of the soil. Lui *et al.* (2006) studied variations of organic acid in root secretions of several rice cultivars. This study reported that acetic acid and formic acid constituted more than 96% of the total concentration of LMWOAs. However, another study showed that rice roots, under Zn deficiency, secrete mainly malate and citrate (Goa, 2007).

The pH in the rhizosphere can be lower than the pH in the bulk soil, due to plant cation uptake releasing protons to solution, and/or release of LMWOAs by plant roots (Shuman, 1997; Jauert *et al.*, 2002). Collins *et al.* (2003) suggested that LMWOAs could alter metal bioavailability in soils via two mechanisms: formation of metal-ligand complexes and acidification around the rhizosphere. Metal-organic acids complexation depends on the types and concentrations of the organic acids and the metals, as well as the soil properties. Oxalic and citric acids, which are two LMWOAs secreted by plant roots, can bridge Cd to mineral surfaces, forming ternary complexes (Krishnamurti *et al.*, 1997; Leslie *et al.*, 2002; Zhou *et al.*, 2003). Min *et al.* (2006) studied the effects of oxalic, acetic, and citric acids on Cd sorption to kaolinite, goethite, and bayerite. This study found that the amounts of Cd sorbed to the minerals were high at low concentrations of the organic acids, and were lower at high concentrations of the LMWOAs. Mench and Martin (1991) found that root exudates promoted desorption of Mn and Cu from soil components.

Several techniques have been used to identify and quantify LMWOAs from root exudates, such as gas chromatography (GC), Fourier Transform Infrared (FTIR) spectroscopy, and High Performance Liquid Chromatography (HPLC). The latter is one of the most popular techniques for determining LMWOAs secreted from plant roots (Chen *et al.*, 2004; Angeles *et al.*, 2006; Mucha *et al.*, 2010).

1.4 Batch technique vs stirred-flow technique

The distribution of Cd between the soil solid phase and the soil solution is more controlled by sorption than mineral precipitation mechanisms (McLaughlin and Singh, 1999). Traditional batch and stirred-flow methods are principally used to measure metal sorption and desorption kinetics in soils (Yin *et al.*, 1997; Strawn and Sparks, 2000; Sukreeyapongse *et al.*, 2002; Shi, 2006; McNear, 2006). The traditional batch approach has been widely used because of its experimental easiness, and provides reproducible results, in a short time period. However, the batch technique does not always provide proper mixing of the solid suspension in the batch vessel. Too much mixing may cause abrasion of the solid phase, changing its surface area. Conversely, insufficient mixing may enhance mass transfer and transport processes. Additionally, a major portion of the reaction is often completed before the first data point can be collected with this technique (Sparks, 2003). Another disadvantage is the possibility of reverse reactions, e.g. re-adsorption or secondary precipitation reactions. Lastly, the increase in metal concentrations in solution during desorption batch experiments can inhibit further release of adsorbate in the desorption process (Shi, 2006). Therefore, the batch method may not be suitable for studying metal desorption in soils over short time scales. Conversely, the stirred-flow technique reduces diffusion effects and re-adsorption phenomena, because of the continuous mixing in the reaction chamber, and removal of the products in the effluent solutions. Flow rates, desorption agents, and mixing rates can be modified during one stirred-flow experiment (Sparks, 2003). However, the blockage of the filter, which can affect the flow rate, can sometimes occur with this technique (Sparks, 1999).

1.5 X-ray Absorption Spectroscopy (XAS) and data analysis

X-ray absorption spectroscopy (XAS) is a widely used in situ technique for determining the local geometric and/or electronic structure of matter. X-ray radiation is produced at synchrotrons, which provide intense and tunable x-ray beams. With this technique, x-rays are absorbed by an atom, at energies near and above the core-level binding energies of the atom (Fendorf, 1992; Sparks, 2003; Newville, 2004). X-ray absorption spectroscopy does not alter the sample, which can be in the solid, liquid, and/or gaseous phase(s). Heterogeneous samples, such as soils, can be analyzed with this technique, containing the element of interest at concentrations of a few milligrams per liter (O'Day *et al.*, 1993; Sparks 2003). An XAFS experiment can be carried out in transmission and/or fluorescence mode(s). Concentrated samples should be measured in transmission mode, while fluorescence mode is used for low concentrated samples.

A XAFS spectrum is divided in two main parts: the x-ray absorption near-edge structure spectroscopy (XANES) region and the extended x-ray absorption fine structure spectroscopy (EXAFS) region. The term x-ray absorption fine structure (XAFS) refers to these two regions. The typical XAFS region in energy (eV) for the Cd-K edge is shown in Figure 1.4.

The XANES region is strongly sensitive to oxidation state and coordination chemistry (e.g., octahedral, tetrahedral coordination) of the atom analyzed. From the inflection point of the first derivative of the white line, one can determine the oxidation states of a metal present in a sample (Morra *et al.*, 1997;

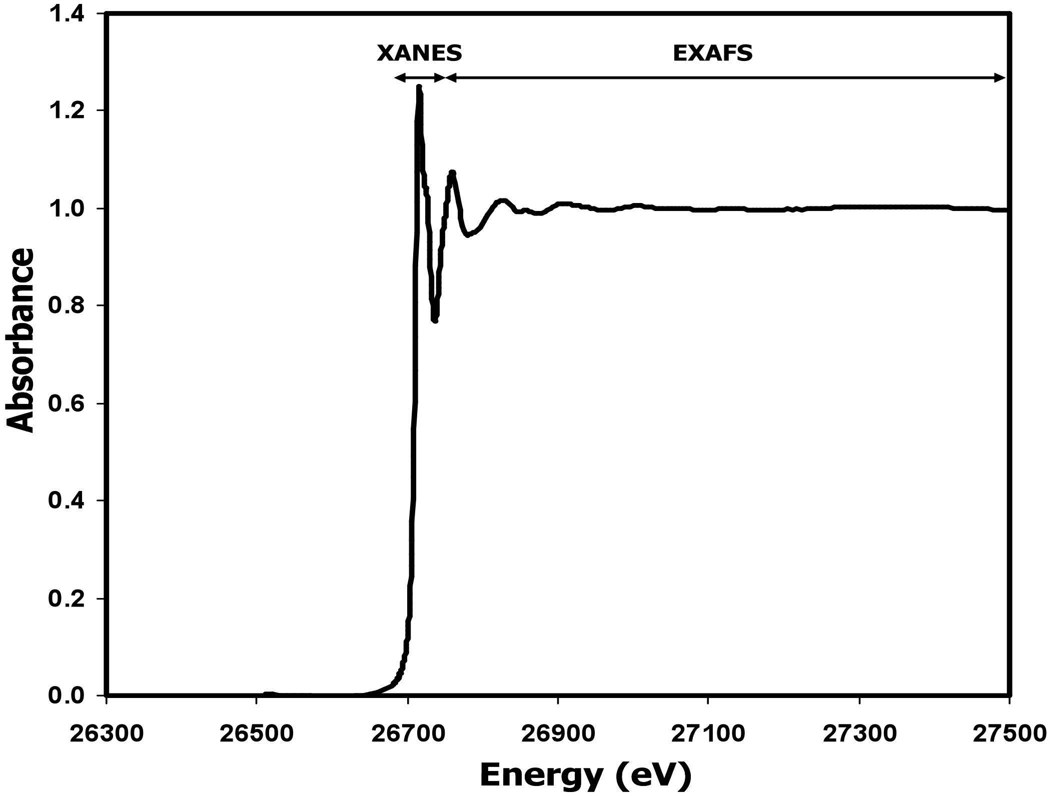

Figure 1.4 XANES and EXAFS regions of $CdCO_3$ XAFS spectrum collected at the Cd K-edge, and in transmission mode.

Beauchemin *et al.*, 2002; Seiter *et al.*, 2006). The EXAFS region is used to determine the coordination numbers and distances between the absorption atom and its neighboring atoms, as well as the identity of the atoms (Newville, 2004). Information obtained from the EXAFS region can be used to study sorption, desorption, and precipitation mechanisms. For example, Nachtegaal and Sparks (2004) used EXAFS analysis to study Zn sorption mechanisms at the clay mineral/water interface, and

Roberts *et al.* (2003) employed EXAFS to investigate the kinetics and mechanisms of Zn complexation on metal oxides.

Shell-by-shell fitting analysis can be applied to XAFS spectra of homogenous samples to obtain information about the molecular environment of a given atom. However, for samples featuring multi components, such as soils, overlapping of different atomic shells at similar radial distances may occur, which can result in misinterpretations of the data.

Principal Component Analysis (PCA), a statistical-based procedure, Target Transformation (TT), and linear combination are preferred over shell-by-shell fitting for determining species present in a mixed system (Wasserman *et al.*, 1999; Morra *et al.*, 1997; Manceau, 2002; Beauchemin *et al.*, 2002; Seiter *et al.*, 2006; McNear, 2006). The PCA technique describes the data set as weighted sums of a smaller number of components. Target transformation is used to identify the components, by taking a spectrum of a known reference compound, and mathematically removing from the spectrum anything that does not resemble the principle components identified by PCA. Reference spectra are evaluated for their "goodness of the fit" by the SPOIL value. Numbers < 1.5 are considered excellent, 1.5 to 3 good, 3-4.5 fair, 4.5-6 poor, and >6 unacceptable (Malinowski, 1978). After the contributing standard phases are identified, linear combination is used to determine the amount, in percentage, of each standard within the individual sample spectrum. Consequently, one can identify the species present in the sample, and their relative amounts. The accuracy of this fitting approach depends on the data quality of the XAFS spectra, the completeness of the standard data set, and the range of the EXAFS region over which the data are fitted (Manceau *et al.*, 2002).

Although bulk-XAFS has been successfully applied to identify metal speciation in soil samples, this technique averages the XAS signal issued from all individual species present in the sample. Microprobe synchrotron techniques, e.g. micro x-ray Fluorescence (μ-XRF), micro x-ray diffraction (μ–XRD), and micro x-ray absorption spectroscopy (micro-XAS), are alternative methods to constrain elemental distributions, oxidation states, and local molecular environment of multiple species present in a sample at a region of interest, and at the micron scale (Manceau *et al.*, 2000). An experiment carried out at a microprobe beamline often begins by collecting μ-XRF maps, to determine the distribution and association of elements. From these maps, regions that locally feature high concentrations of the element of interest (i.e. hot spots) are selected, and further analyzed by μ–XAS to constrain the oxidation states or the local molecular environment of an element, and/or μ–XRD, to identify the mineral phases in which an element is present. However, the quality of the data obtained by μ–XRD declines as grain size decreases to submicron dimensions (Scheidegger *et al.*, 2006).

1.6 Research objectives

Cadmium contamination in paddy soil at Mae Sot poses a serious health threat to the population living in this district of Thailand. People living around the contaminated sites are exposed to Cd via the consumption of local agricultural products. If remedial actions to clean up Cd are not undertaken at these sites, one must employ other strategies that can at least minimize the transport of Cd in the soil and its uptake by plants. This implies understanding Cd and Zn speciation beforehand, as well as the desorption behavior of these two metals from the soil components in the paddy fields of Mae Sot. Therefore, the main objectives of this research are

i) To elucidate Cd speciation and release kinetics in an alkaline paddy soil under various flooding and draining conditions.

ii) To investigate the transformation of Cd speciation and release kinetics in the alkaline soil after acidification, under various flooding and draining conditions.

iii) To study Zn speciation and release kinetics from the soils used in i) and ii), since these soils are co-contaminated with Cd and Zn.

iv) To determine LMWOAs secreted from two local rice cultivars from Mae Sot under Cd stress, and to determine the effect of these root exudates on Cd and Zn release kinetics from the soils used in i) and ii).

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Chapter 2

SPECIATION AND RELEASE KINETICS OF CADMIUM IN AN ALKALINE PADDY SOIL AT VARIOUS FLOODING PERIODS AND DRAINING CONDITIONS

2.1 Abstract

Cadmium contamination in paddy soils of rice-producing countries has been reported at locations near Zn mining and smelting plants. Cadmium in these soils can contaminate rice and subsequently enter the human food chain. Understanding the chemical forms in which Cd is present in paddy soils is needed to develop efficient and cost-effective strategies to clean up the soils, and/or minimize Cd uptake by rice. This study aims to determine Cd speciation and release kinetics in an alkaline paddy soil, at various flooding periods and draining conditions, by employing synchrotron-based techniques, and a stirred-flow kinetic method. Results revealed that varying flooding periods and draining conditions affected Cd speciation and release kinetics. Linear Least-Squares Fitting (LLSF) of bulk-EXAFS spectra of the air-dried soil sample, and the 1 day-flooded soil sample, showed that the EXAFS spectrum of Cd bound to organic sorbent (humic acid) contributed to at least 50% of the fits. However, this standard was not dominant in the fit for the soil flooded for 7 days and longer periods. The EXAFS spectrum of cadmium carbonates (either Cd-$CaCO_3$, or/and $CdCO_3$) significantly contributed to the LLSF fits for all soil samples. At all experimental conditions, Cd release kinetics were characterized by two steps: an initial rapid release, followed by a slow release. About 25% of Cd was released after a two-hour desorption experiment. Results obtained by micro x-ray fluorescence (μ-XRF)

spectroscopy showed that Cd was less associated with Zn than Ca, in most soil samples. This is the first study that addressed the speciation of Cd in a paddy soil with a synchrotron x-ray microprobe technique.

2.2 Introduction

Cadmium is toxic to humans, animals, and plants. Humans can be exposed to this metal via the consumption of Cd-contaminated food, particularly rice grain (Ideka *et al.*, 2004; Simmons *et al.*, 2005; Chaney, 2010). Although some minerals can naturally contain Cd, sources of this metal present at high concentrations in soils often come from anthropogenic activities, such as zinc mining and smelting operations (Alloway, 1995; Simmons and Pongsakul, 2003; Chaney, 2010). The International Water Management Institute (IWMI) reported in 2003 that Zn-mining activities in the Mae Sot district, northern part of Thailand, have resulted in Cd contamination in irrigation water, paddy soils and rice grain. Villagers in this district risk developing severe chronic health issues from Cd exposure via these contaminated soils and crops (Nogawa *et al.*, 1983; Simmons *et al.*, 2005; Swaddiwudhipong *et al.*, 2007). A number of in-situ and ex-situ remediation methods (e.g., excavation, land-filling, solidification, stabilization, thermal treatment, electro-reclamation, phytoextraction, and soil washing) have been employed to minimize Cd bioavailability in agricultural soils (Chaney *et al.*, 1996; Maejima *et al.*, 2006; Murakami *et al.*, 2007; Makino *et al.*, 2007). However, applying these remediation methods at the field scale is intricate and often expensive. To optimize their effectiveness, understanding metal speciation in contaminated soils is needed.

The lowland rice culture employs flooding and draining cyclic processes, which modify the biological and chemical properties of the soil, especially soil pH and

Eh. Therefore, the soils are mostly in oxidizing or reducing conditions, during draining or flooding cycles, respectively. These modifications in soil properties may affect Cd speciation present in the contaminated paddy soils. Synchrotron-based X-ray Absorption Spectroscopy (XAS) has been used to speciate inorganic contaminants in soil and minerals. Bulk XAFS has been successfully applied to characterize metal speciation in soil samples (Sparks, 2003). Micro-focused XAS and micro X-Ray Fluorescence spectroscopy (μ-XRF) have been used to elucidate metal speciation and elemental distribution in heterogeneous samples, at the micro-scale (Manceau *et al.*, 2000). These techniques were used in a few studies to determine the speciation of Cd in minerals and soils (Papelis *et al.*, 1996; Parkman *et al.*, 1999; Karlsson *et al.*, 2005; Grafe *et al.*, 2007; Vasconcelos *et al.*, 2007), but no study has addressed the speciation of Cd in contaminated paddy soils.

This research aims to investigate Cd speciation and release kinetics in a Cd-Zn contaminated alkaline paddy soil, subjected to various flooding periods and draining conditions. Bulk-XAFS and micro-XAS techniques were employed to determine Cd speciation, distribution, and association with other elements in the soil. A stirred-flow technique was used to measure Cd release kinetics. Results reported in this study will be useful for choosing the best strategy to remediate Cd in the paddy soils of the Mae Sot district, or at least minimize the uptake of this metal by rice plants in the contaminated fields.

2.3 Materials and methods

2.3.1 Study site and soil characterization

Surface samples (0-15 cm) were collected from Zn-Cd contaminated paddy soils, at a site located near a Zn mining area, in the Mae Sot district, Tak

Province, Thailand. The study site was selected based on Cd concentrations reported in a survey by the International Water Management Institute (Simmons *et al.*, 2005). Basic soil physicochemical properties, such as soil texture, soil pH, elemental concentrations, and organic matter content, were determined by standard procedures (Bouyoucos, 1962; Schofield and Taylor, 1955; U.S. EPA, 1986; Nelson and Sommers, 1982).

2.3.2 Incubation experiment: Flooding and draining conditions

Alternate flooding and draining conditions were employed to mimic lowland rice culture. Four periods of flooding and two draining conditions were used to study variations in Cd speciation and release kinetics. The four periods of flooding were 1, 7, 30, and 150 days, based on the understanding that the chemical properties of paddy soils usually reach apparent equilibrium within 30 days (Kyuma, 2004), and the longest period of flooding in lowland rice does not exceed 150 days. The draining cycles were carried out to saturation (Sat), i.e. ~75 % soil moisture content, and to field capacity (FC), i.e. ~58 % soil moisture content. These two moisture contents are relevant to those found in Thai paddy soils during draining periods. Incubation experiments were carried out by adding 20 g of air-dried paddy soil (particle size <2 mm) into 100 ml columns, which featured at their bottom a 0.45-µm pore-size filter. The columns were connected to 45 cm-long plastic tubing, which was used to adjust the moisture content in the columns to field capacity value. Soils in the columns were flooded with 60 ml of water for 1, 7, 30, or 150 days, by keeping the level of water about 10 cm above the soil surface, at room temperature. At the end of the flooding period, samples were drained to the saturation or to field capacity. The soil samples were then immediately mounted on sample holders for XAS analyses, or used for desorption experiments. The pH and Eh of the soil suspensions were measured throughout the flooding periods, using a pH and Eh-meter, respectively.

2.3.3 Desorption Experiments

Cd desorption kinetics were conducted using a stirred-flow technique, with soil samples flooded for different periods and draining conditions. The experimental set up was similar to the one used in Strawn and Sparks (2000). Diethylenetriamine Pentaacetate (5 mM)–Triethanolamine (100 mM) $CaCl_2$ (10 mM) (DTPA-TEA-Ca) was used as a desorption agent, at pH 7.3 (Lindsay and Norvell, 1978). The experiments were initiated by purging the tubing connected to the stirred-flow chamber with the DTPA-TEA-Ca solution, then adding 0.4 g of wet soil into the reaction chamber, and quickly filling the chamber with 8 ml of the DTPA-TEA-Ca solution. A 25-mm diameter filter membrane of 0.45 µm pore-size was used to separate the effluent from the solid suspension in the stirred-flow chamber. A 25 mm magnetic bar was used to mix the suspension at 500 rpm. The experiments were run for 120 minutes at a flow rate of 0.8 $ml.min^{-1}$, and the effluent was collected in 12 tubes, one every 10 minutes. Each experiment was run in duplicate, and Cd concentrations were measured using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), to subsequently calculate cumulative Cd desorption percentages (Shi, 2006).

2.3.4 Cadmium speciation in soil samples and Cd standards

2.3.4.1 Cd standard preparations

Cadmium standards considered for XAS analyses included some Cd phases, i.e. $CdCO_3$, CdS, $Cd(OH)_2$, $Cd(NO_3)_2$, $CdSO_4$, and Cd sorbed to some mineral phases, i.e. Cd-ferrihydrite, Cd-goethite, Cd-gibbsite, Cd-humic acids, Cd-montmorillonite, Cd-kaolinite, Cd-$CaCO_3$, and Cd-illite.

The Cd-$CaCO_3$ standard was prepared by adding a solution of $CdCO_3$ into a $CaCO_3$ suspension at pH 6.5, using a Cd:Ca molar ratio of 1:140. The reaction was equilibrated for 72 hours at room temperature. After equilibration, excess water was removed from the reaction vessel, and the wet paste was air-dried at room temperature for a week. The sample was then mounted on a sample holder for XAFS analyses.

The standards of Cd sorbed to ferrihydrite, goethite, humic acids, and clay minerals were conducted at pH 6.5, with 1 mM Cd (ionic strength = 0.01 M $NaNO_3$), under a N_2 environment for 48 hours. After each sorption experiment, the suspension was centrifuged and washed two times with 1 mM $NaNO_3$, at pH 6.5, and a third time with DDI water. After removing the supernatant during the last centrifugation, wet-pastes of samples were mounted on sample holders, and subsequently analyzed by bulk-XAFS. Ferrihydrite and goethite used for sorption experiments were prepared following the procedure described in Schwertmann and Cornell (2000). The two standards representing Cd sorbed to organic matter in soils, i.e. Cd-humic acid 1 and Cd-humic acid 2, were respectively prepared from humic acid No. H1, 675-2, Sigma-Aldrich, and Pahokee peat humic acid, International Humic Substance Society (IHSS). Montmorillonite No 20, Husband Mine (Bentonite), Mississippi, kaolinite No 7, Dixie Rubber Pit Bath, South Carolina, and illite, from Sanford, North Carolina, were used to prepare standards of Cd sorbed to clay minerals.

Solid compounds of CdS, $Cd(OH)_2$, $Cd(NO_3)_2$, $Cd(SO_4)$, and $CdCO_3$, ACS reagent grade, were also mounted on sample holders for XAFS analyses, after mixing each of them with 95% boron nitride.

2.3.4.2 Bulk-XAFS

2.3.4.2.1 Standards

The Cd standards were analyzed by bulk-XAFS at the Cadmium K-edge (26711 eV), at beamline 10-ID, Advanced Photon Light Source (APS), Chicago, IL, and at beamlines Bio 7-3 and 11-2, Stanford Synchrotron Radiation Laboratory (SSRL), Stanford University, CA. The energy was calibrated from the first inflection point of the first derivative of a Cd metal foil XANES spectrum (K-edge, 26711 eV). Each XAFS spectrum was collected with an energy range from 150 eV below the Cd absorption edge, to k values of 12 $Å^{-1}$ above the absorption edge, in fluorescence mode for Cd-sorption standards, and transmission mode for CdS, $Cd(OH)_2$, $Cd(NO_3)_2$, $Cd(SO_4)$, and $CdCO_3$ samples.

Sulfur XANES analyses (using the references samples: CdS, $CdSO_4$ and Cd-humic acids) were performed at beamline X15B at the National Synchrotron Light Source (NSLS), Broohaven National Laboratory, Upton, NY. The samples were placed in a small hutch box filled with He, and equipped with a Ge fluorescence detector. The energy range of the incident x-ray beam was from 2380 to 2550 eV (S-K edge: 2472 eV).

2.3.4.2.2 Soil samples

Air-dried and wet soil samples prepared from the incubation experiments were analyzed by bulk-XAFS in fluorescence mode, at room temperature, at beamline 10-ID (APS), and beamlines Bio 7-3 and 11-2 (SSRL). Each sample was analyzed by collecting 5-10 scans, to obtain an adequate signal to noise ratio. For sulfur speciation, only S-XANES of air-dried soil was analyzed at beamline X15B (NSLS). Each raw bulk-XAFS spectrum was normalized to obtain the EXAFS function, using Athena version 8.0.054 and the SixPack software package (Ravel and Newville, 2005; Webb, 2005). The mineral phases in which Cd is present in the soils were identified and quantified with Sixpack, using Principal Component Analysis (PCA), Target Transformation (TT), and Linear Least Squares Fitting (LLSF) procedures. These tools have been used to identify element speciation in heterogeneous samples (Wasserman *et al.*, 1999; McNear, 2006; Seiter *et al.*, 2008; Jacquat *et al.*, 2008). The energy calibrations for LLSF analyses of EXAFS functions of soil and reference samples were set at the same value (26711 eV). The PCA and LLSF were performed using the EXAFS function, which was k^3 weighted, with k values between 2 to 8.5 $Å^{-1}$. SPOIL values obtained from Target transformation (TT) analysis were used to select principal components for LLSF analysis.

2.3.4.3 Micro X-Ray Fluorescence (μ-XRF) and micro-focused X-Ray Absorption Near Edge Structure Spectroscopy (μ-XANES)

Micro x-ray fluorescence and μ-XANES analyses were conducted at beamline 13-ID-C (APS), to determine elemental distributions, and Cd speciation in the contaminated paddy soils. The μ -XRF maps were 200 μm^2 in size, with a 2 μm step size, and 1 second dwell time. The beam energy was set to 29000 eV for μ-XRF analyses of Cd, Ca, Cu, Fe, K, Zn, and Mn distributions in the soil samples. Micro XANES spectra were then collected, from about -100 eV below to about 200 eV above the Cd-K edge, at local regions observed in μ-XRF maps, where Cd was relatively highly concentrated. Three scans were taken per location, to minimize the signal to noise ratio.

2.4 Results and discussion

2.4.1 Soil analysis

Bulk physicochemical properties of the alkaline Thai paddy soil are shown in Table 2.1. The total concentration of Cd in the soil was 142 ppm, which is nearly 1,000 times higher than the background Cd concentration found in Thai soils (i.e. 0.15 ppm, Zarcinas *et al.*, 2003). Soil organic matter content was 4.3 %, and the total Zn and S concentrations were 3,050 and 282 ppm, respectively. The variations in pH and Eh in the soil suspension during 30 days of flooding period are shown in Figure 2.1 a and b, respectively. The high soil pH is reported to result from application of limestone to the fields by farmers to reduce adverse effects of excessive soil Zn on soybean and rice yields.

2.4.2 Cadmium release kinetics

Desorption experiment results showed that a small amount of Cd was released, although the soils were subjected to various flooding periods and draining conditions. The cumulative Cd desorption in most treatments was less than 20%, except for one day of flooding and draining to the to saturation, which was ~25%. Small variations in amounts of Cd released from the samples drained to the saturated point or field capacity were observed when the period of flooding was varied (Figures 2.2 a and b, respectively). Varying the flooding period seemed to affect Cd release from the soils only to a minor extent, and varying draining conditions had even less effect on Cd desorption. These results support those reported in Gestel and Diepen (1997), who showed that Cd sorption to soil components was not significantly affected by soil moisture content. The small variations in Cd desorption amounts observed in our

Table 2.1 Physicochemical properties of the soil used in this study.

Particle Size			**Soil pH**			**Total concentration (ppm)**							
% sand	% silt	% clay	Water	Buffer	O.M (%)	Cd	Zn	Fe	Ca	Cu	Mn	Mg	S
12	49	39	7.3	8.1	4.3	142	3,050	18,328	34,360	25	878	5793	281
Silty clay loam													
Total soluble salts			0.3 mmhos/cm										
Nitrate			1.7 ppm										

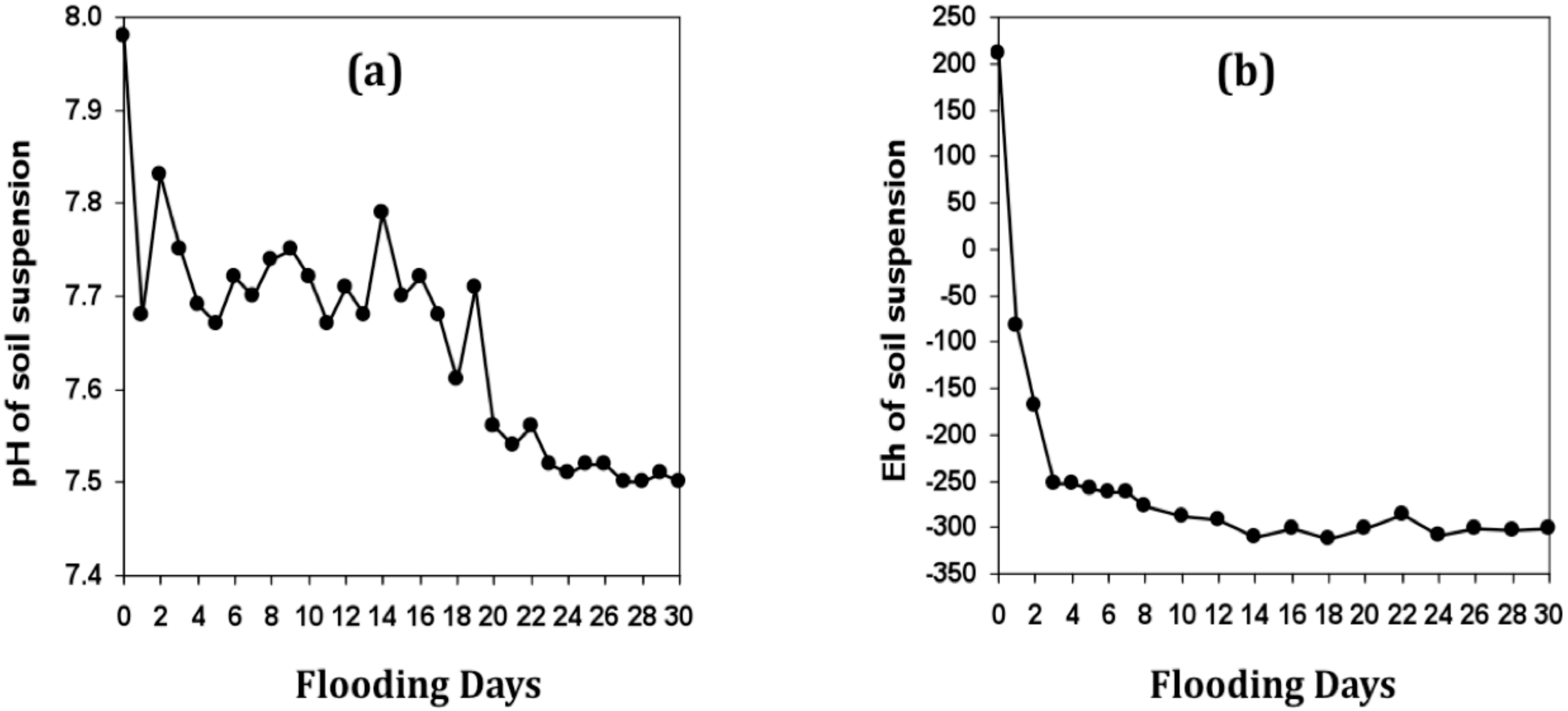

Figure 2.1 **(a)** Soil suspension pH and **(b)** soil suspension Eh of the alkaline Thai paddy soil over 30 days of flooding.

results may be due to the effect of flooding periods on soil pH and Eh (Figures 2.1 a and b). After one day of flooding, soil suspension pH decreased from ~8 to ~7.5. Studies have shown that Cd adsorption increases with increasing pH and decreases with decreasing pH (Christensen, 1984; Gray *et al.*, 1998; Casagrande and Martins, 2004; Itami and Yanai, 2006). Our results showed that Eh in the soil suspension decreased dramatically from ~ +210 to ~ -80 mV after one day of flooding, and reached almost -300 mV after 7 days of flooding. These variations in pH and Eh during flooding could promote some Cd desorption from soil components. Cadmium sorbed to soil organic matter, which decomposes as soils become more reduced during flooding periods, could be released to the soil solution (Candelaria and Chang, 1997). Additionally, Fe^{2+} and Mn^{2+} ions are often released to solution, from the reduction of ferric and manganic compounds (Kuyma, 2004). These ions either sorb to soil particles or exchange with Ca^{2+}, Fe^{2+}, Mn^{2+} and Mg^{2+} in soil components. Therefore, Ca^{2+} could also exchange with Cd^{2+} in mineral phases, since these two elements have similar hydrated ionic radii (Cd, 97 pm, and Ca 99 pm). This is supported by the results from McBride (1980), which showed that Ca^{2+} is replaced by Cd^{2+} in calcite, and Christensen (1984), where high Ca^{2+} concentrations in a sandy loam soil implied low sorption of Cd^{2+} to soil components.

2.4.3 Cadmium speciation and elemental distribution

2.4.3.1 Bulk XAFS analysis

The extracted EXAFS spectra of the soil samples and Cd standards are shown in Figures 2.3 and 2.4, respectively. Results from Principal Component

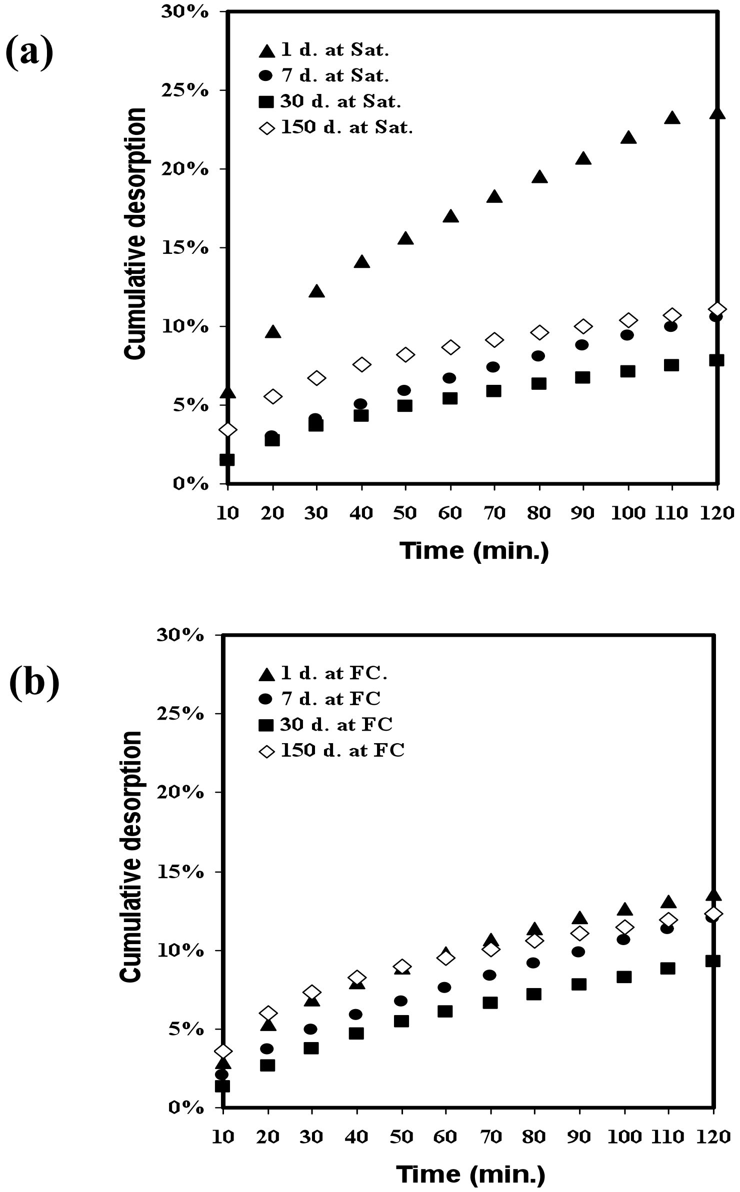

Figure 2.2 Cumulative Cd desorption (%) by DTPA-TEA-Ca, after 1, 7, 30 and 150 days of flooding and draining to **(a)** the to saturation (Sat), and **(b)** to field capacity (FC).

Table 2.2 Target Transformation SPOIL values of selected reference spectra obtained by PCA analysis. SPOIL values <1.5 indicate an excellent fit, 1.5–3 good, 3–4.5 fair, 4.5–6 poor, and>6 are unacceptable (Malinowski ,1978).

References	**SPOIL* Values**
CdS	**2.23**
$CdSO_4$	**4.87**
$CdCO_3$	**2.82**
$Cd(OH)_2$	**6.23**
$Cd(NO_3)_2$	**2.61**
Cd-$CaCO_3$	**1.58**
Cd-humic acid 1	**1.84**
Cd-humic acid 2	**2.92**
Cd-ferrihydrite	**2.68**
Cd-goethite	**2.86**
Cd-kaolinite	**3.06**
Cd-montmorillonite	**3.71**
Cd-illite	**2.91**

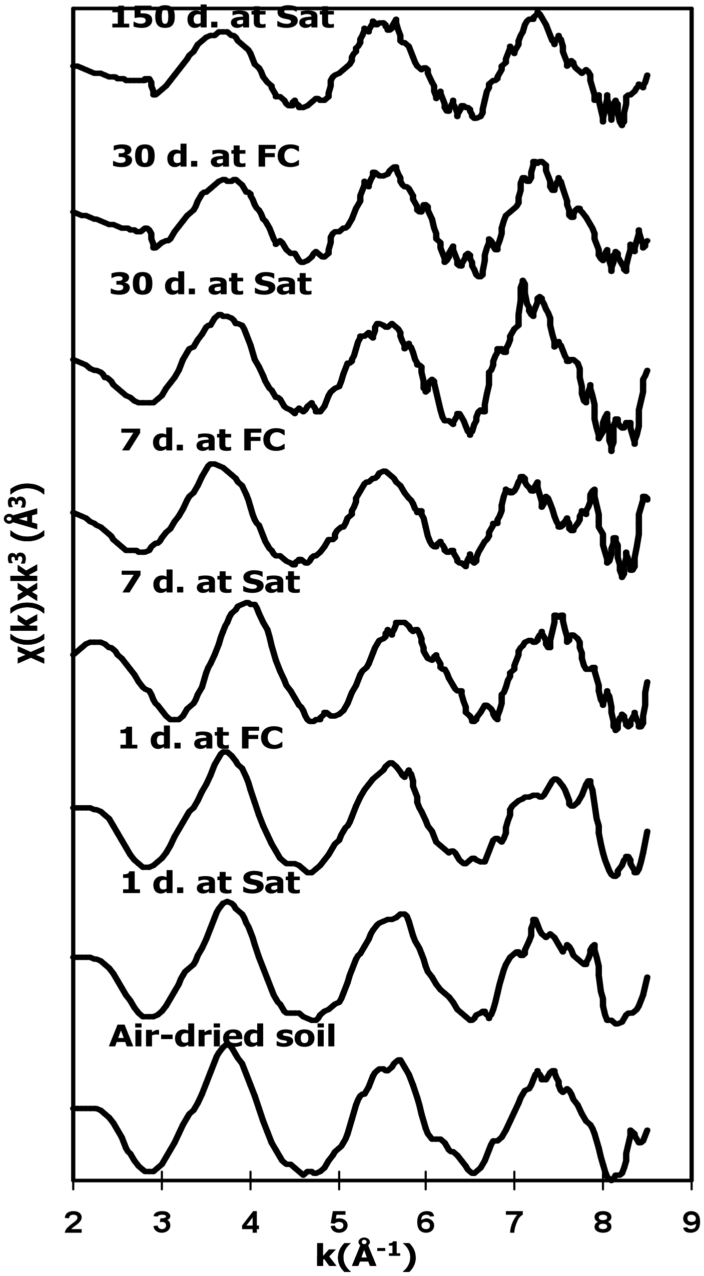

Figure 2.3 The bulk-EXAFS k^3 weighted spectra of an alkaline Thai paddy soil, under air-dried condition, subjected to different flooding periods (d: days), and draining conditions (Saturation; Sat. and field capacity; FC).

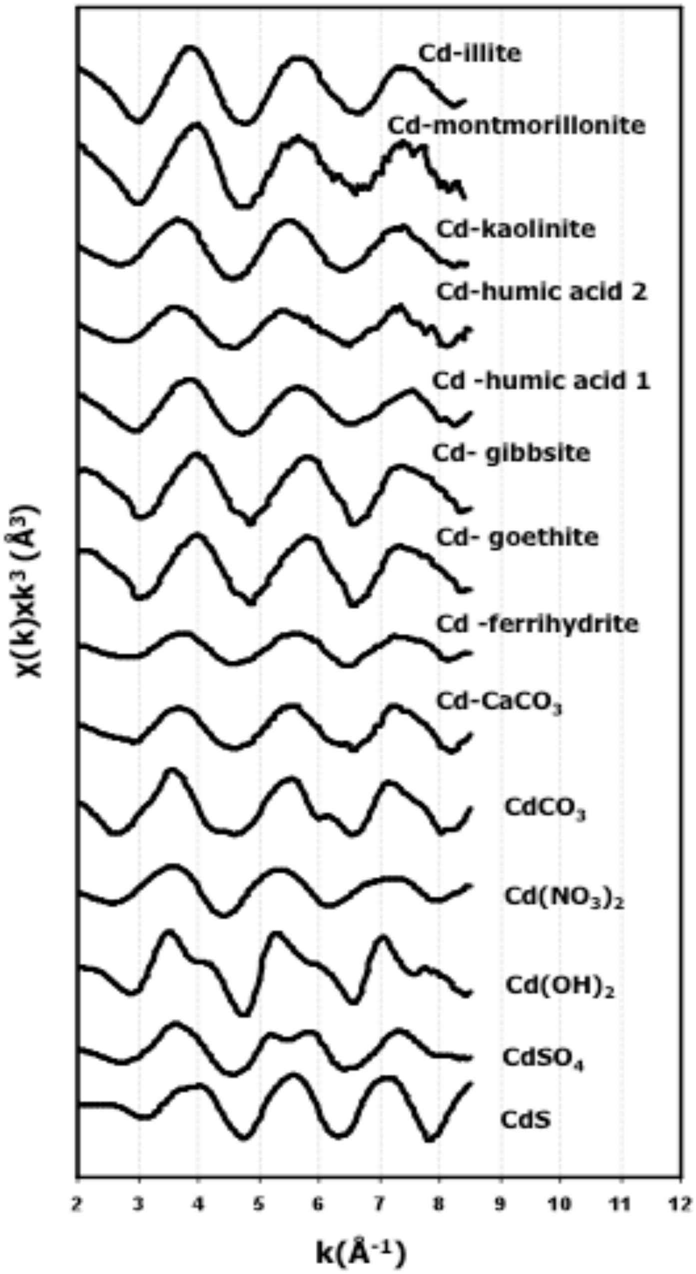

Figure 2.4 Bulk EXAFS k^3 weighted spectra of Cd reference samples.

Analysis (PCA) showed that three components contributed 81 % of the total experimental variance. The SPOIL values of the standard samples obtained from Target Transformation (TT) analysis are shown in Table 2.2. The standards that gave excellent and good SPOIL values were selected to perform Linear Least Squares Fitting (LLSF) analyses.

Several standards gave similar SPOIL values from 1.5 to 3, i.e. $Cd(NO_3)_2$, Cd-goethite, $CdCO_3$, Cd-kaolinite, Cd-illite, Cd-ferrihydrite, and Cd-humic acid 2. Each of these standards were individually used for LLSF fitting of each bulk-XAFS spectrum of the soil samples, to determine which three standards gave the lowest LLSF fit's Reduced Chi Square values (R) per soil sample. Linear least squares fitting was then performed with the set of three standards identified for each bulk-XAFS spectrum of the soil samples. The LLSF fitting results revealed that Cd speciation varied when the soil was subjected to different flooding periods and draining conditions (Figure 2.5). For the air-dried soil and the soil flooded for one day, the Cd-humic acid 1 standard significantly contributed to the fits, up to 50%. After the soil was flooded for 7 days, the contribution of this Cd standard in the fit was no longer noticeable, and Cd carbonate species, i.e Cd-$CaCO_3$ and $CdCO_3$, became the most dominant. These two standards also dominated the fit for the soil flooded for 30, and 150 days, and a small amount of CdS also contributed to the fit. The standard representing Cd bound to kaolinite contributed at least 22% of the fit for all spectra shown in Figure 2.5 of the soils flooded for different periods. Buanuam *et al.* (2005) found high correlation between the spatial distribution of Cd and Fe in 79 contaminated soil samples from Mae Sot, and concluded that Cd was mostly sorbed to

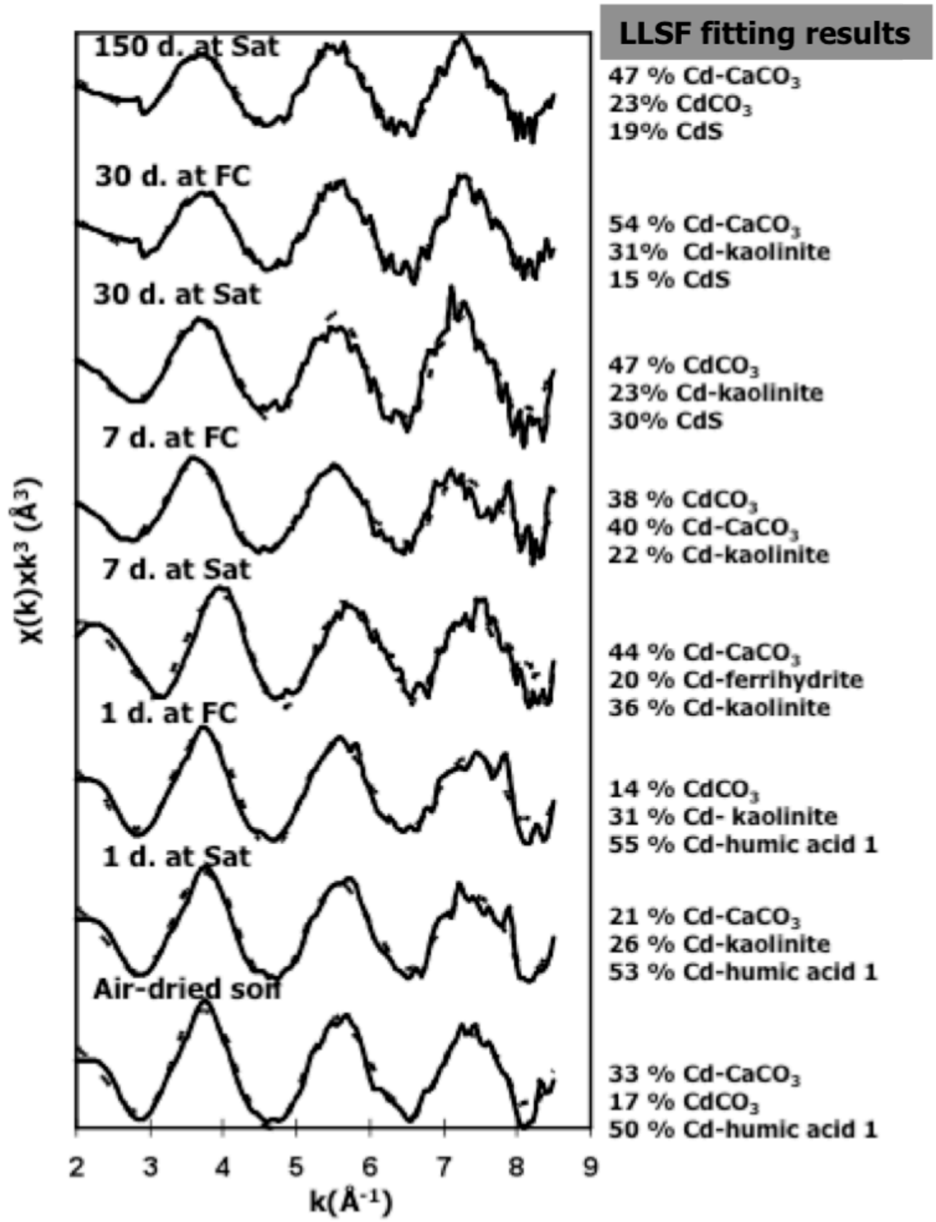

Figure 2.5 Linear least squares fitting results for bulk-XAFS spectra of the alkali Thai paddy soil under air-dried and various flooding periods and draining conditions. Solid lines represent the k^3 weighted χ-spectra and the dotted line the best fits obtained using linear least squares fitting. The percentages represent the quantity of each species represented in a particular spectrum.

Fe-oxides, based on elemental atomic ratio plot extractograms obtained from continuous-flow desorption experiments, using different extracting reagents in the influent solution. The results reported in our study show that Cd bound to ferrihydrite was only noticeable in the LLSF fit for the soil flooded for 7 days and drained to field capacity, and gave a relative small contribution to the fit (20%). Therefore, although Fe-oxides may contain Cd in our soil sample, they are not the principal Cd sorbents in the alkaline paddy soil analyzed in this study. Using extracting reagents to determine the compartmentalization of one element in a soil is often inaccurate, because re-absorption and redistribution of the element can occur, which can over or under estimate its concentration (Ostegren *et al.*, 1999). Additionally, extraction procedures only determine the pools in which an element is present in the soil, e.g. the exchangeable, acid soluble, reducible, and oxidizable phases (Ostegren *et al.*, 1999), but do not identify the mineral phases in which the element is present. Results from our study show that Cd is distributed in several mineral phases during flooding periods in the paddy soils, including carbonates, kaolinite, ferrihydrite, humic acid, and CdS. This differs from previous studies that suggested that Cd is mainly present as CdS in paddy soils during flooding periods (Bingham *et al.*, 1975; Chaney *et al.*, 1996, Simmons and Pongsakul, 2003).

Previous studies have shown that humic acids enhance Cd sorption to hematite, goethite, or kaolinite (David and Bhatnagar, 1995; Arias *et al.*, 2002; Lai *et al.*, 2002). Humic acids can directly bind metals via their functional groups (Sparks, 2003), and sorb to oxides and clay minerals, allowing the formation of ternary surface complexes (Weber, 1988; Arias *et al.*, 2002). Cadmium bound to humic acids is not stable, especially at lower pH values (Zachara *et al*., 1992; Candelaria and Chang, 1997). Therefore, some amount of Cd bound to humic acids could be released to solution during our flooding experiments, since pH measured in our soil samples decreased with increasing flooding period (Figure 2.1). The amount of Cd released from humic substances could bind to other adsorbents, such as kaolinite (Bthattachryya and Gupta, 2007; Grafe *et al.*, 2007), the most dominant clay mineral in the paddy soils used in this study, and to carbonate minerals. This is supported by our LLSF results, which showed that the Cd-kaolinite, Cd-$CaCO_3$, and $CdCO_3$ standards contributed significantly to the fits for the soils flooded for long periods of time. Metals can sorb to carbonate minerals in alkaline soils (Pickering, 1982; Li and Thornton, 2001). Cadmium and calcium can compete for adsorption sites on calcite, since Cd^{2+} and Ca^{2+} have similar hydrated ionic radii (McBride, 1980; Pickering, 1982; Garin *et al.*, 2003). Mononuclear Cd^{2+} can chemisorb to the calcite surface at low Cd concentrations, and/or precipitate, to form $CdCO_3$, at high Cd^{2+} loadings (McBride, 1980; Stipp *et al.*, 1992). Because the dominant Cd species identified by LLSF analysis included Cd-humic acid 1, Cd-kaolinite, and Cd-$CaCO_3$, Cd^{2+} is mainly sorbed to soil components in our paddy soils, and it is geogenically included in mineral phases only to a minor extent. Therefore, the main sources of Cd in our paddy soils could be anthropogenic, and not natural. This is also supported by the high concentrations of Cd measured in our soil samples, which were higher than the background concentration of Cd in Thai paddy soils, by at least three orders of magnitude. Similarly, three previous studies reported that the main source of Cd in the contaminated paddy soils of Mae Sot district came from Zn mines located near the sampling sites. (Simmons *et al.*, 2005; Buanuam *et al.*, 2005; Akkait and Tongcumpou, 2010).

Bulk XANES taken at the S K-edge featured multi white-lines in the air-dried soil sample spectrum, indicating that S in the soil is present in multi oxidation states (Figure 2.6). Because multiple oxidation states of S are also observed at the same energy positions in the bulk XANES spectrum of the Cd-humic acid 1 standard (Figure 2.6), sulfur may be associated with humic acids in the air-dried soil sample. However, sequential extraction methods were employed in these two studies, which could have resulted in re-adsorption and redistribution of Cd in the soil (Ostergren *et al.*, 1999; Ahnstrom and Parker, 1999). Additionally, no direct microscopic evidence, probing Cd in situ in the soil, was reported in these two studies. Our results are supported by those reported in Morra *et al.* (1997), which showed that S was distributed mainly with humic acid in their soil samples, and Martinez *et al.* (2002), who suggested that reduced S sorbed to organic compounds in soil can bind to Cd. Additionally, Solomon *et al.* (2003) suggested that S sorption to organic matter typically occurs in both temperate and tropical soils. Therefore, sulfur sorption to humic acid may occur in the paddy soils of Mae Sot district.

2.4.3.2 Micro -XRF and μ -XANES analyses

Micro-XRF maps of Cd, Ca, Zn, and Fe distributions in the air-dried soil and the soils flooded for different periods are shown in Figure 2.7. The brightest color in these maps represents the highest count or highest concentration of an element, while the darkest color represents the lowest count or lowest concentration of an element. These maps show that Cd is distributed both in regions where Cd is diffuse in the soils, and in regions where Cd is locally concentrated (hot spots). Cadmium

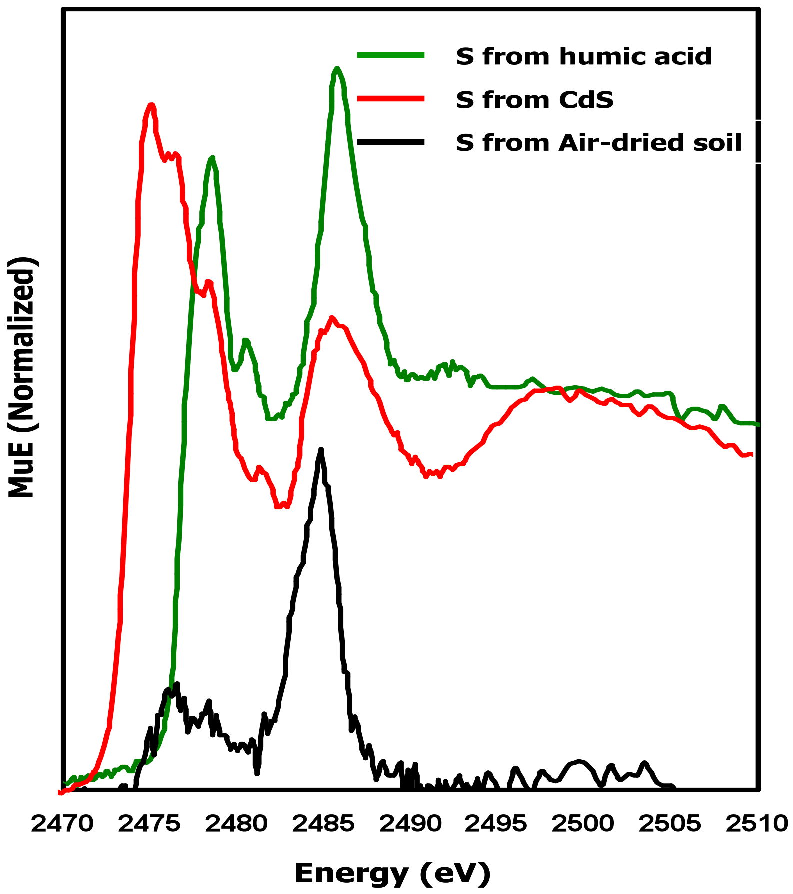

Figure 2.6 Bulk XANES of sulfur-K edge spectrum of the air-dried soil, compared to bulk XANES spectra of CdS and Cd-humic acid 1 standards.

present in the air-dried soil seems to be associated with Zn and Fe at regions where these elements are diffuse, but not at hot spot regions (Figure 2.7). After the soil was flooded for different periods, Cd is less diffuse in the soil and seems to concentrate more locally. The locations of these Cd hot spots are not similar with those of the Zn and Fe hot spots observed in the μ-XRF maps of the flooded soils. However, the locations of some Cd hot spots are similar to those of Ca hot spots observed in the μ-XRF maps of the soils flooded for 7, and 30 days (Figure 2.7). Micro-XANES spectra taken at the Cd K-edge were collected at two Cd hot spots chosen in the μ-XRF map of the soil flooded for 30 days and drained to the to saturation (Figure 2.7 and 2.8). One notices that the shape of these two XANES spectra are different from each other, suggesting that Cd is present in different mineral phases at these two locations, which are separated by only a few hundred micrometers in the μ-XRF map. The shape of these two μ-XANES spectra were compared with those of $CdCO_3$, Cd-$CaCO_3$, CdS, and Cd-kaolinite XANES spectra, i.e. the four Cd phases that were identified by LLSF analysis for the soil flooded for 30 days (Figure 2.5). The shape of the μ-XANES spectrum taken at the first location (spot 1) is similar to those of Cd-$CaCO_3$ and CdS XANES spectra, while the shape of the μ-XANES spectrum taken at the second location (spot 2) is similar to those of $CdCO_3$ and Cd-kaolinite XANES spectra. These results support our LLSF results, which showed that Cd can be distributed in several phases in the contaminated paddy soils taken from Mae Sot. Additionally, our results suggest that Fe phases are not important Cd scavengers, which differ from the results reported in Akkajit and Tongcumpou (2010), and Buanuam *et al.* (2005). These two studies indicated that Cd was mainly sorbed to Fe oxide minerals in the contaminated soils of Mae Sot. However, sequential extraction methods were employed in these two studies, which could have resulted in re-adsorption and redistribution of Cd in the soil (Ostergren *et al.*, 1999; Ahnstrom and Parker, 1999). Additionally, no direct microscopic evidence, probing Cd in situ in the soil, was reported in these two studies.

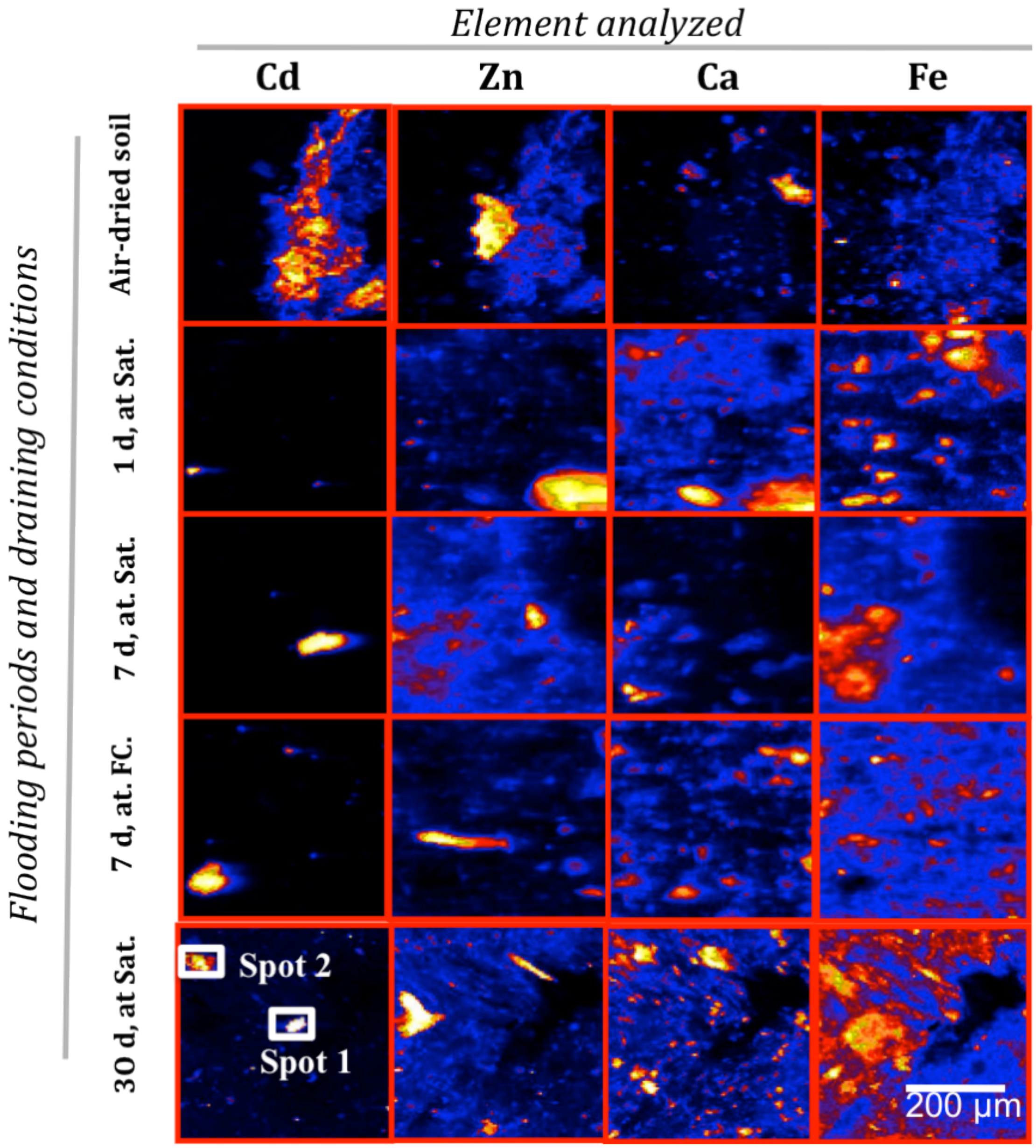

Figure 2.7 Elemental distributions of cadmium (Cd), zinc (Zn), calcium (Ca), and iron (Fe) in air-dried soil, the soil flooded for 1 day, and drained to saturation, the soil flooded for 7 days, and drained to the saturation, the soil flooded for 7 days, and drained to field capacity, and the soil flooded for 30 days, and drained to the saturation, using μ-XRF mapping.

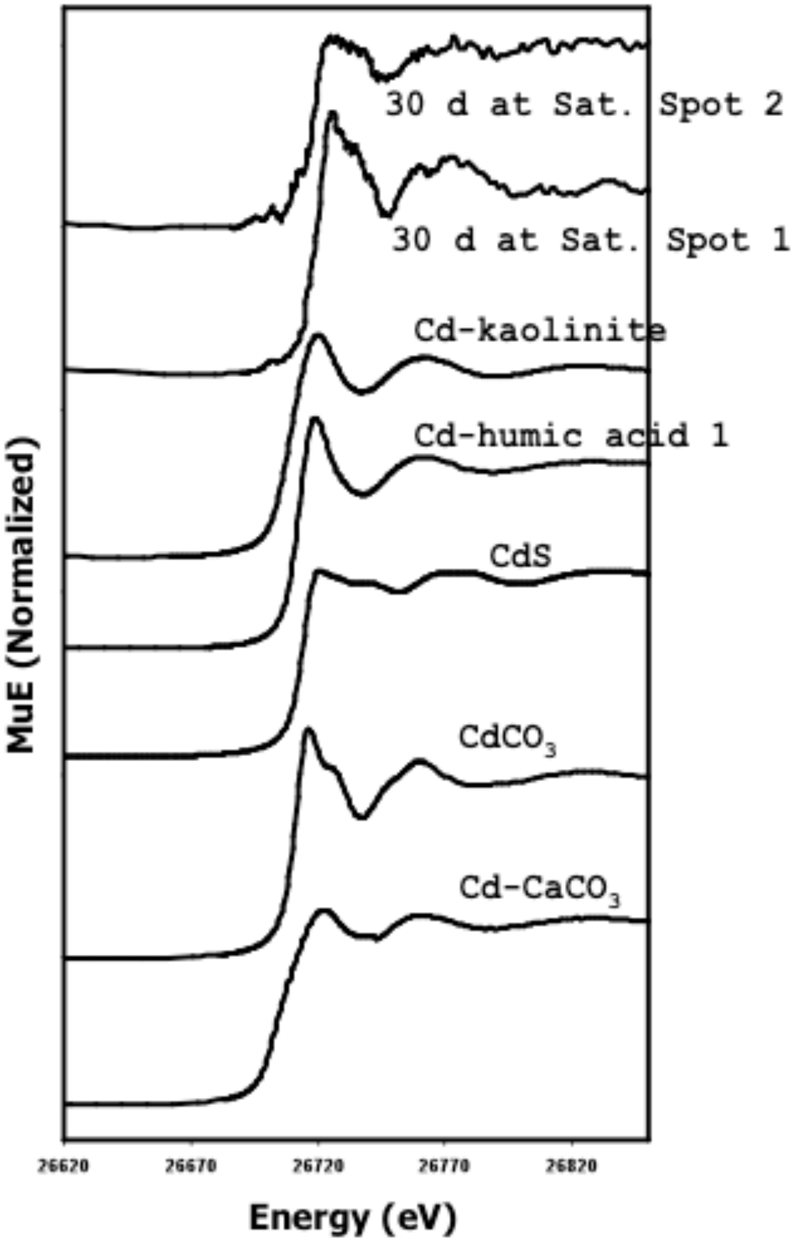

Figure 2.8 Micro-XANES spectra, at the Cd-K-edge, of Cd-CdCO$_3$, CdCO$_3$, CdS, Cd-humic acid 1, and Cd-kaolinite standards, and μ -XANES spectra taken at spots 1 and 2 (Figure 2.7 e).

2.5 Conclusions

Several phases containing Cd, (Cd-carbonates, Cd-humic acid, Cd-kaolinite, Cd-ferrihydrite, and CdS) were found in the Cd-contaminated alkaline paddy soil in Mae Sot district, under various flooding periods and draining conditions. Therefore, Cd-sulfide is not the only main species to control Cd release in the alkaline paddy soil during flooding periods, as was suggested by former studies. About 1 ppm of Cd was desorbed from the alkaline Thai paddy soil using DTPA-TEA-Ca, after two hours of stirred-flow experiments. This amount of Cd that can be desorbed from soil components poses a threat to humans, knowing that less than 0.1 ppm Cd in the soil solution, and subsequently taken up by rice plants, can be toxic to rice consumers (Dabin *et al.*, 1978; Bingham, 1979). This study demonstrates the urgent need for remediation actions to clean reduce Cd risks from the contaminated paddy soils in Mae Sot. The amount of Cd that can be easily desorbed from the soil should be removed by acidify the alkaline soil and/or adding some chemicals to alter Cd phases and make it more mobile. The remaining amount of Cd, which is not readily desorbed from soil components, could be controlled by liming.

2.6 References

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Chapter 3

CADMIUM SPECIATION AND RELEASE KINETICS IN ACIDIFIED PADDY SOIL UNDER VARIOUS FLOODING PERIODS AND DRAINING CONDITIONS

3.1 Abstract

This study investigated Cd speciation and release kinetics from a treated Cd-contaminated paddy soil, taken from the Mae Sot district, Thailand. The soil treatment consisted of acidification of the soil to pH 6 and addition of K_2SO_4. Bulk and micro X-ray Absorption Spectroscopy (XAS) were employed to study Cd speciation and elemental distribution in the treated soil, which was flooded for different periods, and drained to saturation or field capacity. A stirred-flow kinetic technique was employed to study Cd release kinetics from the soil. Cumulative Cd desorption, after 2 hours reached 100% from the air-dried treated soil, and more than 50% when the treated soil was flooded for 30 days and drained to saturation point. This value was less than 25% of what was observed in untreated alkaline soil. Therefore, acidifying the soil and adding K_2SO_4 to it promoted Cd desorption. Results from Linear Least Squares Fittings (LLSF) of bulk-EXAFS spectra for the air-dried soil, acidified and treated with K_2SO_4, showed that Cd-carbonate phases were less present in the soil than CdS and Cd-humic acid phases. After the soil was flooded for different flooding periods, Cd-kaolinite became the most dominant species. Therefore, results from stirred-flow experiments and LLSF analyses suggested that Cd is less strongly sorbed to kaolinite than carbonate species. If leaching techniques are used to

remediation Cd in the contaminated paddy soils in the Mae Sot district, lowering soil pH and adding K_2SO_4 to the soil could be an efficient way to enhance Cd desorption from soil components.

3.2 Introduction

Although Cd is a non-essential element for human metabolism, it can accumulate in the body, especially in the kidney and liver, and cause serious health problems (Elinder and Piscator, 1978; Nogawa *et al.*, 1983; Swaddiwudhipong *et al.*, 2007). Rice grown in Cd contaminated paddy soil can uptake and accumulate Cd into its grain. Therefore, humans can be exposed to soil Cd through the consumption of contaminated rice grains (Ikeda *et al.*, 2004, Simmons *et al.*, 2005). Several in-situ and ex-situ remediation methods have been employed to minimize Cd bioavailability in agricultural soils (Chaney *et al.*, 1996; Maejima *et al.*, 2006; Murakami *et al.*, 2007; Makino *et al.*, 2007). Among these methods, liming, which minimizes Cd solubility, is considered to be one the best practical choices for farmers, due to its low cost and ease of application (Bolan et al, 2003; Hong *et al.*, 2007; Loganathan *et al.*, 2008).

Under lowland rice culture, paddy soil undergoes several cycles of oxidizing and reducing conditions, which alter soil pH and Eh. Additionally, exudates from roots and ion uptake processes can locally decrease the pH in the rhizosphere (Nye, 1981; Hinsinger, 1998 and 2001; Chaignon *et al.*, 2002). These modifications in soil chemical properties can inhibit the efficiency of liming around root zones. In the Mae Sot district of northern Thailand, paddy soils have been contaminated with Cd, due to Zn mining discharges (Simmons *et al.*, 2005; Swaddiwudhipong *et al.*, 2007). Although farmers have used liming to immobilize Cd present at very high concentrations in some contaminated paddy soils in Mae Sot, small amounts of Cd were still desorbed from soil components and taken up by rice. In these paddy soils, featuring very high levels of Cd, the amount of Cd that can easily desorb from soil components might be released to solution using chemical procedures. Afterwards, Cd in solution might be removed from the soils, before liming is used to immobilize the remaining amount of Cd present in the soils. Two examples of chemical procedures that promote Cd desorption from soil components are soil acidification, the addition of sulfate, sodium and calcium chloride to the soil (Benjamin and Leckie, 1982; McLaughlin *et al.*, 1998; Römkens and Vries, 2007; Makino, 2009). The nature of the Cd phases in the soil may vary during the different cycles of low land rice culture, which can modify the physicochemical properties of the soil. These variations in soil properties can in turn affect, Cd speciation, and consequently the effectiveness of chemical procedures to desorb Cd from soil components. Therefore, our research aims to investigate Cd speciation and release kinetics in a Cd-contaminated alkaline paddy soil, which was acidified to pH 6 and spiked with K_2SO_4, under different flooding periods and draining conditions characteristic of the lowland rice culture. This will be accomplished by employing synchrotron-based XAS techniques and a stirred-flow kinetic method. The results provided in this study will be useful in choosing the best strategy for Cd remediation in agricultural soils, especially in paddy soils highly contaminated with Cd and Zn, including those located in the Mae Sot district of Thailand.

3.3 Materials and methods

3.3.1 Study site and soil preparation

Surface samples (0-15 cm) were collected from Zn-Cd contaminated paddy soils, at a site located near a Zn mining area, in the Mae Sot district, Tak Province, Thailand. The study site was selected based on Cd concentrations reported in a survey by the International Water Management Institute (Simmons *et al.*, 2005). Basic soil physicochemical properties, such as soil texture, soil pH, elemental concentrations, soluble salts, nitrate, and organic matter content, were determined by standard procedures (Bouyoucos, 1962; Schofield and Taylor, 1955; U.S. EPA, 1986; Sims and Heckendorn, 1991; Nelson and Sommers, 1982). The soil was acidified with HNO_3 to pH 6 and spiked with K_2SO_4 to increase the amount of sulfur in the soil. Sulfur can bind to Cd, forming Cd-sulfide and/or Cd-sulfate species, and can also enhance Cd release from soil components (Benjamin and Leckie, 1982; McLaughlin *et al.*, 1998). To acidify the soil sample, 500 grams of the alkaline Thai paddy soil were mixed with 250 ml of DDI-water, and titrated to pH 6 with HNO_3. The pH of the soil suspension was manually kept at pH 6 for 5 weeks. The soil was air-dried for 2 weeks under a hood, without draining the water from the soil. About 20 g of air-dried soil was used as the acidified soil sample, but not spiked with K_2SO_4. The remaining amount of air-dried soil (~ 480 g) was spiked with approximately 250 ml of 0.5 M K_2SO_4. The pH of this soil suspension was maintained at pH 6, by gently stirring, for 2 weeks, and was finally air-dried again. The soil sample was ground and sieved through a 2 mm sieve, and digested with acid, to measure total sulfur and metal concentrations (U.S. EPA, 1986).

3.3.2 Incubation experiment: flooding and draining conditions

The periods of flooding and draining conditions were similar to those described in chapter 2. The three periods of flooding were 1, 30, and 150 days. The draining conditions were to saturation point (Sat), and field capacity (FC). Experimental procedures were similar to those described in section 2.3.2.

3.3.3 Desorption experiments

Cadmium release kinetic experiments were conducted with the alkaline soil, the acidic soil before the addition of K_2SO_4, the acidic soil spiked with K_2SO_4, and the acidic soil samples spiked with K_2SO_4 that were flooded for different time periods (3.3.2), using a stirred-flow method. The experimental procedures were similar to those described in section 2.3.3. The extracting solution used for the desorption experiment was DTPA-TEA-Ca (Lindsay and Norvell, 1978).

3.3.4 Cadmium speciation of soil samples and Cd standards

3.3.4.1 Cd standard preparations

Cadmium standards considered for XAS analyses included some Cd phases, i.e. $CdCO_3$, CdS, $Cd(OH)_2$, $Cd\ (NO_3)_2$, $Cd\ (SO_4)$, and Cd sorbed to some mineral phases, i.e. Cd-ferrihydrite, Cd-goethite, Cd-gibbsite, Cd-humic acids, Cd-montmorillonite, Cd-kaolinite, Cd-$CaCO_3$, and Cd-illite. The preparation procedures were explained in section 2.3.4.1.

3.3.4.2 Bulk- X-ray Absorption Fine Structure Spectroscopy (Bulk-XAFS)

Air-dried acid soil samples and wet pastes of soil samples flooded for different time periods were mounted on sample holders. These samples were analyzed at the Cd K-edge (26711 eV), at beamlines Bio 7-3 and 11-2 at Stanford Synchrotron

Radiation Laboratory (SSRL). Fluorescence mode was used to analyze the samples, with a 30-element Ge detector, at room temperature. Ag-filter was placed between the sample and the detector to reduce elastic scatterings. At least 5 scans were collected per sample and averaged together, to obtain an adequate signal to noise ratio. A raw bulk-XAFS spectrum of each soil sample was normalized to obtain the EXAFS function, using Athena (version 8.0.054) and the SixPack software package (Ravel and Newville, 2005; Webb, 2005). The mineral phases in which Cd is present in the soils were identified and quantified with Sixpack, using Principal Component Analysis (PCA), Target Transformation (TT), and Linear Least Squares Fittings (LLSF) procedures. These tools have been used to determine metal speciation in heterogeneous samples (Wasserman *et al.*, 1999; McNear, 2006; Seiter *et al.*, 2008; Jacquat *et al.*, 2008). The energy calibrations for LLSF analyses of EXAFS functions of soil and reference samples were set at the same value (26711 eV). The PCA and LLSF were performed using the EXAFS function, which was k^3 weighted, with k values between 2 to 8.5 $Å^{-1}$. SPOIL values obtained from Target transformation (TT) analyses were used to select principal components for LLSF analysis.

3.3.4.3 Micro-focused X-Ray Fluorescence (μ-XRF) and X-Ray Absorption Near Edge Structure Spectroscopy (μ-XANES)

Micro x-ray fluorescence and μ-XANES analyses were conducted at beamline 13-ID-C at the Advanced Photon Source (APS), to determine elemental distributions, and Cd speciation in the contaminated paddy soils. The μ -XRF maps were 200 μm^2 in size, with a 2μm step size, and a 1second dwell time. The beam energy was set to 29000 eV for μ-XRF analyses of Cd, Ca, Cu, Fe, K, and Zn distributions in the soil samples. Micro XANES spectra were collected, from about -

100 eV below to about 200 eV above the Cd-K edge, at local regions observed in μ-XRF maps, where Cd was highly concentrated. Three scans were taken per location and averaged together, to minimize the signal to noise ratio.

3.4 Results and discussion

3.4.1 Soil analysis

Bulk physicochemical properties of the acidified soil are shown in Table 3.1. The total concentration of Cd in the soil was 142 ppm, which is nearly 1,000 times higher than the background Cd concentration found in Thai soils (i.e. 0.15 ppm, Zarcinas *et al.*, 2003). Soil organic matter content was 4.3 %, and the total Zn and S concentrations were 3,050 and 3,618 ppm, respectively. The amounts of total soluble salts and nitrate in the acidified soil were 60 and 7,200 times higher than in the alkaline soil, respectively. The soil suspension pH slowly increased during 30 days of flooding, from pH 6 to ~pH 7 (Figure 3.1). The soil suspension Eh slowly decreased from ~315 mV to ~240 mV during 30 days of flooding. These Eh values correspond to moderately reduced conditions (Delaune and Reddy, 2005). The slow decrease in Eh is likely due to the high concentrations of nitrate and manganese (IV), which can act as electron acceptors in the soil (Reddy and Patrick, 1974; Ponnamperuma, 1981; Weres *et al.*, 1990; Reddy *et al.*, 1995; Reddy and Delaune, 2008). Reddy and Patrick (1974) showed that the stability of the Eh value, at approximately + 200 mV, and measured over a period of 100 days, was due to nitrate present at high concentrations in the soil studied. The Eh of the alkaline soil studied in Chapter 2, which had much lower soluble manganese and nitrate concentrations in soil solution became negative after the first 24 hours of flooding, and reached about -250 mV after 30 days of flooding.

Ponnamperuma (1981) reported that under flooded conditions and in a pH range from 5 to 6, Mn^{2+} can dissolve into soil solution, while at pH above 7, Mn^{2+} can be precipitated in carbonates, oxides, and /or hydroxide minerals. This could explain the high concentration of manganese in soil solution of the acidified soil sample (Table 3.2). Additionally, high soluble salts, sulfate, and/or nitrate could also inhibited soil microbe activities, and prevented the expected reduction in Eh under flooded conditions. Reddy and Delaune (2008) reported that dissolved sulfate can inhibit and/or minimize microbial activities and sequentially causes a slow decrease of the soil suspension Eh.

3.4.2 Cadmium release kinetics

Cumulative Cd desorption reached 100% in the air-dried soil acidified and spiked with K_2SO_4, while this value was less than 25% in the air-dried alkaline soil (Figure 3.2). This demonstrates that acidifying the soil, and adding K_2SO_4 to it, enhanced Cd release from the alkaline soil. Buanuam *et al.* (2005) studied the desorption behavior of Cd and Zn from seventy-nine soil samples taken from the Mae Sot district, at the same contaminated area as our study. More Cd and Zn were desorbed from the acidic soils than from the alkaline soils. Therefore, the results from Buanuam *et al.* (2005) support those reported in this study, which showed that Cd desorption is enhanced when the soil is acidified.

Cadmium release kinetics from the soils flooded for different periods and drained to different conditions are shown in Figures 3.3. During a 2 hour period of desorption, cumulative Cd desorption reached almost 60% in the soil flooded for 30 days and drained to saturation point, and >30% in the soils flooded for other periods. All these desorption experiments revealed a Cd release pattern characterized by two

Table 3.1 Physicochemical properties of the soil used in this study.

Particle Size				**O.M**	**Total concentration (ppm)**							
% sand	% silt	% clay	**Soil pH**	**(%)**	**Cd**	**Zn**	**Fe**	**Ca**	**Cu**	**Mn**	**Mg**	**S**
12	49	39	6.0	4.3	142	3,050	18,328	34,360	25	878	5793	3,618
Silty clay loam												
Total soluble salts			18 mmhos/cm									
Nitrate			12,300 ppm									

Table 3.2 Elemental concentrations in solution of the acidic soil spiked with K_2SO_4 after different periods of flooding.

Incubation period (day)	**Water soluble element (ppm)**									
	Al	**Ca**	**Cd**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**S**	**Zn**
1 day	0.1	4,196	3.9	0.0	0.0	2,187	286	75	346	25.0
30 days	0.3	5,794	5.1	0.0	0.0	3,045	422	123	359	28.7
150 days	0.1	1,224	0.5	0.0	0.0	426	61	24	353	3.2

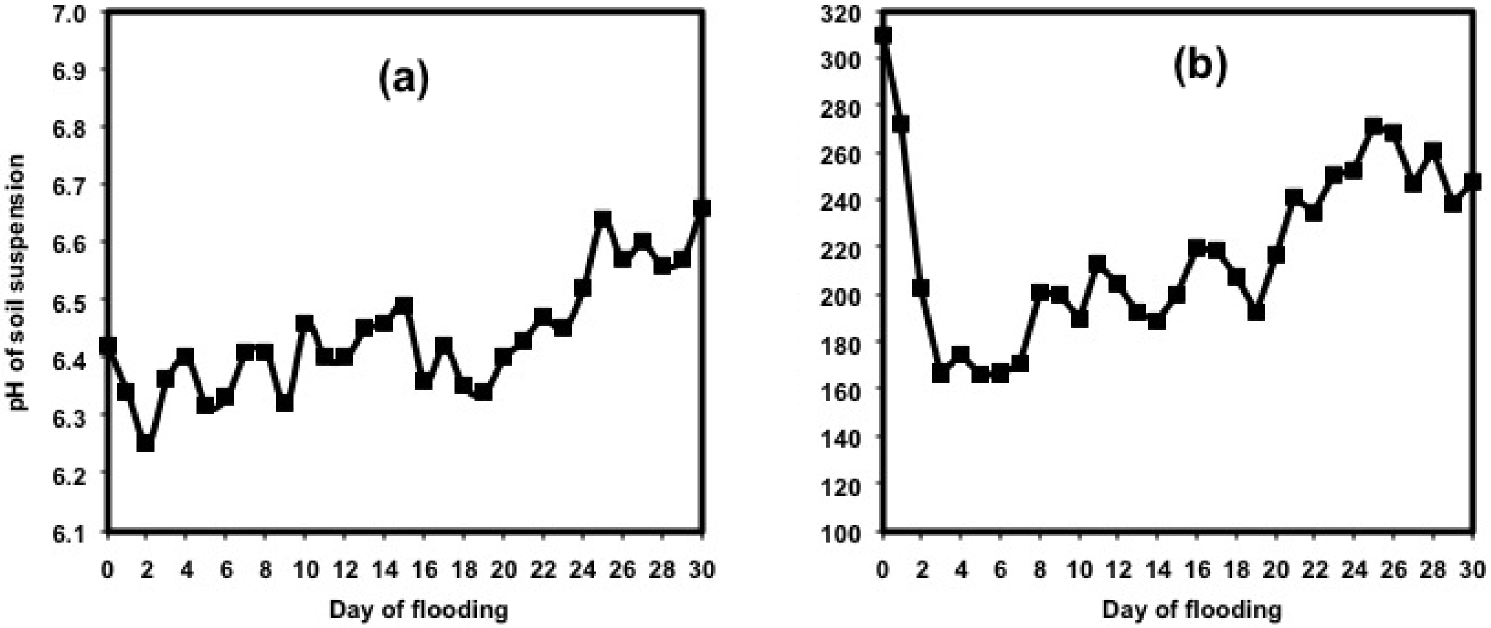

Figure 3.1 **(a)** Soil suspension pH and Eh **(b)** soil suspension Eh of the acidified paddy soil over 30 days of flooding.

steps, i.e. an initial rapid release within the first 30 minutes, followed by a slower release. This release pattern was also reported by Sukreeyapongse *et al.* (2002). During the first 10 minutes, the highest amount of Cd released was found for the soil flooded for 30 days, followed by the soil flooded for 1 day. The lowest Cd release was found for the soil flooded for 150 days. This order was also found in incubation water of the soils flooded for the same three periods, during the incubation experiments (Table 3.1). In the acidic soil, some cations, such as $Ca^{2+,}$ Mg^{2+}, Fe^{2+}, Na^{+}, and K^{+}, could be released from silicate minerals by protons in the soil solution, via a cation exchange mechanism (Schaetzl and Anderson, 2005). Additionally, under flooded conditions and a pH range from 5 to 6, Mn^{2+} can dissolve into the soil solution (Ponnamperuma, 1981). These cations could sorb again to soil components and/or exchange for Cd^{2+} sorbed to soil components, resulting in a variation in Cd release into the soil solution.

3.4.3 Cadmium speciation and elemental distribution

3.4.3.1 Bulk XAFS analysis

Results from Principal Component Analysis (PCA) showed that three components contributed 80 % of the total experimental variance (Table 3.3). The SPOIL values of the standard samples obtained from Target Transformation (TT) analysis are shown in Table 3.4. Although the standards that gave excellent and good SPOIL values were preferred in performing Linear Least Squares Fitting (LLSF) analyses, some standards that gave fair SPOIL values were also considered to optimize the quality of the fits. Each of these standards was individually used for LLSF fitting of each bulk-XAFS spectrum of the soil samples, to determine which three standards

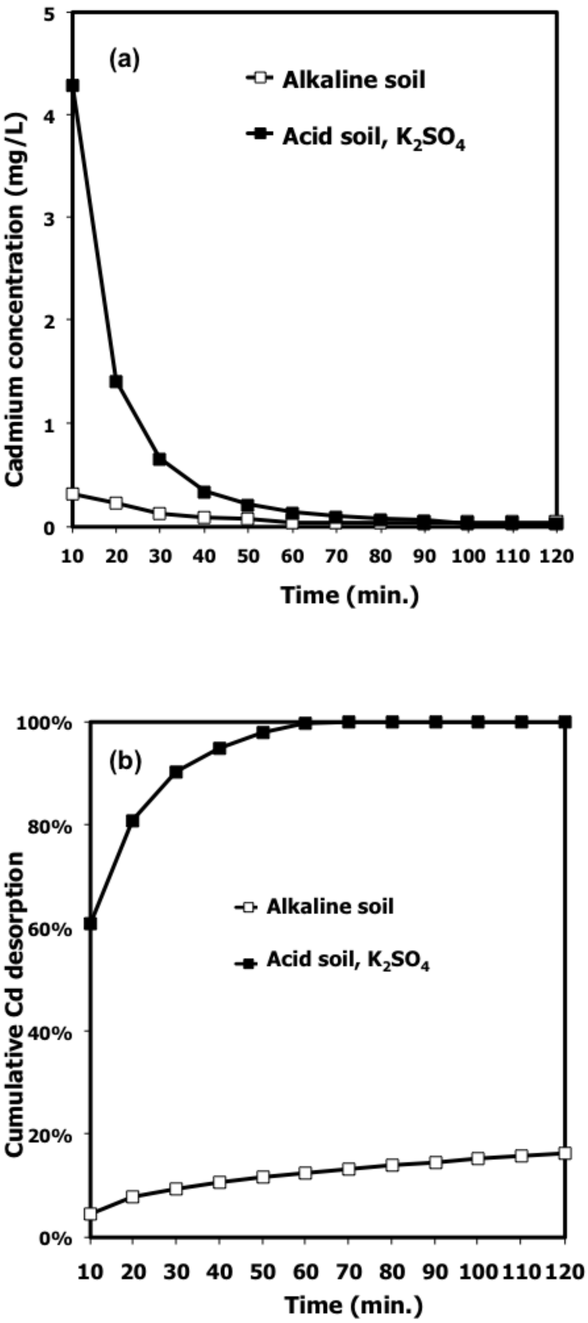

Figure 3.2 **(a)** Cadmium release kinetics and **(b)** cumulative Cd desorption (in %) by DTPA-TEA-Ca, in alkaline air-dried soil, and acidified air-dried soil.

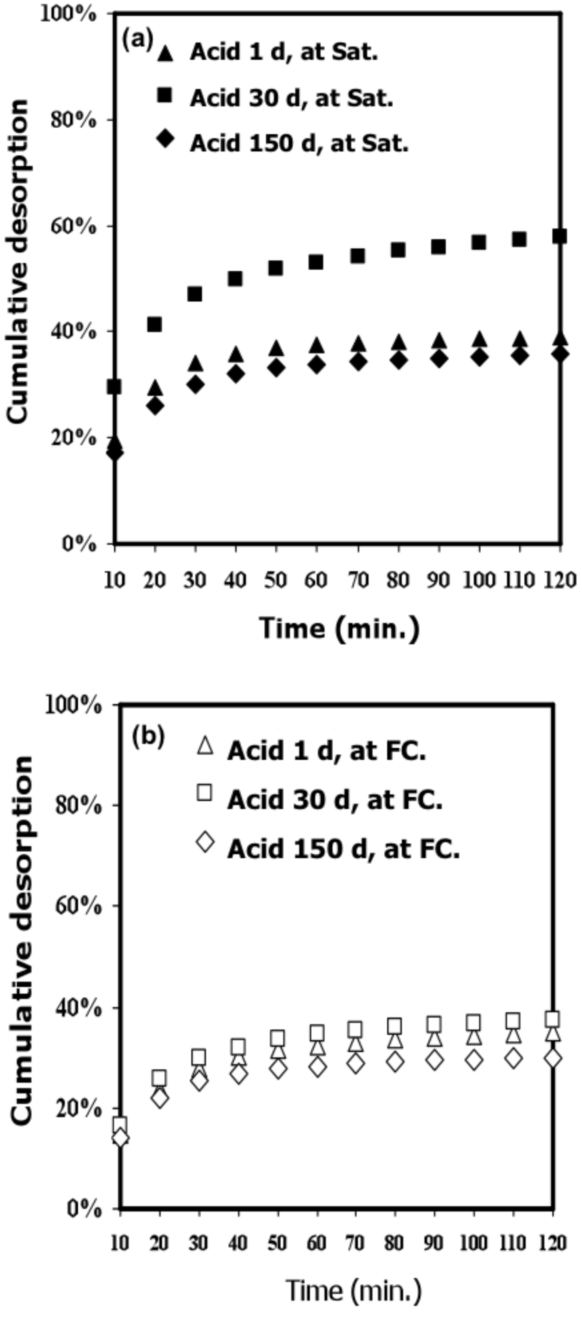

Figure 3.3 Cumulative Cd desorption (%) by DTPA-TEA-Ca, after 1, 30 and 150 days (d=days) of flooding and draining **(a)** to saturation (Sat) and **(b)** to field capacity (FC).

gave the lowest Reduced Chi Square values (R) per each soil sample. Linear least squares fitting was then performed with the set of three standards identified for each bulk-XAFS spectrum of the soil samples. Results from LLSF showed that CdS contributed more to the fit for the air-dried soil spiked with K_2SO_4 than the air-dried soil not spiked with K_2SO_4 (Figure 3.4). However, after the air-dried soil spiked with K_2SO_4 was flooded for different periods, the CdS standard was no longer dominant. Cadmium sorbed to kaolinite became the most dominant species at most flooding periods, except for the soil flooded for 30 days and drained to saturation (Figure 3.4). Cadmium carbonates were also found in the air-dried soils spiked with K_2SO_4 and flooded for different periods. These species were already present in the soil before addition of K_2SO_4 and acidification to pH 6. The presence of $CdCO_3$ in the flooded acidic soil spiked with K_2SO_4 may be due to Cd-$CaCO_3$ dissolution at acidic conditions, which releases Ca^{2+} into the soil solution. However, the presence of $CdCO_3$ in an oxidized environment and at acidic pH values could be also due to the release of Cd^{2+} from soil components, which could have subsequently precipitated with carbonate ions. The total concentration of Cd in the soil solution during the incubation experiments was the highest after 30 days of flooding, and the lowest after 150 days of flooding (Table 3.2). These results support those obtained with LLSF, which showed that $CdCO_3$ precipitate was one of the major Cd species after 150 days of flooding. This Cd phase forms slowly, and at high Cd concentrations (McBride, 1980). The standard representing Cd bound to humic acid also contributed to the fits for the air-dried acidic soil spiked with K_2SO_4, and flooded for 1 and 30 days. Humic acids are major components of organic matter (Schinitzer, 1986). They can enhance or inhibit metal mobility, notably via the formation of metal ternary complexes on mineral surfaces (Floroiu *et al.*, 2001). Therefore, Cd could directly bind to organic matter, which represents 4.3% of the soil components in the paddy soils used in this study, or be included in ternary complexes bound to mineral surfaces, along with humic acids (Mejuto, 2002).

The high extraction of Cd during the stirred-flow experiment measured in the soil flooded for 30 days could be due to the high content of Cd-humic acid species present in the soil sample. Cadmium is weakly bound to humic acid at low pH (Candelaria and Chang, 1997), and thus can be more easily desorbed into solution in an acidic soil. Additionally, divalent ions, especially Ca^{2+} that are found at high concentrations in the solution of the soil samples, can compete with Cd^{2+} for the same binding sites on humic acids (Pellet *et al.*, 2009). Normally, soil pH would rise rapidly during soil flooding as reduction proceeded.

Cadmium sorbed to kaolinite is one of the most abundant species found when the soil was flooded, and its content in the soil increased with increasing flooding periods. Kaolinite is the most dominant clay mineral in the Thai paddy soil used in this study, and can sorb metals, including Cd (Thomas and Wendt, 2003; Bhattacharyya and Gupta, 2007). Farrah and Pickering (1978) found that kaolinite has a higher affinity for Cd than illite and montmorillonite. Grafe *et al.* (2007), who studied the Cd sorption mechanism on kaolinite, showed that more than 75% of Cd is bound via outer-sphere complexation to the clay, at pH 6. At higher pH, Cd can form inner-sphere complexes on the edge sites of kaolinite (Vasconcelos *et al*., 2008). The high amount of Cd desorbed from the acidic soil suggests that the species containing Cd did not transform over time into more stable secondary phases, e.g. phyllosilicates, as has been reported for Zn (Nachtegaal and Sparks, 2004). Cadmium has a large

Table 3.3 Results of the principle component analysis of bulk-EXAFS soil sample spectra.

Components	Eigen value	Variance	Cumulative Variance	IND*
1	54.15	0.564	0.564	0.218
2	13.73	0.143	0.707	0.244
3	8.88	0.092	0.799	0.3159
4	6.87	0.071	0.871	0.476
5	5.78	0.060	0.931	0.821
6	3.53	0.036	0.968	3.00
7	3.00	0.031	1	NA

* Empirical Indicator function

Table 3.4 Target Transformation SPOIL values of selected reference spectra by PCA analyses. SPOIL values <1.5 indicate an excellent fit, 1.5–3 good, 3–4.5 fair, 4.5–6 poor, and>6 are unacceptable (Malinowski,1978).

References	SPOIL* Values
CdS	2.23
$CdSO_4$	4.87
$CdCO_3$	2.82
$Cd(OH)_2$	6.23
$Cd(NO_3)_2$	2.61
Cd-$CaCO_3$	1.58
Cd-humic acid 1	1.84
Cd-humic acid 2	2.92
Cd-ferrihydrite	2.68
Cd-goethite	2.86
Cd-kaolinite	3.06
Cd-montmorillonite	3.71
Cd-illite	2.91

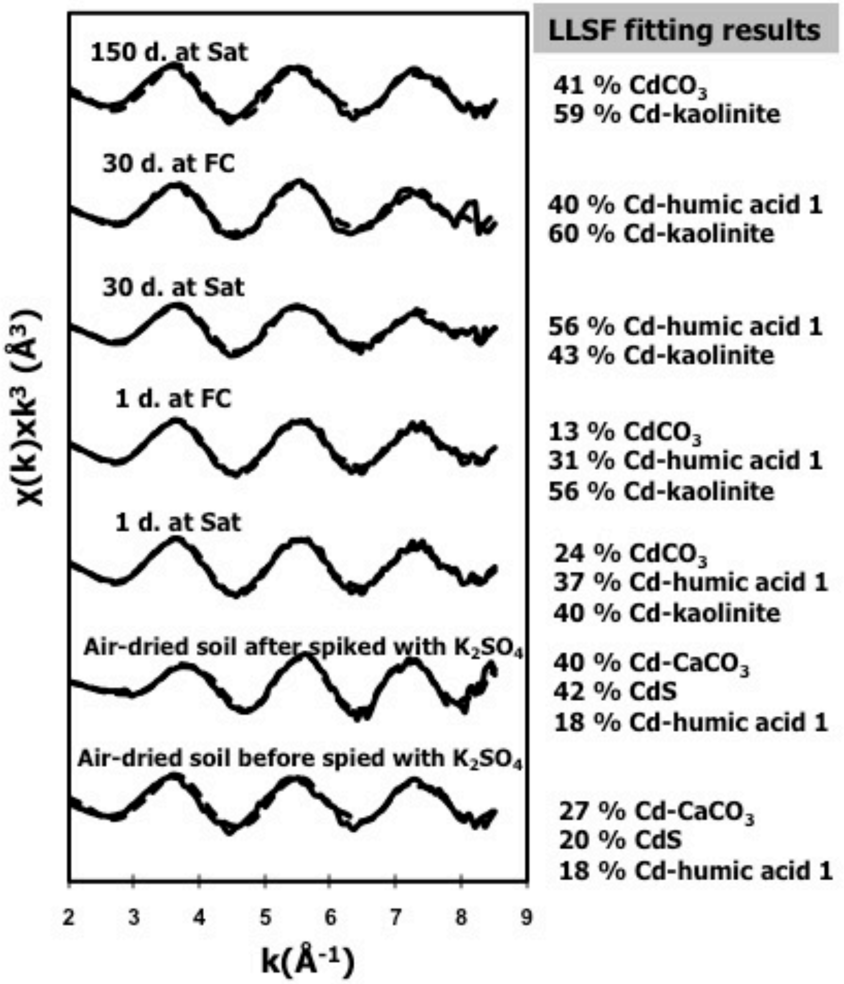

Figure 3.4 Linear least squares fitting results for bulk-XAFS spectra of the acidified soil, air-dried or flooded for different periods (d=days), and drained to saturation (Sat) or field capacity (FC). Solid lines represent the k^3 weighted χ-spectra of soil samples and the dotted line the best fits obtained using linear least squares fitting.

ionic radius, larger than that for Zn^{2+}. Therefore, Cd^{2+} is less likely incorporated into aluminum hydroxide minerals, which subsequently transform in more stable secondary phases (Lothenbach *et al.*, 1997).

3.4.3.2 μ-XRF and μ-XANES Analysis

Micro-XRF maps of Cd, K, Ca, and Fe distribution in the air-dried soil and the soils flooded for different periods are shown in Figure 3.5. The brightest color in these maps represents the highest count or highest concentration of an element, while the darkest color represents the lowest count or lowest concentration of an element. Results showed that the distribution of Cd varied in the air-dried soils and those flooded for different periods. In the air-dried acidic soil not spiked with K_2SO_4, Cd was locally present at high concentrations (hot spots), and did not seem to be associated with other elements represented in the μ-XRF maps. When the soil was spiked with K_2SO_4, Cd was distributed at hot spots, or at regions where Cd was diffuse and Ca, K, and Fe were present at high concentrations (Figure 3.5). Cadmium was most diffuse in the soil flooded for 1 day. Cadmium became more localized in the soil flooded for 150 days (Figure 3.5). Micro-XANES spectra taken at the Cd K-edge were collected at two Cd hot spots. These locations were chosen from three different μ-XRF maps, respectively for the air-dried acidic soil not spiked with K_2SO_4 (spot 1), and the soil flooded for 150 days and drained to saturation (spot 2) (Figure 3.6). The shape of these two μ-XANES spectra were compared with those of $CdCO_3$, Cd-$CaCO_3$, CdS, Cd-humic acid, and Cd-kaolinite XANES spectra, i.e. the five Cd phases that were identified by LLSF analyses (Figure 3.4). The shape of the μ-XANES spectrum taken at the first location (spot 1) is similar to that of the Cd-$CaCO_3$ and the CdS XANES spectra, while the shape of the μ-XANES spectra taken at the second location (spot 2)

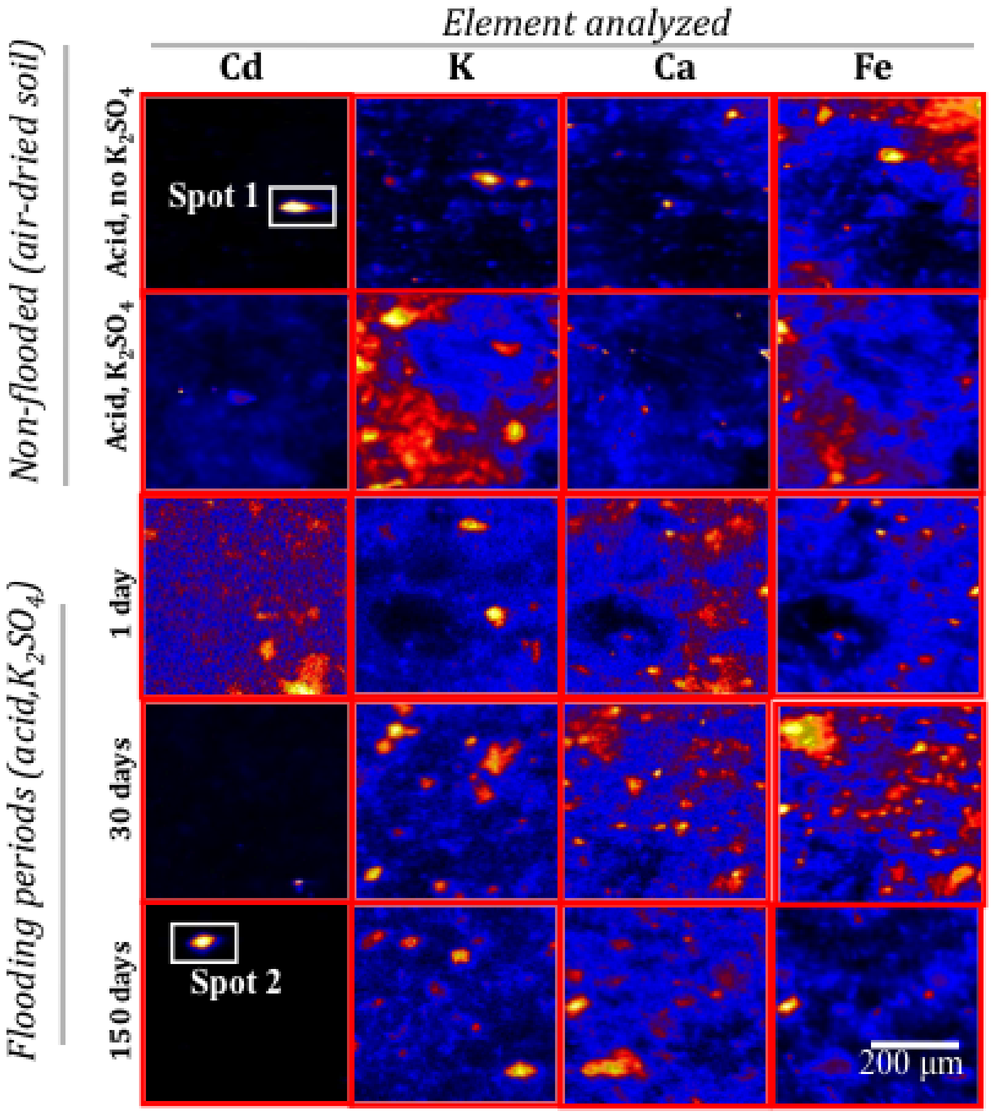

Figure 3.5 Elemental distributions of cadmium (Cd), potassium (K), calcium (Ca), and iron (Fe) in acid air-dried soil before and after adding K_2SO_4, and acidified soil spiked with K_2SO_4 after 1, 30, and 150 days of flooding and draining to saturation, using µ-XRF mapping.

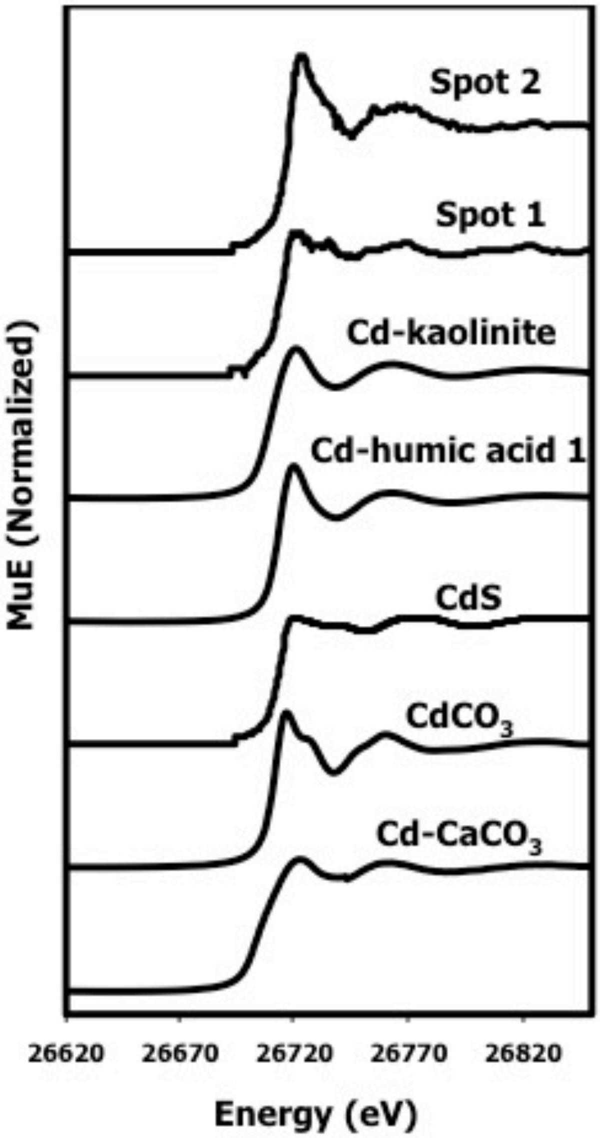

Figure 3.6 Micro-XANES spectra, at the Cd-K-edge, of Cd-$CdCO_3$, $CdCO_3$, CdS, Cd-humic acid 1, and Cd-kaolinite standards, and μ -XANES spectra taken at spots 1 and 2 (Figure 3.5).

is similar to the $CdCO_3$ XANES spectrum. These results support those found with LLSF analyses, which showed that Cd can be distributed in several phases in the contaminated paddy soils taken from Mae Sot. The results in this study differ from those reported in Akkajit and Tongcumpou (2010) and Buanuam et al. (2005). These two studies showed that Cd was mainly sorbed to Fe-oxide minerals in the contaminated soils of Mae Sot, based on elemental correlation plots obtained from sequential extraction. No direct microscopic evidence, probing Cd in situ in the soil, was reported in Akkajit and Tongcumpou (2010) and Buanuam *et al*. (2005). Sequential extraction methods were employed in these two studies, which could have resulted in re-adsorption and redistribution of Cd in the soil (Ostergren *et al.*, 1999; Ahnstrom and Parker, 1999).

3.5 Conclusions

Results from XAFS analyses, reported in a previous study (Chapter 2), showed that Cd was mostly distributed with carbonate species in the alkaline soil, before and after the soil was flooded. However, in this study, Cd was mostly sorbed to kaolinite when the soil was acidified and spiked with K_2SO_4, especially when the soil was flooded for a long period of time. Treating the soil with HNO_3 and K_2SO_4 can maintain aerobic condition during flooding, which can prevent a formation of CdS during flooding, and allow Cd to sorb with other soil components. Results from stirred-flow experiments showed that much more Cd was desorbed from the soil acidified and spiked with K_2SO_4 compared to the untreated soil. Therefore, Cd sorbed to kaolinite can be desorbed more easily than Cd included in carbonate minerals.

Treating the soil with HNO_3 and K_2SO_4 can thus change the nature of the mineral phases in which Cd is distributed in the paddy soil of Mae Sot, and promote Cd desorption from soil components. After treating the soil with HNO_3 and K_2SO_4, Cd could be removed from the soil solution by employing desorption or with leaching chemical agents. The fraction of Cd that could not be desorbed from soil components could be immobilized by liming. Further research is needed to optimize the procedures that could be employed to remediate Cd from the contaminated paddy soils of Mae Sot.

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Chapter 4

SPECIATION AND RELEASE KINETICS OF ZINC IN ALKALINE AND ACIDIFIED PADDY SOILS UNDER VARIOUS FLOODING PERIODS AND DRAINING CONDITIONS

4.1 Abstract

Zinc and cadmium can contaminate agricultural soils of industrialized countries, due to Zn-mining and Zn-smelter activities. Although Zn is an important nutrient, it can be toxic to plants at high concentrations. The solubility and speciation of Zn is controlled by many factors, especially soil pH and Eh. These two soil parameters can vary during the flooding and draining cycles employed in lowland rice culture. This study investigates Zn speciation and release kinetics in an acidified or untreated alkaline paddy soil, under different flooding periods and draining conditions, by bulk and micro x-ray absorption spectroscopy (XAS). The soil treatment consisted in acidification of the soil to pH 6, and addition of K_2SO_4. Zinc release kinetics from the soil samples were also studied, using a stirred-flow approach. Results from XAS analyses and stirred-flow experiments showed almost no change in Zn speciation and release kinetics in the soils, although the treated and untreated soils were subjected to different flooding periods, and drained to field capacity or saturation. After 2 hours of stirred-flow experiment, the cumulative Zn desorption was less than 25% in all soil samples. The mineral phases in which Zn is immobilized in the soil samples were constrained by Linear Least Squares Fitting analyses (LLSF). Only two main phases were identified by LLSF, i.e. Zn-layered double hydroxides (Zn/Mg-hydrotalcite-like,

and ZnAl-LDH) and Zn-phyllosilicates (Zn-kerolite). The information obtained in this study will be useful in finding the best strategy to control Cd and Zn phytobioavailability in contaminated paddy soils in Southeast Asia.

4.2 Introduction

Zinc-mining and smelter activities can result in Zn and Cd contamination of agricultural soils, including paddy soils in Southeast Asia (Manceau *et al.*, 2000; Scheinost *et al.*, 2002; Simmons *et al.*, 2002; Lee, 2006; Chaney, 2010). The degrees of Zn toxicity, bioavailability, and mobility in soils are directly controlled by Zn speciation, which depends on soil pH (Roberts *et al.*, 2002; Jacquat *et al.*, 2009). Variations in soil pH and Eh commonly occur during the flooding and draining cycles employed in the lowland rice culture. These variations could modify Zn distribution in the paddy soil, and the bioavailability of Zn to rice. Under acidic and oxic conditions, Zn is one of the most soluble and mobile trace metal cations (McBride, 1994). Gao (2007) reported that the bioavailability of Zn diminished under aerobic conditions, while Mandal *et al.* (2000) showed that Zn uptake and accumulation in rice were high under anaerobic conditions, i.e. when the soil is flooded.

New analytical tools that use synchrotron radiation allow scientists to investigate in situ metal speciation in soils, minerals, and sediments. Roberts *et al.* (2002) reported that Zn speciation in smelter-contaminated soils was primarily associated with Fe in discrete phases, using μ-XAFS and μ-XRF spectroscopy. Manceau *et al.* (2000) used x-ray diffraction (XRD), x-ray texture goniometry, powder and polarized extended x-ray absorption fine structure (EXAFS) spectroscopy, as well as micro-x-ray fluorescence (μ-XRF) and micro extended x-ray absorption fine structure (μ-EXAFS) spectroscopy to study Zn speciation in soils. This study found that franklinite ($ZnFe_2O_4$), willemite (Zn_2SiO_4), hemimorphite ($Zn_4Si_2O_7(OH)_2{\cdot}H_2O$) and Zn-bearing magnetite ($[Fe,Zn]Fe_2O_4$) were the dominant Zn species present in the soils. Other species, e.g. Zn-LDH, Zn-kerolite, and sphalerite, in contaminated soil have been also reported (Manceau *et al.*, 2000, Voegelin *et al.*, 2002; Scheinost *et al.*, 2003; Nachategaal and Sparks, 2004). However, only one study has addressed the speciation of Zn in paddy soils (Manceau *et al.*, 2005).

In the Mae Sot district, located in the northern part of Thailand, around 2,200 hectares of paddy soils have been heavily contaminated by Cd and Zn, due to Zn mining activities (Simmons *et al.*, 2005; Swaddiwudhipong *et al.*, 2007). The total concentrations of Zn at these contaminated sites can reach 3,000 ppm, which is twenty times higher than the total Cd concentrations at the same locations. However, most rice plants cultivated at these contaminated sites grow without displaying physical damages that could be signs of Zn toxicity. Although the concentrations of this element distributed in soil components are high, Zn concentration in soil solution is not high enough to be toxic to rice plants, which could be due to the low solubility of Zn in the alkaline paddy soils of Mae Sot. Therefore, our research aims to characterize Zn desorption from soil components of the contaminated paddy soils in the Mae Sot district, and identify the phases in which Zn is distributed in the soil. Zinc speciation and release kinetics are investigated, before and after the alkaline soil was acidified to pH 6, spiked with K_2SO_4, and flooded for different periods, using synchrotron based techniques, and a stirred-flow kinetic approach. The information provided in this study will be useful in finding the best strategy to control Zn mobility and solubility in Cd & Zn co-contaminated paddy soil in the Mae Sot district.

4.3 Materials and methods

4.3.1 Study site and soil characterization

Surface samples (0-15 cm) were collected from a Zn-Cd contaminated paddy soil, at a site located near a Zn mining area, in the Mae Sot district, Tak Province, Thailand. This site was selected based on Cd concentrations measured by the International Water Management Institute in contaminated paddy soils at different locations in Mae Sot (Simmons *et al.*, 2005). The soil was treated by acidification, and addition of K_2SO_4, to increase the amount of sulfur in the soil. The untreated soil is referred in this study as the alkaline soil. To acidify the soil sample, 500 grams of the alkaline paddy soil were mixed with 250 ml of DDI-water, and titrated to pH 6 with HNO_3. The pH of the soil suspension was manually kept at pH 6 for 5 weeks. The soil was air-dried for 3 weeks under a hood, without draining the water out from the soil. The air-dried soil was spiked with approximately 250 ml of 0.5 M K_2SO_4. The pH of this soil suspension was maintained at pH 6, under stirring, for 2 weeks, and was finally air-dried again. Basic soil physicochemical properties, such as soil texture, soil pH, elemental concentrations, and organic matter content, were determined by standard procedures (Bouyoucos, 1962; Schofield and Taylor, 1955; U.S. EPA, 1986; Nelson and Sommers, 1982), and results are reported in Chapter 1 &2 (Table 2.1 and 3.1.).

4.3.2 Incubation experiments: Flooding and draining conditions

The flooding periods and draining conditions were similar to those studied in chapter 2. The two periods of flooding were 1 and 30 days. The two draining conditions were saturation (Sat) or field capacity (FC). Experimental procedures were similar to those described in 2.3.2.

4.3.3 Desorption experiments

Zinc release kinetic experiments were conducted with the alkaline and treated soils, which were air-dried or flooded for different time periods, using a stirred-flow method. The experimental procedures were similar to those described in 2.3.3.

4.3.4 Zinc speciation in soil samples and Cd standards

4.3.4.1 Zn standard preparations

Zinc standards considered for XAS analyses included Zn-$CuCO_3$, Zn-hydrotalcite, Zn/Mg hydrotalcite-like phase, Zn-CuS, Zn-$CuCO_3$, Zn-hydrous manganese oxide (Zn-HMO), Zn-humic acids, Zn-fulvic acid, Zn-kaolinite, Zn-illite, Zn-vermiculite, Zn-montmorillonite, Zn-kerolite, ZnAl -layered double hydroxide (ZnAl-LDH), Zn-gibbsite, Zn-ferrihydrite, Zn-goethite; willemite (Zn_2SiO_4), hemimorphite ($Zn_4Si_2O_7(OH)_2.H_2O$), sphalerite (ZnS from XLS Ruby sphalerite, Joplin, MO), smithsonite ($ZnCO_3$), hydrozincite ($Zn_5(CO_3)_2(OH)_6$), ZnS, $ZnCO_3$, ZnO, $Zn(SO_4)$, ZnFeO, and a solution of 1 mM Zn^{2+}.

The Zn-$CuCO_3$ and Zn-CuS were prepared by adding $ZnCO_3$ into $CuCO_3$ or ZnS into CuS suspension at pH 6.5, respectively, using a Zn:Cu molar ratio of 25:1. The reaction was equilibrated for 72 hours at room temperature. After equilibration, excess water was removed from the reaction vessel, and the wet paste was air-dried at room temperature for a week. The sample was mixed with ~85% boron nitride, and mounted for XAFS analyses.

The two standards representing Zn sorbed to organic matter in soils, i.e. Zn-humic acid 1 and Zn-humic acid 2, were respectively prepared from humic acid No. H1, 675-2, Sigma-Aldrich, and Pahokee peat humic acid, International Humic Substance Society (IHSS). Montmorillonite No 20, Husband Mine (Bentonite), Mississippi, kaolinite No 7, Dixie Rubber Pit Bath, South Carolina, and illite, Sanford, North Carolina, were used to prepare standards of Zn sorbed to clay minerals. To prepare these standards, sorption experiments were conducted for 48 hours with 1 mM Zn, 10 g/L of clay suspension or 5 g/L of humic acids, at pH 6.5, and using an ionic strength of 0.01 M $NaNO_3$, under a N_2 environment. After each sorption experiment, the suspension was centrifuged and washed two times with 1 mM $NaNO_3$, at pH 6.5, and a third time with DDI water. After removing the supernatant during the last centrifugation, wet-pastes of samples were mounted on sample holders, and subsequently analyzed by bulk-XAFS.

Zn hydrotalcite was synthesized using the method of Trainor *et al.* (2000), with a Zn/Al ratio of 3:1, under ambient environment. For Zn/Mg-hydrotalcite-like, the preparation was conducted in two steps. Firstly, Mg-hydrotalcite, with a Mg:Al ratio of 2.7:1, was synthesized by a method similar to the one used to prepare Zn-hydrotalcite. Secondly, Mg^{2+} was substituted by Zn^{2+} in Mg-hydrotalcite, by conducting a sorption experiment, with a Zn:Cu molar ratio of 1:16. To prevent local precipitation, dissolved $Zn(NO_3)_2$ was slowly dropped into the Mg-hydrotalcite suspension, and the pH was controlled at 6.5 under ambient environment. After 48 hours, the sample was filtered, air-dried at room temperature, and mounted for XAFS analyses.

Other Zn standards included ZnS, $ZnCO_3$, ZnO, $Zn(SO_4)$, ZnFeO (ACS reagent grade). These standards were mixed with 95% boron nitride, and then mounted for XAFS analyses. Willemite, hemimorphite, Zn-kerolite, Zn-LDH, Zn-gibbsite, Zn-ferrihydrite, Zn-goethite and Zn fulvic acid spectra were synthesized in three previous studies carried out in our laboratory (Roberts *et al.*, 2002; Scheinost *et al.*, 2002; Nachategaal and Sparks, 2004)

4.3.4.2 Bulk- x-ray Absorption Fine Structure Spectroscopy (bulk-XAFS)

4.3.4.2.1 Standards

Bulk-XAFS was used to characterize the reference samples. Spectra were collected at the Zn K-edge (9659 eV), at beamline X-11A at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, Upton, NY. This beamline features a monochromator with two Si (111) crystals, with an entrance slit of 0.5 mm. The beam energy was calibrated to the first inflection point of the first derivative of the Zn absorption edge (9659 eV) of a Zn(0) metal foil XANES spectrum. Bulk-XAFS spectra were collected at room temperature, from 150 eV below the absorption edge energy, to k values of 12.5 $Å^{-1}$. At least three scans were collected per sample, to improve the signal to noise ratio. Both fluorescence and transmission modes were used to collect spectra, depending on the concentration of the Zn standards. A 6 μm-Cu filter was placed between the sample and the detector to reduce elastic scattering.

4.3.4.2.2 Soil samples

Air-dried and wet soil samples prepared from the incubation experiments were analyzed by bulk-XAFS in fluorescence mode, at room temperature, at beamline X-11A (NSLS). Each sample was analyzed by collecting 3-5 scans, to obtain an adequate signal to noise ratio. Each raw bulk-XAFS spectrum was normalized to obtain the EXAFS function, using Athena version 8.0.054 and the SixPack software package (Ravel and Newville, 2005; Webb, 2005). The mineral phases in which Zn is present in the soils were identified and quantified with Sixpack, using Principal

Component Analysis (PCA), Target Transformation (TT), and Linear Least Squares Fitting (LLSF) procedures. These tools have been used to identify metal speciation in heterogeneous samples (Wasserman *et al.*, 1999; McNear, 2006; Seiter *et al.*, 2008; Jacquat *et al.*, 2008). The energy calibrations for LLSF analyses of EXAFS functions of soil and reference samples were set at the same value (9659eV). The PCA and LLSF were performed using the EXAFS function, which was k^3 weighted, with k values from 2 to 10 $Å^{-1}$. SPOIL values obtained from Target transformation (TT) analysis were used to select principal components for LLSF analysis.

4.3.4.3 Micro X-Ray Fluorescence (μ-XRF) maps.

Micro x-ray fluorescence analyses were conducted at beamline 13-ID-C (APS), to determine elemental distributions in the contaminated paddy soils. The μ - XRF maps were 200 μm^2 in size, with a 2 μm step size, and a 1 second dwell time. The beam energy was set to 29000 eV for μ-XRF analyses of Cd, Ca, Cu, Fe, K, Zn, and Mn distributions in the soil samples.

4.4 Results and discussion

4.4.1 Zinc release kinetics

The amounts of Zn desorbed from the treated (acidified to pH6 and spiked with K_2SO_4) and untreated alkaline air-dried soils were small. After 2 hours of desorption experiment using the DTPA-TEA-Ca solution, the highest amount of Zn

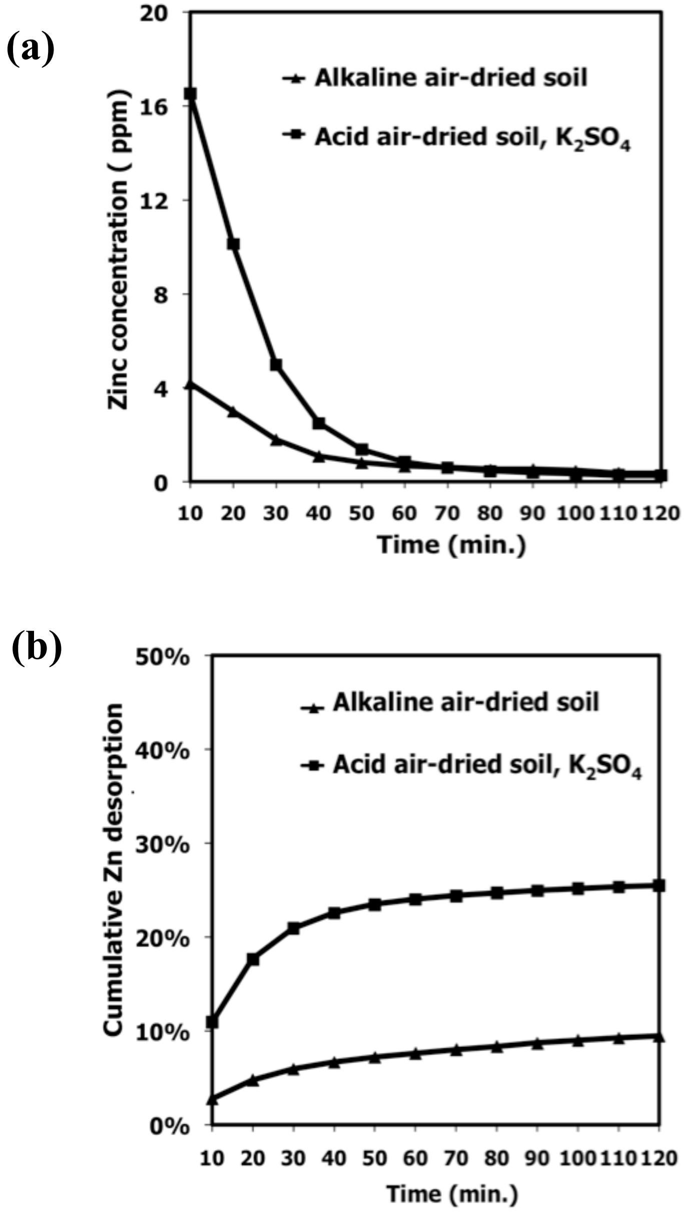


Figure 4.1 **(a)** Zinc release kinetics and **(b)** cumulative Zn desorption (in %) by DTPA-TEA-Ca from the alkaline air-dried soil and acidified air-dried soil.

was released from the acidic soil spiked with K_2SO_4, i.e. ~25% of cumulative Zn desorption (Figure 4.1).

Similarly, less than 25% of cumulative Zn desorption was measured from stirred-flow experiments performed with soils flooded for different periods (Figure 4.2). These results suggest that Zn is effectively immobilized in the alkaline paddy soil at Mae Sot. Only a small amount of Zn was released, although the soil was acidified and spiked with K_2SO_4. Small release of Zn from the paddy soil could be due to the transformation of the mineral phases in which Zn is distributed in the soil into more stable secondary phases, after a long period of time (Lothenbach *et al.*, 1999; Voegelin *et al.*, 2002; Nachtegaal and Sparks, 2004). Voegelin *et al.*, 2002, and Nachtegaal & Sparks, 2004 reported that ZnAl-LDH phase can form in soils, and greatly reduce Zn mobility. Results from XAFS analyses presented in the next section suggest that, indeed, Zn- LDH phases are present in our soil samples.

4.4.2 Zinc speciation and elemental distribution

4.4.2.1 Bulk-XAFS analysis

The extracted EXAFS spectra of the Zn standards and soil samples are shown in Figures 4.3, 4.4, and 4.5, respectively. Results from Principal Component Analysis (PCA) showed that in the alkaline soil, the first component contributed to 82% of the total experimental variance (Table 4.1). In the acidic soil, the first two components contributed to 84% of the total variance. The SPOIL values of the standard samples obtained from Target Transformation (TT) analysis are shown in

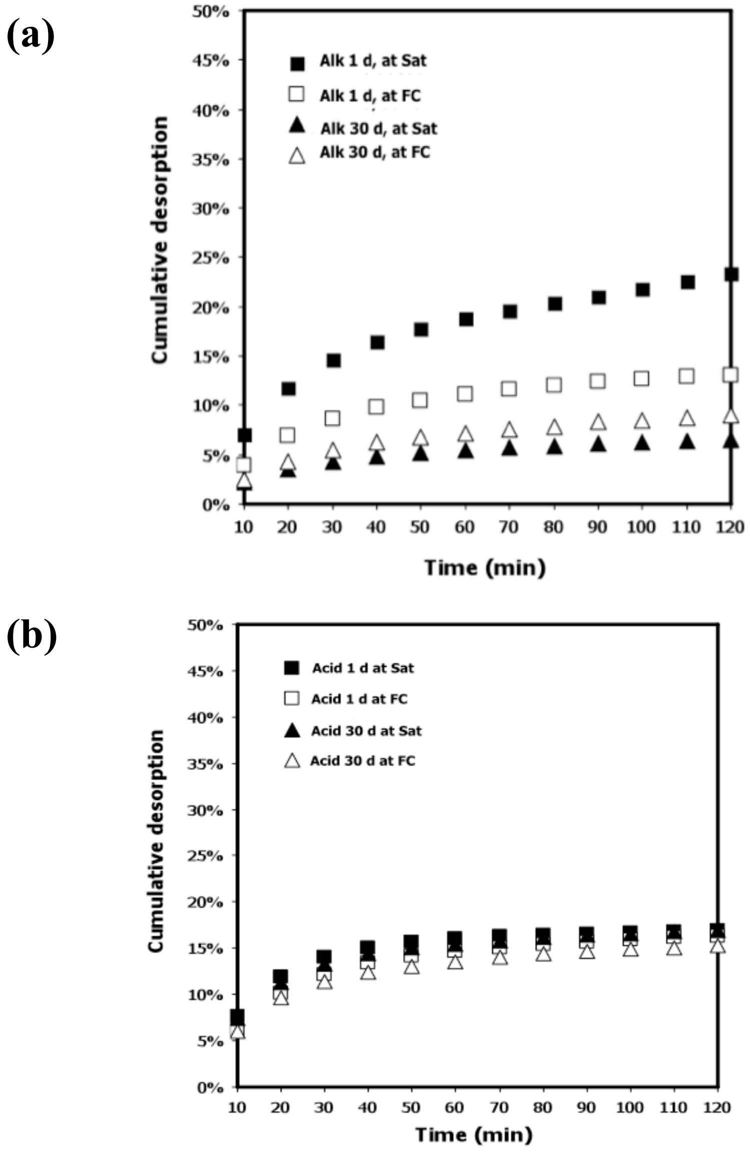


Figure 4.2 Cumulative Zn desorption (in %) by DTPA-TEA-Ca from **(a)** the alkaline soil (Alk) and **(b)** the acidified soil (Acid), after 1 and 30 days (d) of flooding, and drained to saturation (Sat) or field capacity (FC).

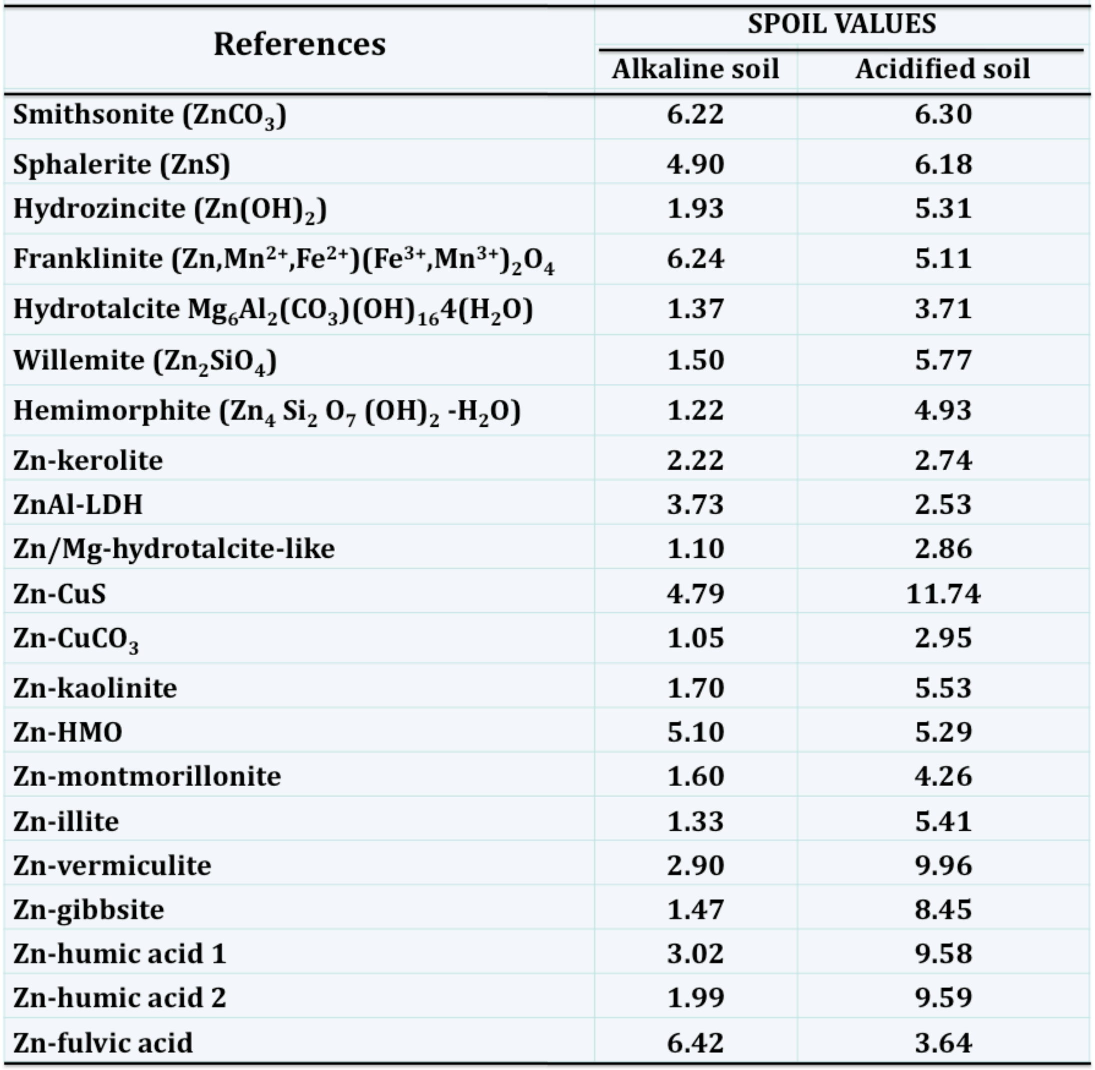
Table 4.1 Results of principle component analysis performed with the bulk-EXAFS spectra of the alkaline (Alk) and acidified (Acid) soil samples.

Component	Eigen value		Variance		Cumulative Variance		IND*	
	Alk	Acid	Alk	Acid	Alk	Acid	Alk	Acid
1	105.87	94.92	0.825	0.689	0.825	0.689	0.197	0.432
2	7.65	21.11	0.059	0.153	0.885	0.842	0.247	0.368
3	5.62	8.95	0.043	0.064	0.929	0.907	0.356	0.492
4	4.51	5.94	0.035	0.043	0.964	0.950	0.570	0.857
5	2.56	3.76	0.019	0.027	0.984	0.977	1.961	3.053
6	1.96	3.05	0.015	0.022	1.0	1.0	NA	NA

Table 4.2. Because Zn standards had similar SPOIL values, they were individually used for LLSF fitting of each bulk-XAFS spectrum of the soil samples, to determine the LLSF fit's Reduced Chi Square values (R) per soil sample. Results revealed that in both alkaline and acidified soils, air-dried or flooded, only two main Zn phases were found, i.e. Zn-layered double hydroxides (Zn-LDH), and Zn-phyllosilicates. The most dominant Zn-LDH phase found in all soil samples was a Zn/Mg-hydrotalcite-like phase, and the most dominant Zn-phyllosilicate was Zn-kerolite. Although the soils were flooded for different periods and drained to field capacity or saturation, the nature of the phases in which Zn is distributed in the soils did not vary (Figures 4.6 and 4.7). Additionally, the same Zn-phases were identified in two soil samples, taken within a two-year interval from the same location in a contaminated soil of Mae Sot.

Shape similarities were observed in the Fourier Transform EXAFS spectra for all soil samples (Figure 4.8). This suggests that the local molecular environments of Zn in all soil samples are similar to each other. The shape similarities of the Fourier

Table 4.2 Target Transformation SPOIL values of selected reference spectra obtained by PCA analysis. SPOIL values <1.5 indicate an excellent fit, 1.5–3 good, 3–4.5 fair, 4.5–6 poor, and>6 are unacceptable (Malinowski ,1978).



References	SPOIL VALUES	
	Alkaline soil	Acidified soil
Smithsonite ($ZnCO_3$)	6.22	6.30
Sphalerite (ZnS)	4.90	6.18
Hydrozincite ($Zn(OH)_2$)	1.93	5.31
Franklinite ($(Zn,Mn^{2+},Fe^{2+})(Fe^{3+},Mn^{3+})_2O_4$	6.24	5.11
Hydrotalcite $Mg_6Al_2(CO_3)(OH)_{16}4(H_2O)$	1.37	3.71
Willemite (Zn_2SiO_4)	1.50	5.77
Hemimorphite ($Zn_4\ Si_2\ O_7\ (OH)_2\ \text{-}H_2O$)	1.22	4.93
Zn-kerolite	2.22	2.74
ZnAl-LDH	3.73	2.53
Zn/Mg-hydrotalcite-like	1.10	2.86
Zn-CuS	4.79	11.74
Zn-$CuCO_3$	1.05	2.95
Zn-kaolinite	1.70	5.53
Zn-HMO	5.10	5.29
Zn-montmorillonite	1.60	4.26
Zn-illite	1.33	5.41
Zn-vermiculite	2.90	9.96
Zn-gibbsite	1.47	8.45
Zn-humic acid 1	3.02	9.58
Zn-humic acid 2	1.99	9.59
Zn-fulvic acid	6.42	3.64

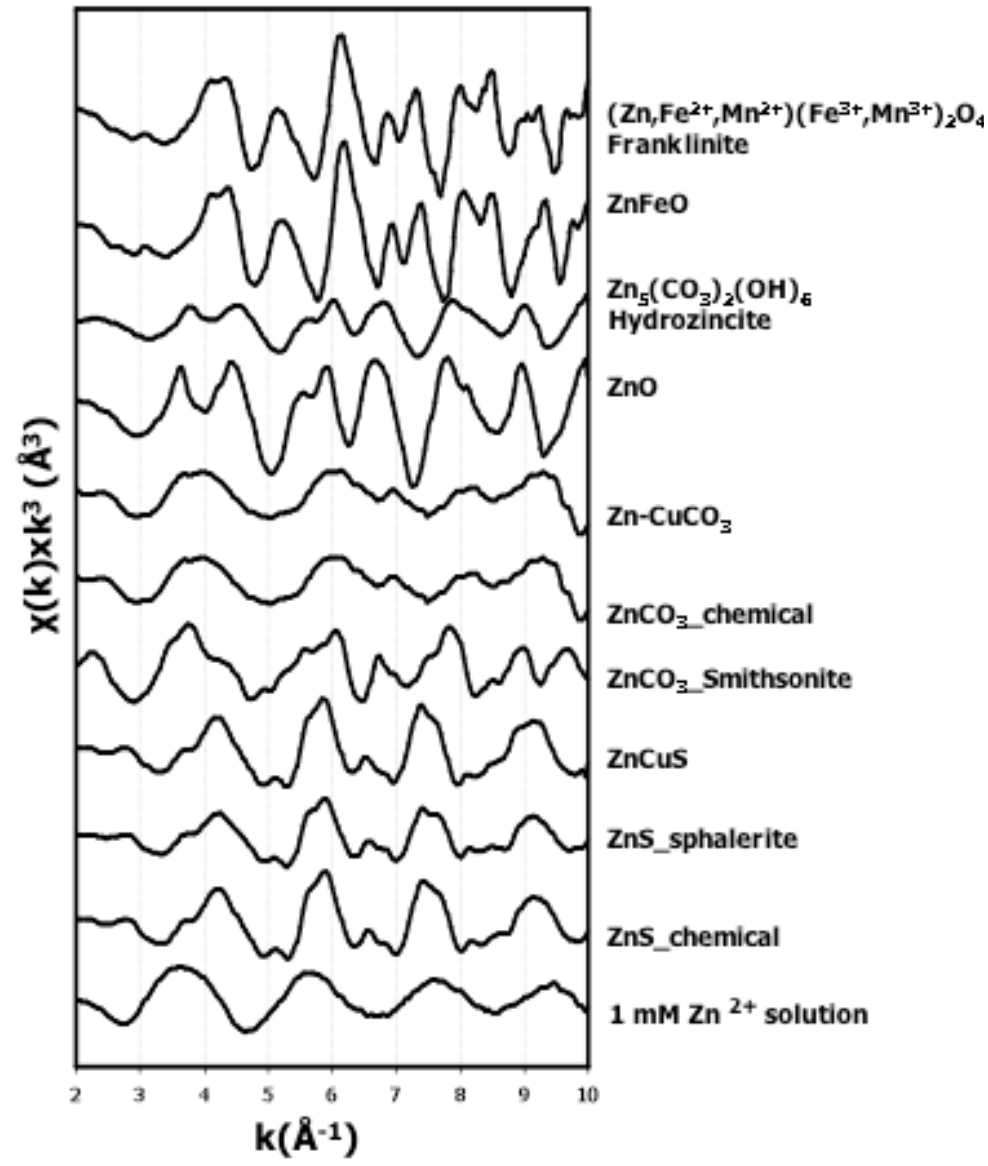


Figure 4.3 Bulk EXAFS spectra of Zn reference samples, k^3 weighted.

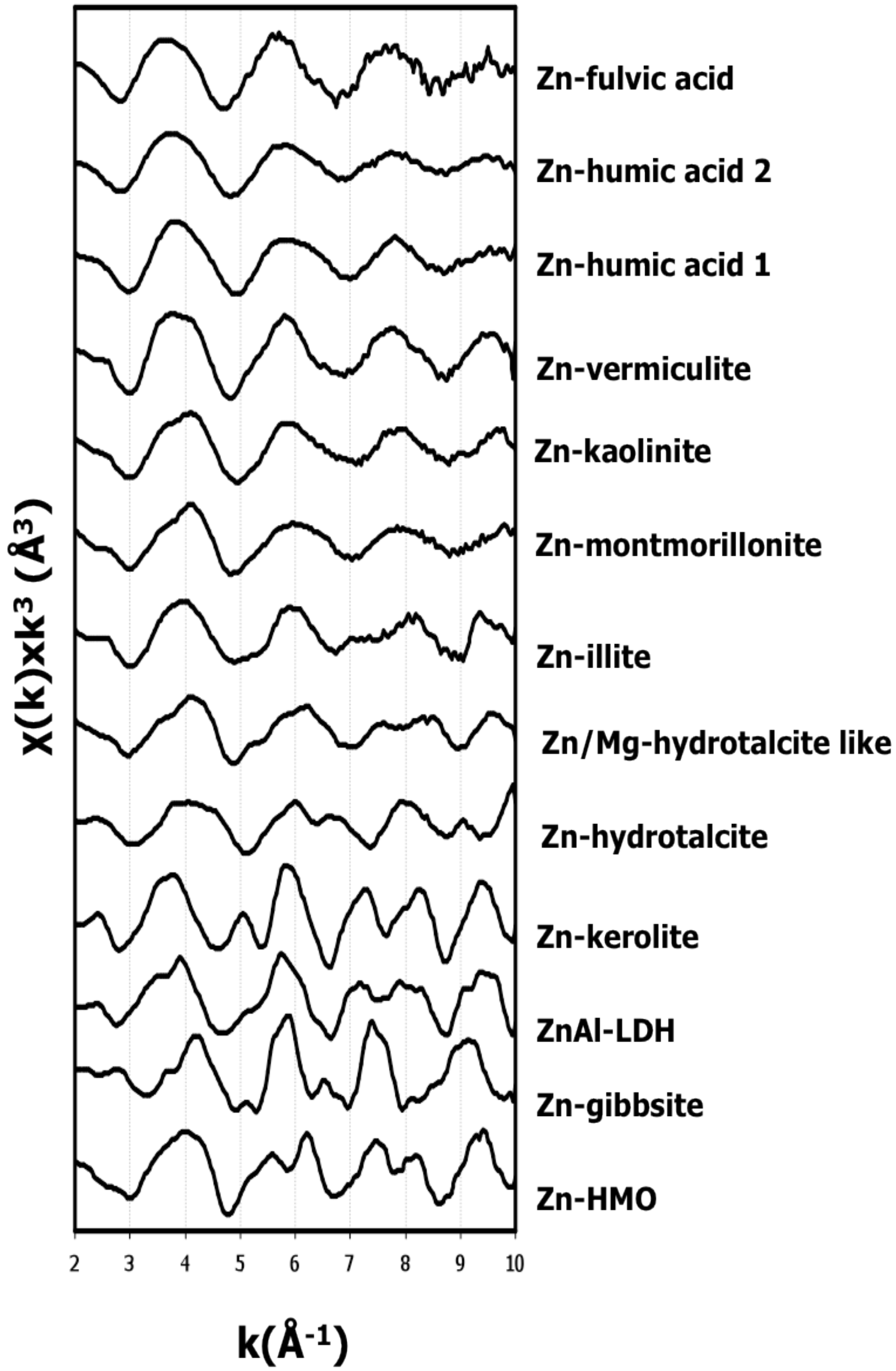


Figure 4.4 Bulk EXAFS spectra of Zn reference samples, k^3 weighted.

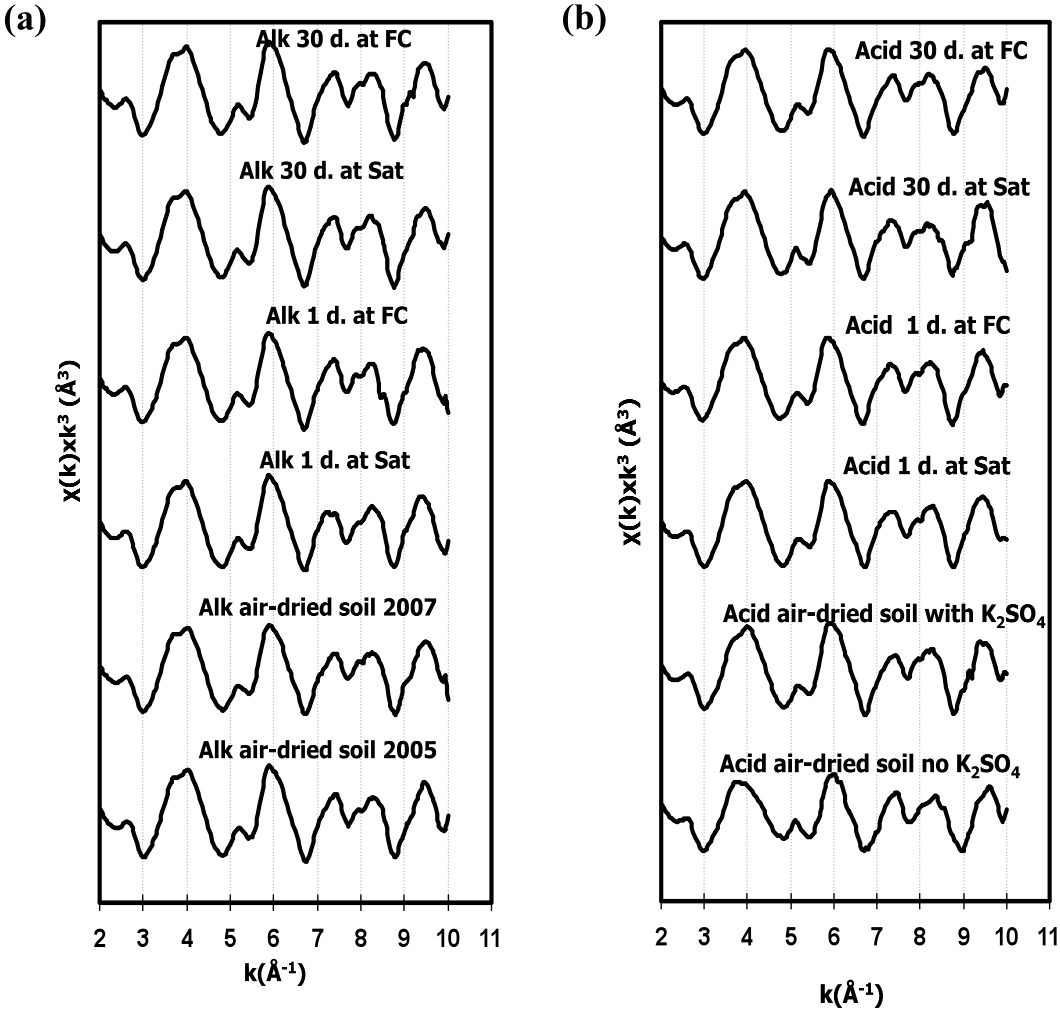


Figure 4.5 k^3 weighted χ-spectra of **(a)** alkaline soil (Alk) and **(b)** acidified soil (Acid), air-dried or flooded for different periods (d=days), and drained to saturation (Sat) or field capacity (FC).

Transform EXAFS spectra for all soil samples also support our results obtained by LLSF analysis, which indicated that the nature of the phases in which Zn is present in

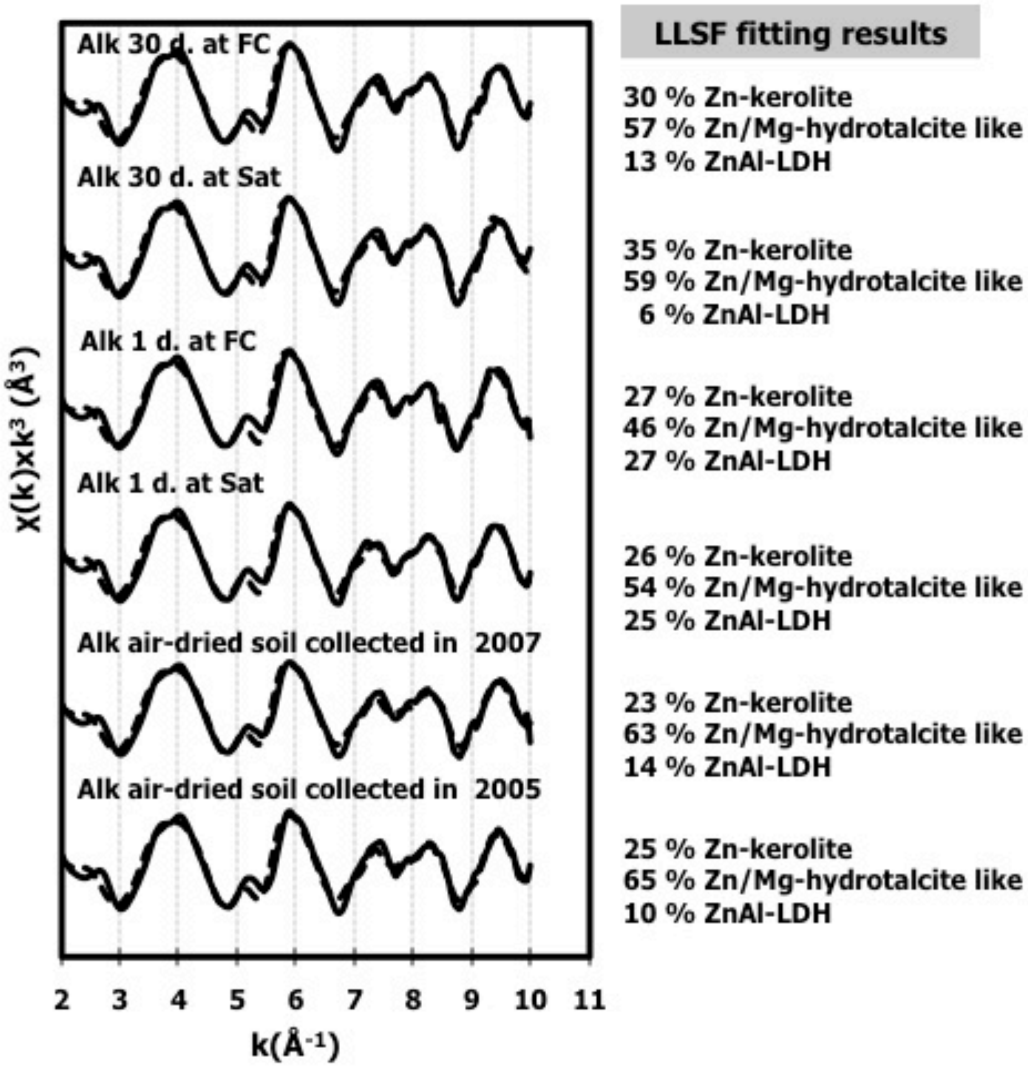


Figure 4.6 Linear least squares fitting results for bulk-XAFS spectra of the alkaline soil samples, air-dried or flooded for different periods (d=days), and drained to saturation (Sat) or field capacity (FC). The solid lines represent the k^3 weighted χ-spectra, and the dotted lines represent the best fits obtained using linear least squares fitting.

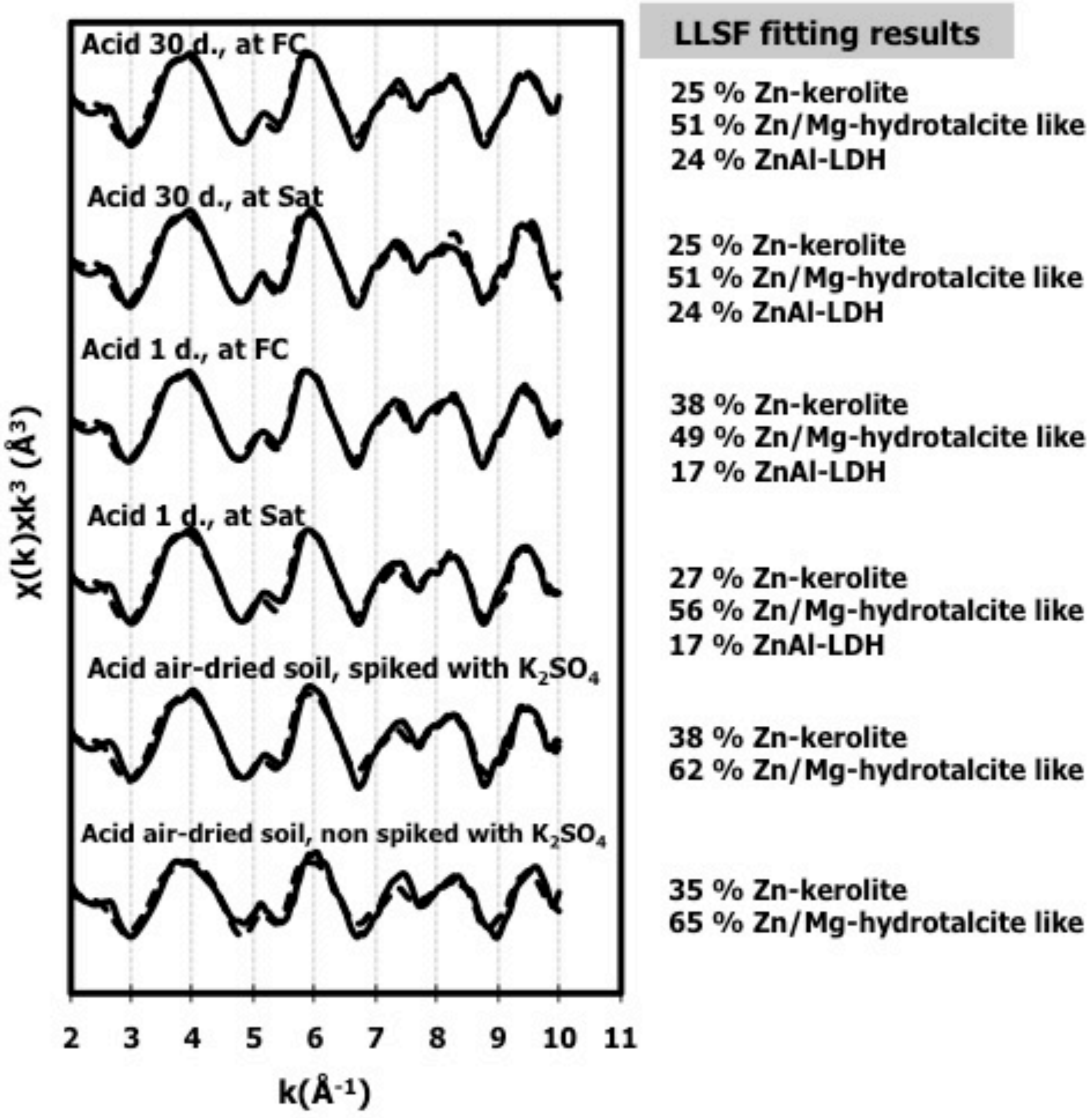


Figure 4.7 Linear least squares fitting results for bulk-XAFS spectra of the acidified soil samples, air-dried or flooded for different periods (d=days), and drained to saturation (Sat) or field capacity (FC). The solid lines represent the k^3 weighted χ-spectra, and the dotted lines represent the best fits obtained using linear least squares fitting.

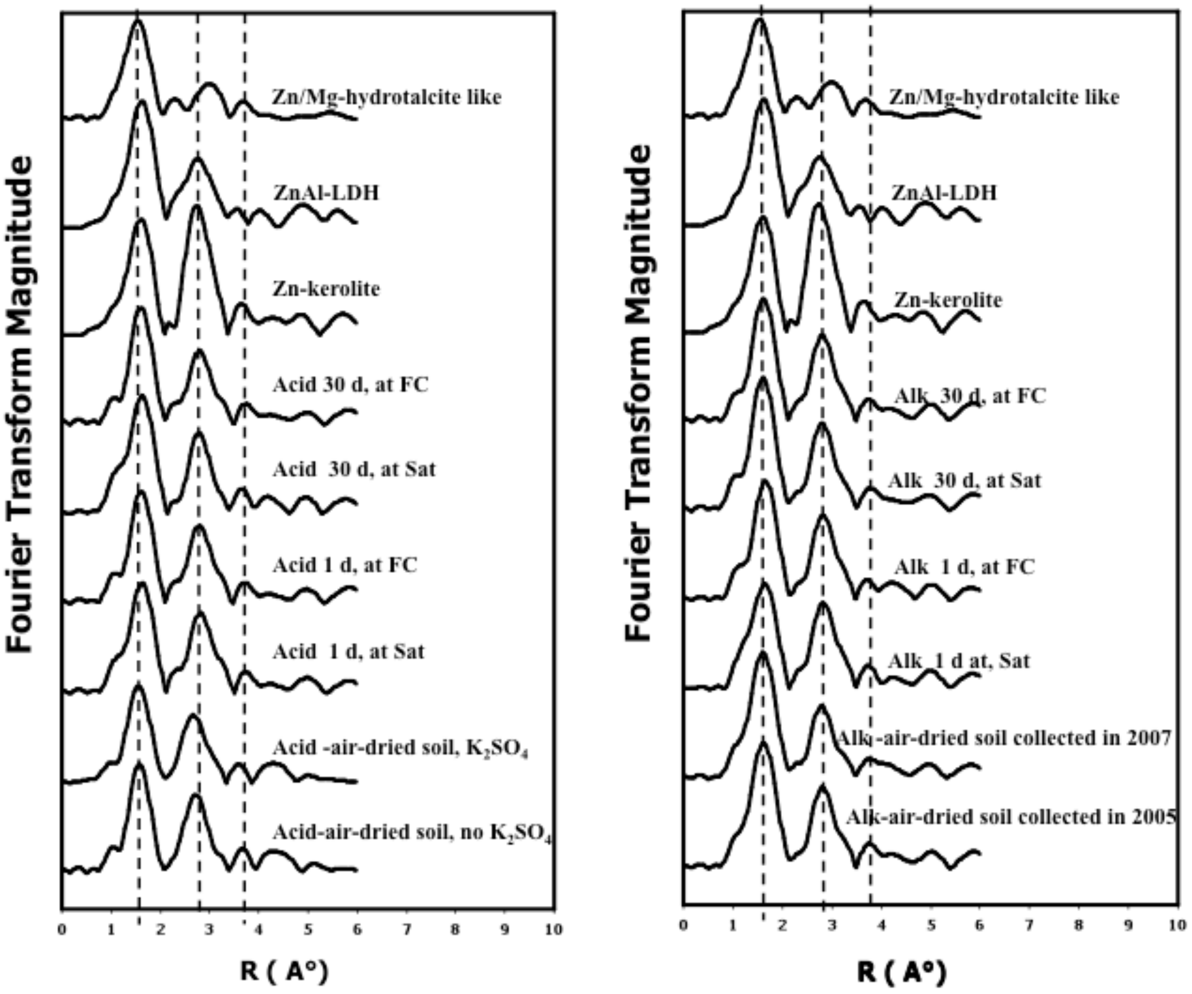


Figure 4.8 Comparison between the Fourier Transform EXAFS spectra of Zn-kerolite, Zn-LDH, Zn/Mg-hydrotalcite-like and the Fourier Transform EXAFS spectra of the alkaline soil (Alk) or the acidified soil (Acid), air-dried or flooded for different periods (d=days), and drained to saturation (Sat) or field capacity (FC).

the soil do not change when the soil was flooded, acidified, and spiked with K_2SO_4. Three main shells are present in each spectrum. The first shell, located at ~2 Å, is similar to the distances of Zn-O in ZnAl- LDH (2.07 Å), Zn-kaolinite (2.02 to 2.07 Å), and Zn-goethite (2.02 to 2.06 Å) (Nachetegaal and Sparks, 2004).

The second shell at ~ 3 Å, is close to the Zn-Al, Zn-Zn, or Zn-Fe distances found in Zn-kaolinite and ZnAl-LDH (3.06 to 3.19 Å) (Scheinost *et al.*, 2002;Voegelin *et al.*, 2002; Nachetegaal and Sparks, 2004). However, this distance also corresponds to Zn-Zn or Zn-Mg found in phyllosilicates (Schlegel *et al.*, 2001). The third shell, between ~3.7 and 4 Å, is similar to the distance of Zn-Fe when Zn is sorbed to iron oxides (Nachetegaal and Sparks, 2004), or the distance of O backscattering in O-Si shells, as found in Zn-kerolite (Schlegel *et al.*, 2001).

Previous studies have suggested that $Zn_5(OH)_5(CO_3)_2$, $Zn(OH)_2$, and $ZnCO_3$ may be the primary species controlling Zn stability in alkaline soils, at high Zn concentrations (Pickering, 1982; Papadopoulos and Rowell, 1989). In soils that have a pH between 6 to 8, and very high concentrations of Zn, franklinite and sphalerite can form (Robert *et al.*, 2002; Scheinost *et al.*, 2002; Sarret *et al.*, 2004; Isaure *et al.*, 2005; Panfili *et al.*, 2005). However, all these minerals are not dominant Zn phases in our soil samples. Franklinite, sphalerite, ZnS, $ZnCO_3$, or $Zn(OH)_2$ used as Zn standards in LLSF analyses of the soil samples did not contribute to the fits. The formations of Zn-phyllosilicate clays and Zn-LDH phases in soils, at various pH values, have been reported in several studies (Manceau *et al.*, 2000, 2004, and 2005; Juilot *et al.*, 2003; Voegelin *et al.*, 2005, Nachtegaal *et al.*, 2005). The LDH phases have a general structure formula of $[Me^{2+}{}_{1-x}\ Me^{3+}{}_{x}\ (OH)_2]^{x+}.(x/n)A^{n-}.\ mH_2O$, where Me^{2+} and Me^{3+} represent divalent and trivalent cations, respectively; x is equal to the ratio Me^{3+} / (Me^{2+} +Me^{3+}) and An- is the interlayer anionic species, with charge n- neutralizing the excess positive charge (Ford and Sparks, 2000; Grunewald *et al.*, 2008). Hydrotalcite, a Mg-Al layered double hydroxide, with a formula of $[Mg_6Al_2(OH)_{16}][CO_3].4H_2O$, is one of the most abundant LDH phases occurring in alkaline soils, and the most common hydroxide compound containing Al (Taylor, 1984; Grunewald *et al.*, 2008). Juillot *et al.* (2003) showed that a Zn/Al-hydrotalcite phase was dominant in Pb & Zn smelter-impacted soils. Kelorite is a poorly crystalline, hydrated, non-expanding, talc-like trioctahedral phyllosilicate, and found in various depositional carbonate environments, e.g. basaltic caves, smelter impacted soils (Leveille *et al.*, 2000 & 2002; Manceau *et al.*, 2000). The Zn-bearing kerolite standard used in our study, which has a general formula of $[Si_4(Mg_{3-x}\ Zn_x)O_{10}(OH)_2.\ n\ H_2O]$, was the same standard used in Manceau *et al.*, 2000. This mineral phase was found to be dominant in Zn contaminated soils in a former Zn-smelter site (Manceau *et al.*, 2000). Therefore, it is possible that Zn-LDH phases, i.e. Zn/Mg hydrotalcite-like & ZnAl-LDH, and Zn-kerolite form in the paddy soil at Mae Sot. These phases are more stable than hydrozincite, $Zn(OH)_2$ and $ZnCO_3$ (Robert *et al.*, 2002; Juillot *et al.*, 2003; Jacquat *et al.*, 2008), and can thus greatly reduce the bioavailability of Zn in soils. This could explain why a small amount of Zn was desorbed from the soil samples during stirred-flow experiments, even when the soil was acidified to pH 6, spiked with K_2SO_4, and flooded for different periods (Figure 4.1 and 4.2).

4.4.2.2 Micro -XRF maps

Micro-XRF maps of Zn, Cu, Fe, and Mn distributions in the alkaline soil, and acidified soil spiked with K_2SO_4 and flooded for different periods, are depicted in multi-color maps (Figure 4.9 and 4.10). In each map, the brightest color (white) represents the highest count or highest concentration of an element, while the darkest color represents the lowest count or lowest concentration of an element. Results showed that Zn was associated with Cu in all soil samples. Additionally, Zn was associated with Fe and Mn in the soil samples, to a minor extent (Figure 4.9 and 4.10). Two studies showed that Zn was mainly sorbed to Fe and Mn oxides in the paddy soils of Mae Sot, based on elemental correlation plots obtained from sequential extractions (Akkajit and Tongcumpou, 2010; Buanuam *et al.*, 2005). Although it is likely that Fe and Mn oxides can indeed retain some amount of Zn in our soil samples, they are not the main phases that immobilize Zn, based on the LLSF and μ-XRF results. Small correlations between Zn and Fe or Mn were observed in the μ-XRF maps of our soil samples. Additionally, results from LLSF analyses performed on all soil samples showed that the Zn-standards containing Fe, Mn, and/or Cu (i.e. ZnFeO , Zn-goethite, Zn-ferrihydrite, Zn-hydrous manganese oxide (HMO), Zn-CuS, Zn-$CuCO_3$, Zn-humic acid, and Zn-fulvic acid), did not significantly contribute to the fits (Figure 4.6 and 4.7). This suggests that Zn was not significantly sorbed to pure mineral phases of Cu, Mn and/or Fe. The associations between Zn with Cu, Mn, or Fe observed in μ-XRF maps could be rather due to the sorption of these elements to clay minerals, e.g. on the clay surfaces or in their interlayers, or the incorporation of these elements in the clay structures (Ryan and Gschwend, 1994; Nachtegaal, and Sparks, 2004; Manceau *et al.*,

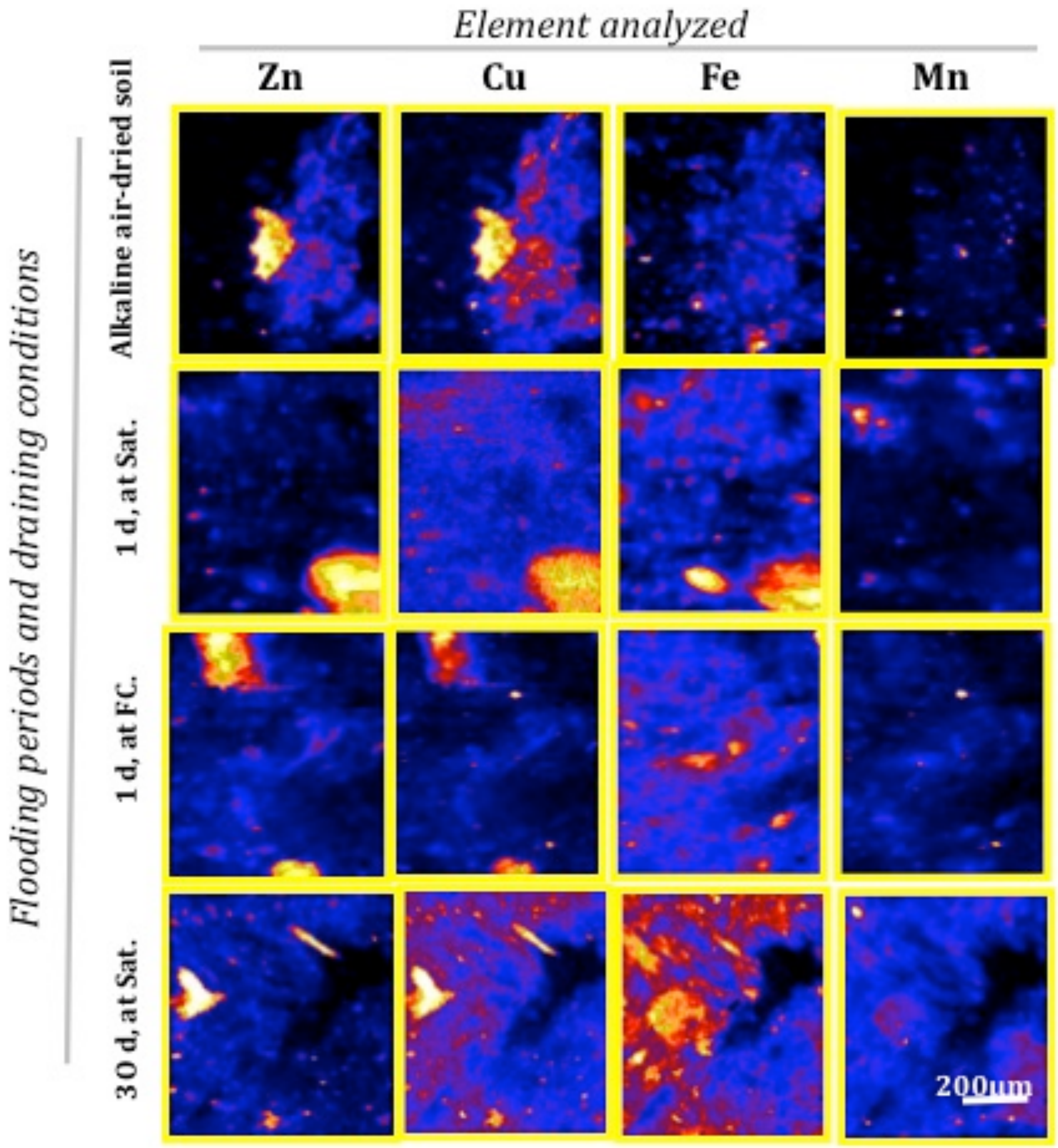


Figure 4.9 Micro XRF maps, showing the elemental distribution of Zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) in the alkaline air-dried soil, flooded for 1 and 30 days and drained to saturated (Sat) or field capacity (FC).

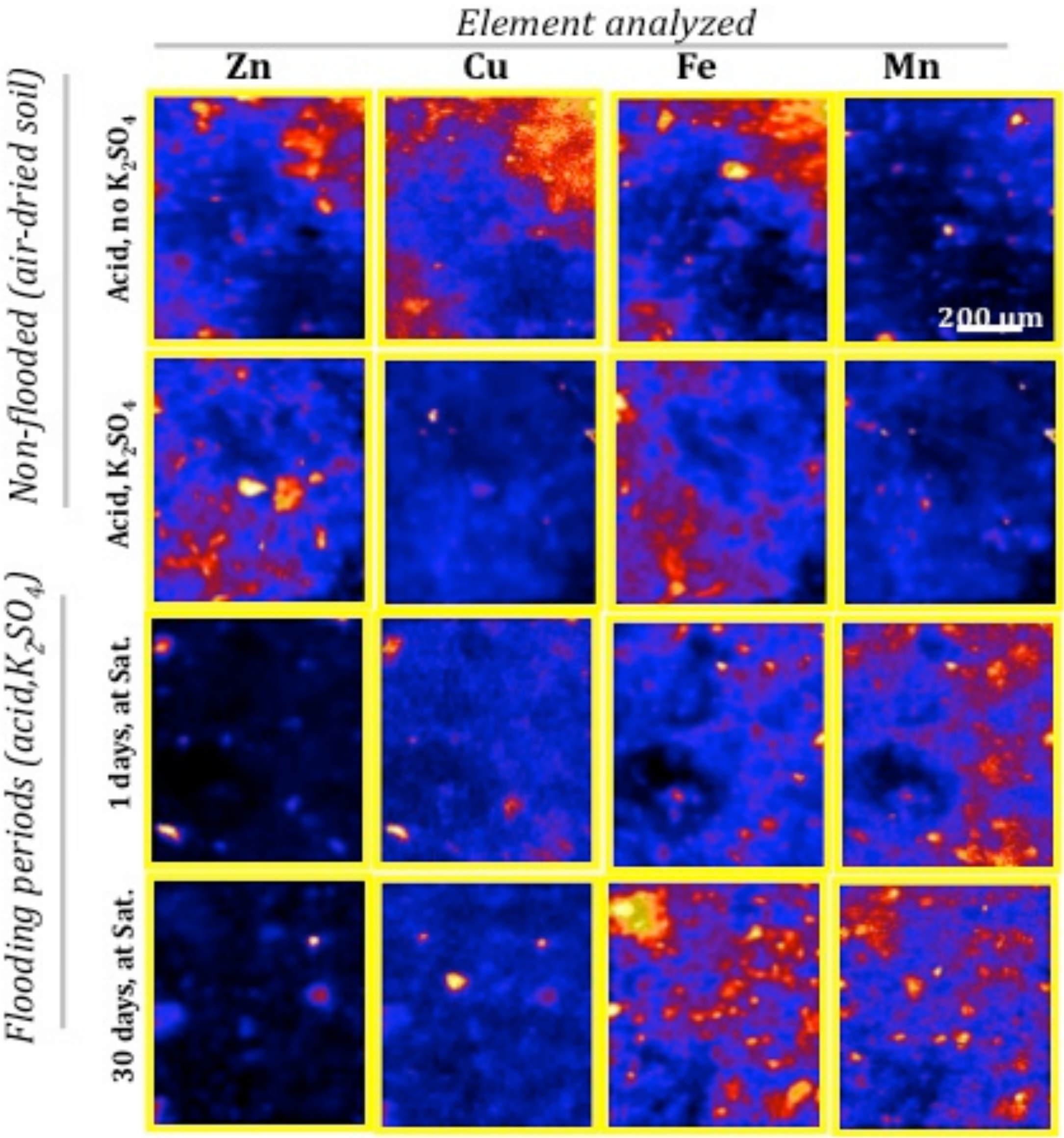


Figure 4.10 Micro XRF maps, showing the elemental distribution of Zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) in the acidified air-dried soil, not spiked with K_2SO_4, the acidified air-dried soil spiked with K_2SO_4, and the acidified soil spiked with K_2SO_4 flooded for 1 and 30 days and drained to saturated (Sat).

2004 & 2005; Wu *et al.*, 2006). Therefore, results from μ-XRF elemental distributions support those obtained by LLSF analyses, which showed that Zinc-LDH and Zn-phyllosilicates are the primary Zn phases that retain Zn in our soil samples. These results are also supported by Zn desorption experiments, which showed that Zn is immobilized in the phases containing this element in the soil, similar to when zinc is incorporated in LDH or phyllosilicate phases (Lothenbach *et al.*, 1999; Voegelin *et al.*, 2002; Nachtegaal and Sparks, 2004). Our results differ from those reported in Manceau *et al.* (2000) and Roberts (2001), which showed that iron oxides were ones of the main Zn sorbents in the soil studied, based on bulk XAFS analyses. Additionally, high correlations between Zn and Fe, or Mn were observed in the μ-XRF maps reported in these two studies.

4.5 Conclusions

This study showed that the primary Zn phases in the alkaline paddy soil, contaminated by Cd and Zn, of Mae Sot were Zn-LDH and Zn-phyllosilicate phases, mainly as a Zn/Mg hydrotalcite-like phase and a Zn-kerolite, respectively. Zinc is immobilized in these phases, since only small amounts of Zn were released after two hours of desorption experiments using DTPA-TEA-Ca. Additionally, acidifying the soil to pH 6, spiking it with K_2SO_4, and flooding it for different periods did not change the nature of the phases in which Zn was distributed in the soil. The presence of Zn in LDH and phylosilicate phases, which are stable minerals, explains why Zn is not easily dissolved to soil solution, and why this element, although present in the soil at very high concentrations, is not toxic to rice plants in the paddy soils of Mae Sot. The information provided in this study will help us find the best strategy to clean up Cd in the Cd and Zn co-contaminated paddy soils located in Mae Sot.

4.6 References

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Chapter 5

THE EFFECTS OF ORGANIC ACID SECRETED FROM RICE (*ORYZA SATIVA* L.) ROOTS ON CADMIUM AND ZINC RELEASE IN PADDY SOILS

5.1 Abstract

The type and amount of low molecular weight organic acids (LMWOA) secreted by plant roots are dependant on the plant species and the soil properties. This study investigated the variations of pH in the rhizosphere and indentified the types of LMWOAs secreted from two rice cultivars, when different concentrations of $CdNO_3$ are in the media. Additionally, this study investigated the effect of these LMWOAs on the kinetics of Cd and Zn desorption from soil components in paddy soils, contaminated by Cd and Zn, using a stirred-flow technique. Results showed that the pH in the rhizosphere was not dependent on Cd concentration, but decreased when each rice cultivar was introduced in the media. Additionally, the types and concentrations of LMWOAs secreted by the two rice cultivars were not dependent on Cd concentration. Both rice cultivars secreted three main LMWOAs, in the following order: oxalic > citric > maleic acids. The amounts of Cd and Zn released were dependent on the types and concentrations of the organic acids, and soil pH. Citric acid showed the greatest ability to desorb Cd and Zn from soil components. This study demonstrated that the LMWOAs released from rice roots can acidify the soil pH and enhance Cd and Zn release in the paddy soil.

5.2 Introduction

Metal bioavailability in the rhizosphere can be modified by root exudates, especially low molecular weight organic acids (LMWOAs) (Brady, 1990). The types and amounts of LMWOAs secreted by roots are dependent on the plant species, cultivars, and environmental conditions (White, 1981; Lui *et al.*, 2006; Fang *et al.*, 2008). The LMWOAs may affect the desorption capacities of metals from soil components, depending on the nature of the metal, the soil properties, as well as the types and concentrations of LMWOAs (Mench and Martin, 1991; Chen *et al.*, 2003). The solubility of Cd and Zn around the rhizosphere generally increases with increasing concentration of LMWOAs (Cieslinski *et al.*, 1998; Nigram *et al.*, 2001; Chiang *et al.*, 2006). Gao *et al.* (2003) reported that the presence of citrate at ≤0.5 mmol/l inhibited Cd desorption from two Cd contaminated soils, whereas the presence of this organic acid at ≥2 mmol/l promoted Cd desorption. Yuan *et al.* (2007) reported that citric acid demonstrated great enhancement on the desorption of Cu but negligible enhancement on the desorption of Cd; oxalic acid enhanced the desorption of Cu only at pH around 6.4 and enhanced the desorption of Cd in the pH range from 6.4 to 10.7. These studies demonstrated that the secretion of LMWOAs by plant roots are vary and can have different effects on each metal desorption in soils. Therefore, it is important to understand roles of the LMWOAs secreted by plant roots on metal desorption from soil components in metal contaminated soils (Chiang *et al.*, 2006). Our research aims to determine the types and amounts of LMWOAs secreted from two rice cultivars, i.e. jasmine and glutinous rice, exposed to different Cd concentrations. This study also investigates the effect of these LMWOAs on the kinetics of Cd and Zn desorption from soil components in paddy soils contaminated by these two metals, using a stirred-flow technique. The results will help understand the role of rice in Cd and Zn desorption from soil components. This knowledge is needed to find the best strategy to control the uptake of Cd by rice cultivated in paddy soils contaminated by Cd and Zn .

5.3 Materials and methods

5.3.1 pH changes in the rhizosphere

A 0.8% solid Murashige & Skoog, 1962 (MS)-media was prepared with bromocresol purple 5',5"-dibromo-o-cresolsulfophthalein. Solid bromocresol MS-media was then divided into two portions. The first portion was used for the no-Cd treatment, and a Cd^{2+} solution prepared from $Cd(NO_3)_2$ salt was added in the second portion, to obtain a Cd^{2+} concentration of 150 ppm in the media. After the pH of the media was adjusted to pH 6.25, the samples were sterilized in an autoclave, at 120 C˚. The samples were then used to grow two Thai rice cultivars that had germinated and grown for 6 days, i.e. jasmine and glutinous rice, the two most cultivated rice cultivars in Cd contaminated paddy soils at Mae Sot district, Tak Province, Thailand. The pH variation in the rhizosphere was monitored at 0, 6, 24, and 48 hours, based on changes in color of the rice plant rhizosphere. If the pH of the rhizosphere decreased lower than 6.25, the color of bromocresol purple MS-media would change from an initial dark purple color (indicating pH 6.25) to light yellow (indicating pH lower than 6.25).

5.3.2 Types and amounts of organic acids secreted by rice roots subjected to various Cd concentrations

The total concentrations of selected elements in the rice grains of the two rice cultivars were measured by total acid digestion, using U.S. EPA method 3050 (reported in Table 5.1). Rice seeds from the two rice cultivars were surface-sterilized with 50 % bleach (Clorox, regular bleach) for 10 minutes, and washed three times with deionized-water. Sterilized seeds were transferred into germination plates containing 10 % solid MS-media, and were allowed to germinate and grow for 6 days. The uniform 6-day-old seedlings were transferred into 5 ml of 1 % liquid-MS media at pH 5.8. A solution of Cd^{2+}, prepared from $Cd(NO_3)_2$ salt, was added to each sample, to reach a final Cd concentration of 0, 50, 100, 150, 300, or 750 ppm. Each of these treatments was replicated six times. After the samples were placed for 10 days in a shaker that operated at 40 rpm, and continuously exposed to a ~70 watts/m2 light density, at room temperature, the root exudates were collected. Each sample was dissolved with 2.5 mM K_2HPO_4, at pH 2.5. Five milliliters of each sample were frozen at –20 C° for 24 hours, and freeze-dried for 48 hours. The samples were then dissolved with 400 µl of 2.5 mM K_2HPO_4, at pH 2.5. The exudates were then quantified and identified by High-Performance Liquid Chromatography (HPLC) with a C18, 5 µm column (120 Å and 4.6 x 150 mm, at a wavelength of 220 nm). To identify the types of LMWOAs, the chromatographic retention times of the organic acids secreted by roots in our samples were compared with the retention times of standards (i.e., succinic acid, formic acid, acetic acid, oxalic acid, maleic acid, malic acid, citric acid, and phthalic acid). The amount of each organic acid was calculated by comparing its peak area with those of known standards (Cawthray, 2003).

5.3.3 Soil characterization

The study site was selected based on Cd concentrations measured at different locations in the Mae Sot district, Tak Province, Thailand, and reported by the International Water Management Institute (Simmons and Pongsakul, 2005). Surface samples (0-15 cm) were collected in a paddy soil, contaminated by Zn and Cd. The soil was acidified to pH 6 using HNO_3, and spiked with K_2SO_4. This treated soil was referred as the "acidified soil" in this study, while the soil not acidified with HNO_3 and spiked with K_2SO_4 was referred as the "alkaline soil". These two soil samples will allow us to determine the effect of soil pH on the ability of LMWOAs to desorb Cd and Zn from soil components in the contaminated paddy soil at Mae Sot. Basic physicochemical properties of the alkaline soil were determined using standard procedures (Schofield and Taylor, 1955; U.S. EPA, 1986; Nelson and Sommers, 1982), and results are reported in Table 5.2.

5.3.4 Cadmium and Zinc release kinetics by LMWOAs

Cadmium and zinc release kinetic experiments in alkaline and acidified soils were conducted using a stirred-flow technique. The stirred-flow set up was similar to the one used in Strawn and Sparks (2000). Oxalic acid, citric acid, and maleic acid were chosen as the extracting solutions, at 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid. These solutions were adjusted at pH 6 with NaOH, and the ionic strength was set at 0.01 M with $NaNO_3$. The experiments were initiated by purging the tubing connected to the stirred-flow chamber with each organic acid solution, adding 0.4 g of acidified soil or alkaline soil into the reaction chamber, and quickly filling the chamber with 8 ml of each organic acid solution. A

25-mm diameter filter membrane of 0.45 µm pore-size was used to separate the effluent from the solid suspension in the stirred-flow chamber. A magnetic bar was used to mix the suspension at 500 rpm. The experiments were run for 120 minutes, at a flow rate of 0.8 $ml.min^{-1}$, and the effluent was collected in 12 tubes, one every 10 minutes. Cadmium and Zn concentrations were measured using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), to subsequently calculate cumulative Cd and Zn desorption percentages (Shi, 2006). Each stirred-flow experiment was run in duplicate.

5.4 Results and discussion

5.4.1 pH changes in the rhizosphere

Results revealed that the pH of the media decreased in all samples, treated or not treated with Cd (Figure 5.1). The color of the rice plant rhizosphere areas changed from dark purple (indicating pH at 6.25) to a bright, yellow color, indicating acidification in the root zones (pH lower than 6.25). The intensity of the yellow color in all treatments was very similar, and increased with time. This showed that the acidification was occurring in the rhizosphere of the two rice cultivars. Haynes (2006) reported that the HCO_3^- ion released by plant roots, due to ion uptake by plants, can acidify the rhizosphere. Additionally, plant respiration and root exudates, especially LMWOAs, can also acidify the pH in the rhizosphere (Marschner, 1995; Jauert *et al.* 2002; Hinsinger *et al.*, 2004; Lui et al, 2006, Dong *et al.* 2007). The acidification of the pH in the rhizosphere observed in our samples could result from the presence in the soil solution of LMWOAs secreted by rice roots, which may affect Cd and Zn

Table 5.1 Total concentrations of selected elements in rice grains.

Samples	Total Concentration (ppm)					
	Ca	**Cd**	**Cu**	**Fe**	**Zn**	**S**
Jasmine rice	**328**	**0.27**	**2.65**	**179**	**33**	**1000**
Glutinous rice	**174**	**0.04**	**2.24**	**91**	**38**	**1178**

Table 5.2 Physicochemical properties of the alkaline paddy soil.

Soil	pH	O.M (%)	Total Concentration (mg/kg)				
						S	
			Cd	**Ca**	**Zn**	**Before addition of K_2SO_4**	**After addition of K_2SO_4**
Alkaline soil	7.5	4.3	142	34,360	3,050	282	3,618

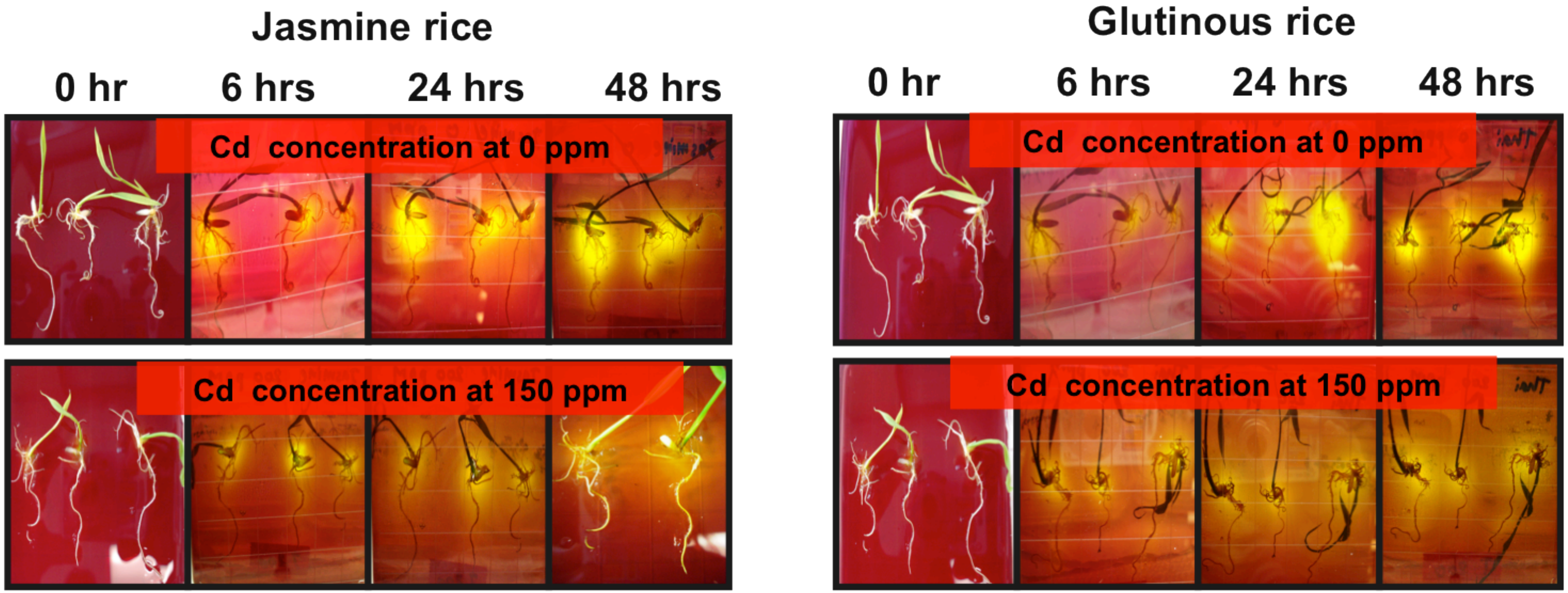

Figure 5.1 The temporal changes of pH in the MS media with 90% bromocresol purple by jasmine rice and glutinous rice, at Cd concentrations of 0 or 150 ppm. The bright yellow color indicates acidification by rice roots of the media to values lower than pH 6.25.

bioavailability in the Thai paddy soil. Therefore, we next investigate the involvement of LMWOAs secreted from the two Thai rice cultivars in the next section.

5.4.3 Organic acids secreted by rice roots subjected to various cadmium concentrations

Results from HPLC analysis indicated that both rice cultivars secreted the same types and similar quantities of LMWOAs. At all Cd concentrations studied, oxalic acid, citric acid, and maleic acid were the predominant LMWOAs secreted by the two rice cultivars, in the following order: oxalic > citric > maleic acids (Figures 5.2). These results are different from those reported in Lui *et al.* (2006), who showed that acetic and formic acids constituted more than 96 % of the total concentration of LMWOAs released from rice exposed to Cd. Other studies have shown that citric and oxalic acids are commonly found in the rhizosphere of monocotyledons, including rice (Cieslinski *et al.*, 1998; Liao, 2006; Goa, 2007). The presence of maleic acid was reported in the rhizosphere of durum wheat, tobacco, and sunflower under Cd stress, as well as wheat under Mn stress (White, 1981; Chiang *et al.*, 2006; Fang *et al.*, 2008). Additionally, the presence of malic acid, a compound similar to maleic acid, has been reported in rice under Zn deficiency (Gao, 2007). Studies have shown that different types and concentrations of LMWOAs can enhance or inhibit metal desorption in soils (Gao *et al.*, 2003; Yuan *et al.*, 2007). The secretion of citric oxalic and maleic acids found in the two Thai rice cultivars could affect phyto-bioavailability of Cd and Zn in paddy soils at Mae Sot that contaminated with Cd and Zn. Therefore, it is important to understand the effects of citric, oxalic, and maleic acids secreted by rice roots on Cd and Zn desorption in paddy soils from Mae Sot.

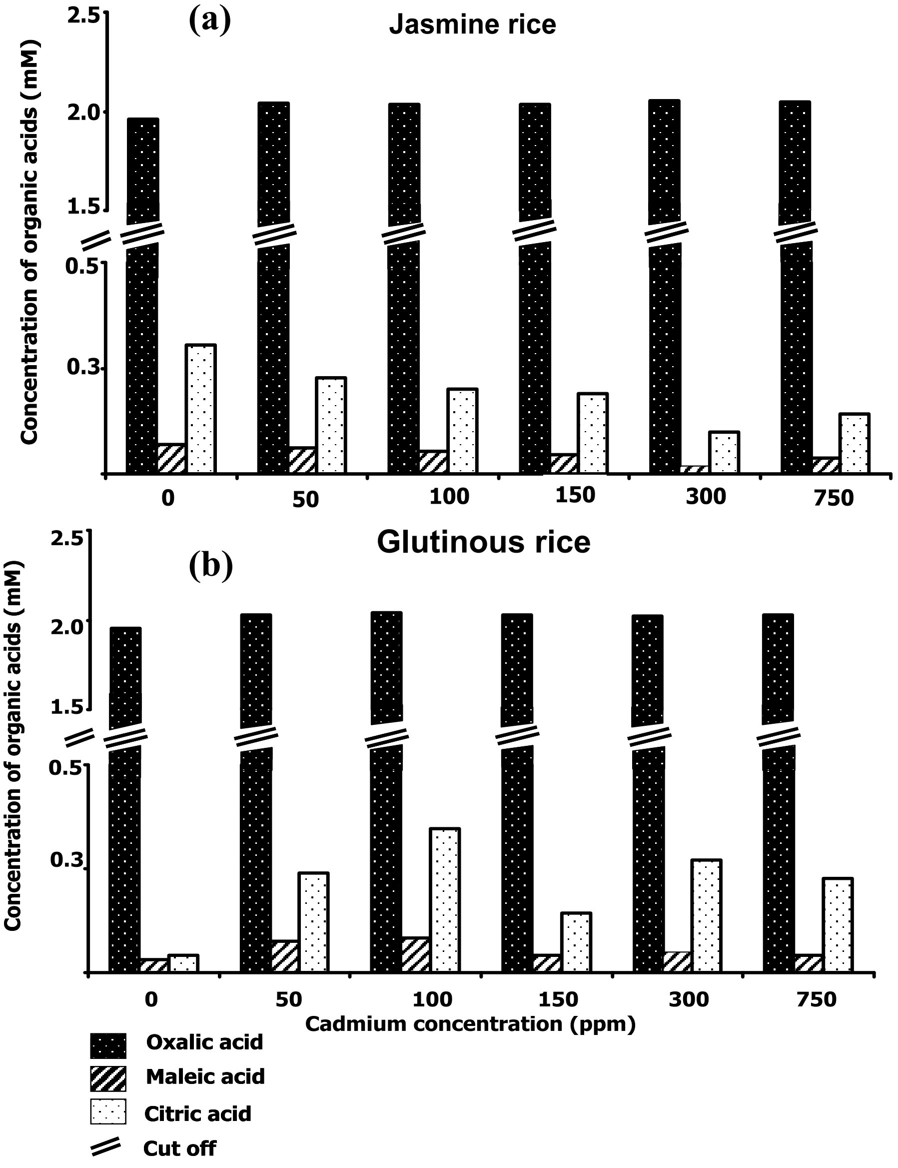

Figure 5.2 Predominant organic acids secreted from **(a)** jasmine rice, and **(b)** glutinous rice, subjected to various concentrations of Cd. The x axis represents the concentrations of Cd (ppm) and the y axis represents the concentrations of oxalic, maleic and citric acids (mM).

5.4.4 Cadmium and zinc release kinetics

The types and concentrations of the extracting solutions, i.e. 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid, were chosen based on the determination of the types and concentrations of LMWOAs present in our samples (section 5.4.3). Results from stirred-flow experiments showed that the amounts of Cd and Zn released in the paddy soils are dependent on soil pH, as well as the types and concentrations of LMWOAs. The amounts of Cd and Zn released in the alkaline soil were lower than in the acidified soil for all stirred-flow experiments conducted with each extracting solutions. Our results thus support those reported in previous studies, which showed that metal desorption reactions occurring in soils are highly pH dependent, and are promoted when the soil pH decreases (Gray *et al.*, 1999; Appel and Ma, 2002; Gao *et al.*, 2003; Itami and Yanai, 2006). In most samples, Cd and Zn were desorbed in the highest quantity when 3.5 mM citric acid was used as the desorption solution, while the lowest quantity were measured when oxalic acid was used (Figure 5.3 to 5.6). Lui *et al.* (2006) reported that LMWOAs could modify Cd bioavailability by two main mechanisms: acidification of the soil, and formation of metal-organic complexes. The fact that citric acid desorbed higher amounts of Cd and Zn from soil components than oxalic and maleic acids could be due to the higher ability of citric acid to acidify soil pH compared to the two other organic acids. When citric acid was used as the extracting solution during the stirred flow experiments, lower pH values were measured in the effluent solution than when oxalic and maleic

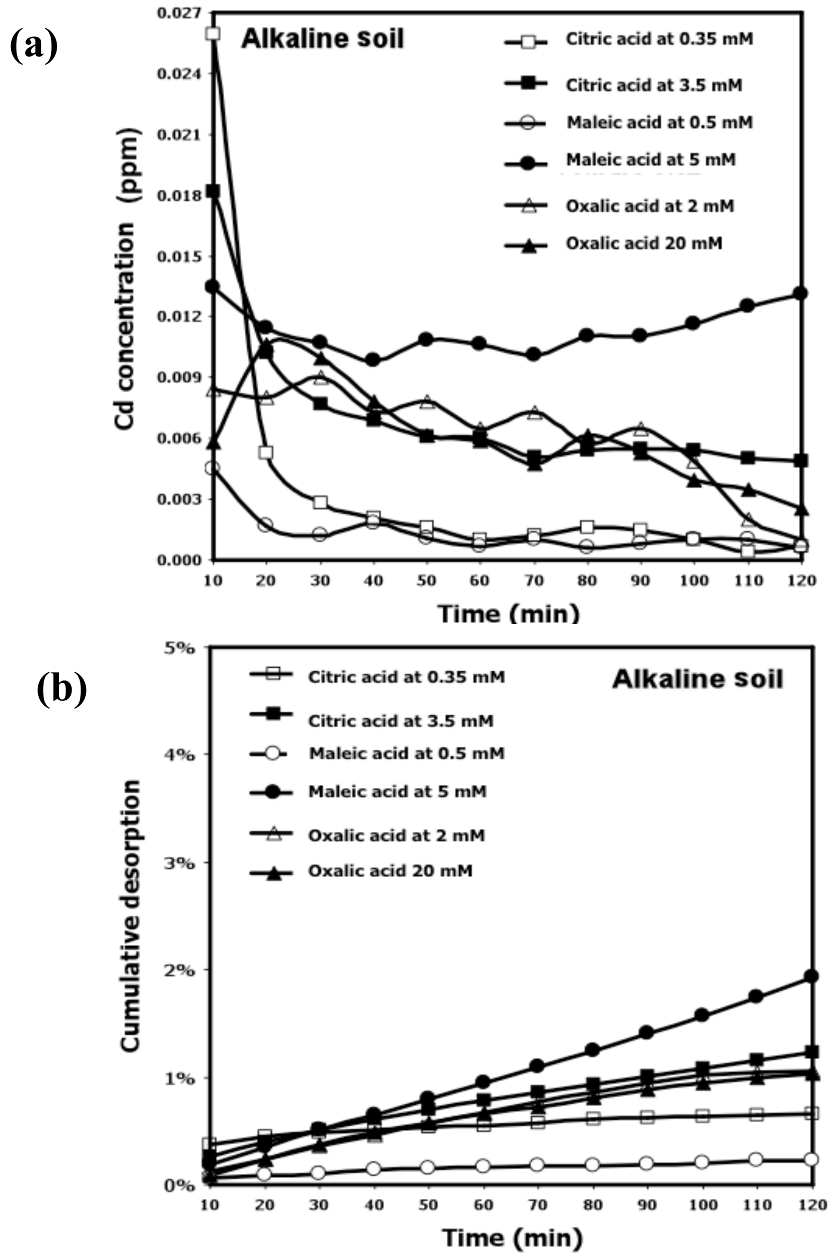

Figure 5.3 **(a)** Cd release kinetics, and **(b)** cumulative Cd desorption (in%), in the alkaline soil by oxalic acid, citric acid, and maleic acid at 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid.

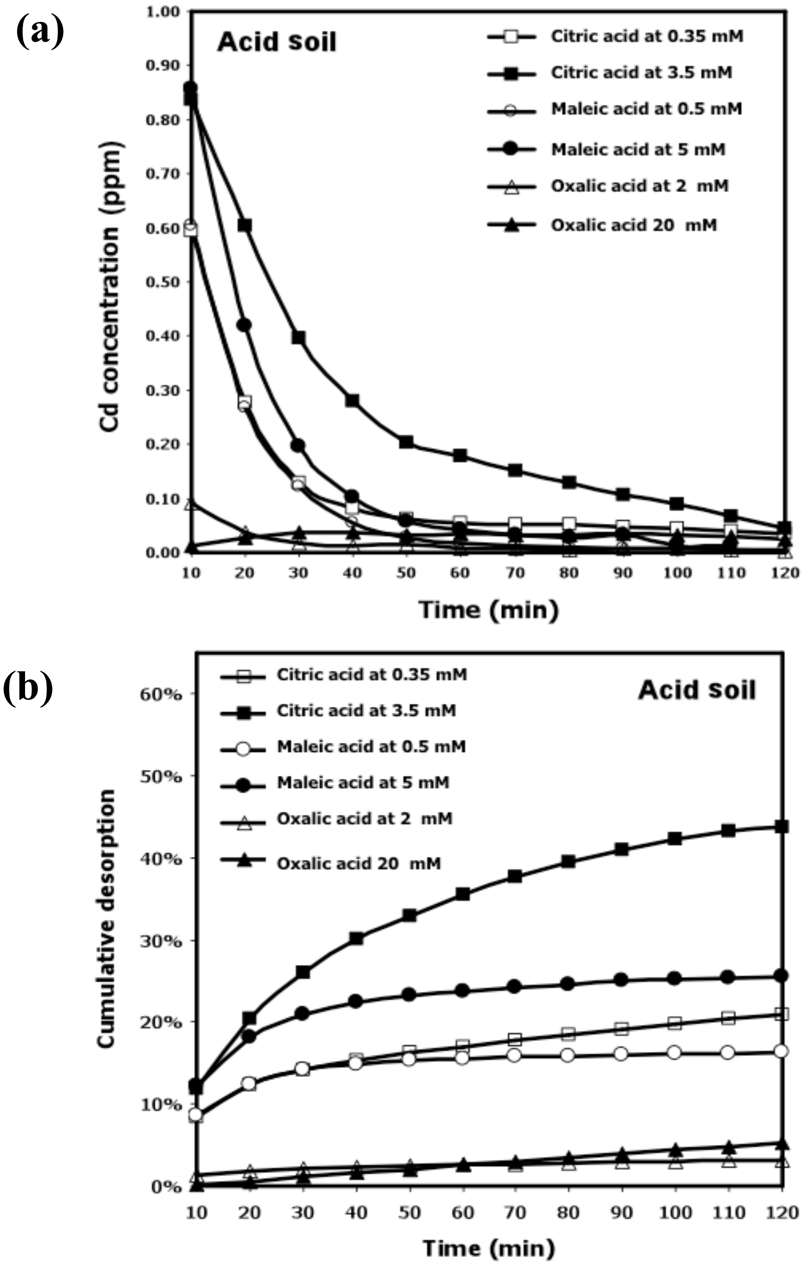

Figure 5.4 **(a)** Cd release kinetics, and **(b)** cumulative Cd desorption (in %), in the acidified soil by oxalic acid, citric acid, and maleic acid at 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid.

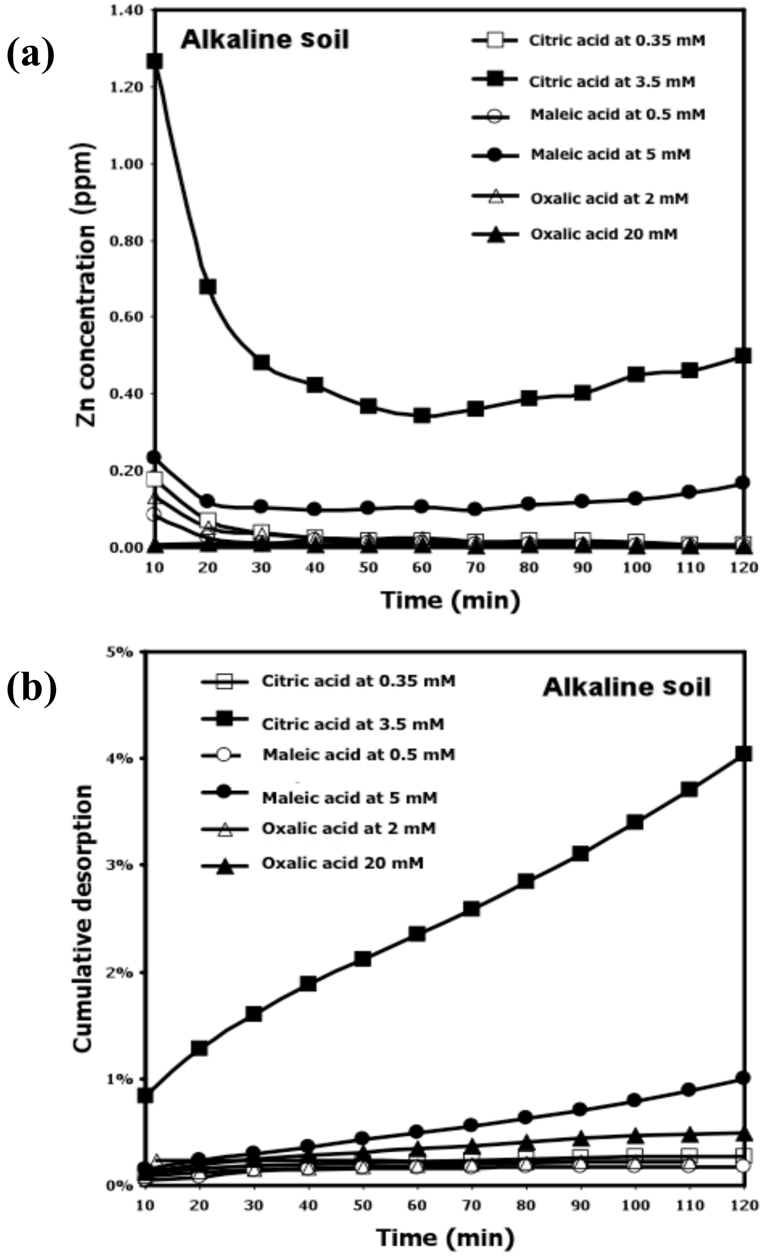

Figure 5.5 **(a)** Zn release kinetics, and **(b)** cumulative Zn desorption (in %), in the alkaline soil by oxalic acid, citric acid, and maleic acid at 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid.

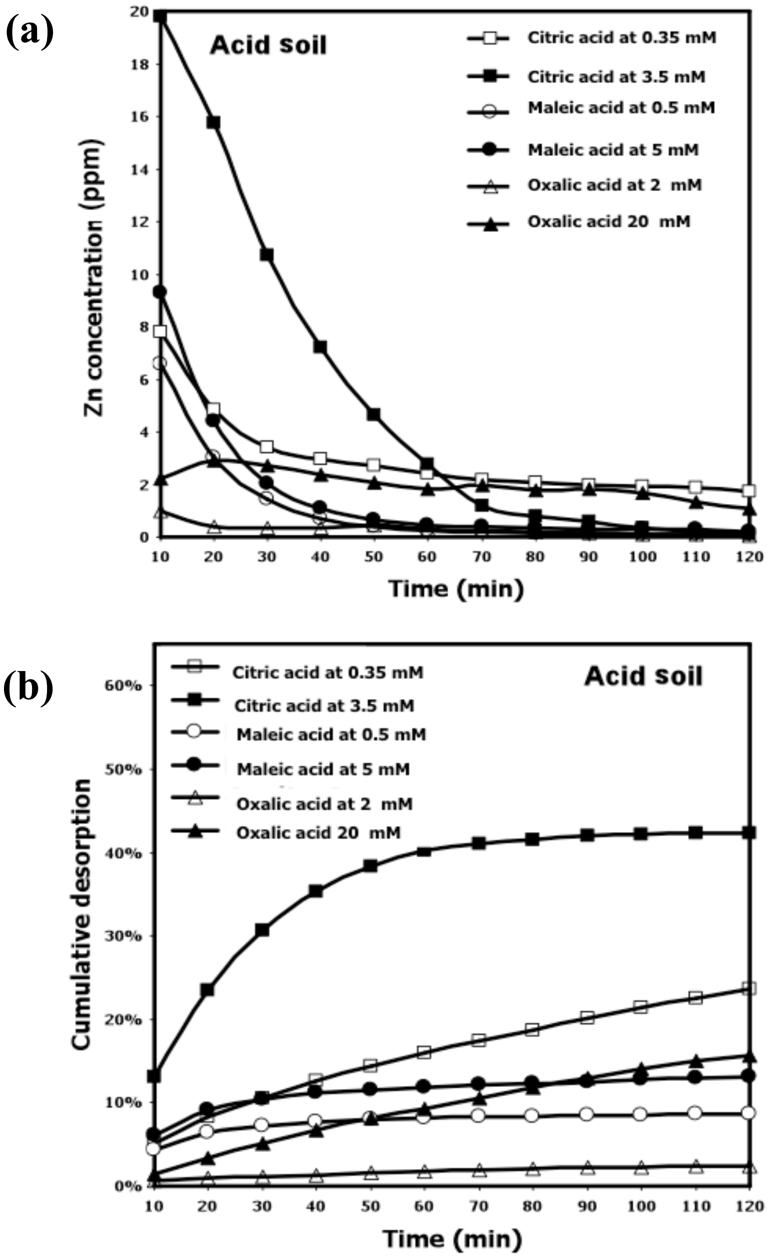

Figure 5.6 **(a)** Zn release kinetics, and **(b)** cumulative Zn desorption (in%), in the acidified soil by oxalic acid, citric acid, and maleic acid at 0.35 or 3.5 mM citric acid, 0.5 or 5 mM maleic acid, and 2 or 20 mM oxalic acid.

acids were employed as the extracting solutions. Citric acid has three pKa values, while oxalic and maleic acid have only two pKa values. In a system with pH initially above pH 6, more protons are released to solution from the citric acid molecule than the maleic and oxalic molecules, based on the value of each organic acid pKa shown in Figure 5.7. These protons could promote mineral dissolution, causing detachment of metals into the soil solution (Sparks, 2003). In addition to acidification, several studies suggested that citric acid could enhance metal release into soil solution via the formation of metal-ligand complexes (White, 1981; Nigam *et al.*, 2001; Zhou *et al.*, 2003). Zhou *et al.* (2003) reported that the presence of citric acid significantly inhibited Cd adsorption in soil, due to the formation of soluble Cd-organic complexes. Therefore, the release of Cd and Zn from the alkaline and acidified soils is particularly high when citric acid is used as the extraction solution. The small amounts of Cd and Zn released by oxalic acid could be attributed to the formation of metal-oxalate precipitation. The precipitation of Ca-oxalate complexes can occur in alkaline soils, containing high exchangeable Ca (Cieslinski *et al.*, 1998; Szmigielska *et al.*, 1996). Additionally, Glover *et al.* (2002) showed that oxalate can also form ternary complexes with Cd at the goethite surface, resulting in lower amounts of Cd desorbed from goethite. The paddy soil used in this study has very high concentrations of Ca and oxides. Precipitation of Ca & oxalic acid, and ternary complexes, involving Cd, oxalic acid, and mineral surfaces, may occur in the paddy soil, resulting in small releases of Cd into the soil solution.

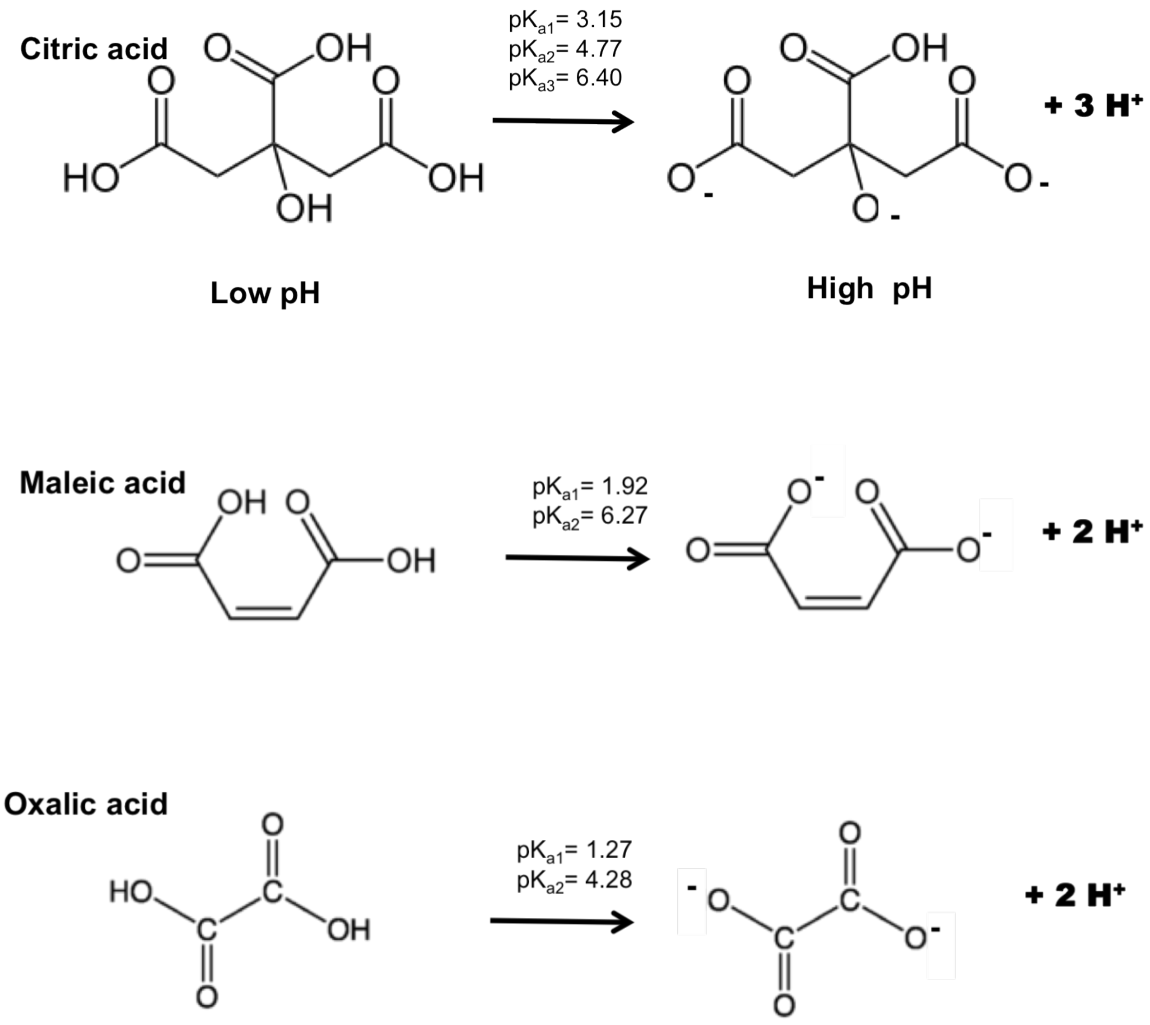

Figure 5.7 Structural formula and pKa values of citric, maleic, and oxalic acids.

5.6 Conclusions

Results from HPLC analysis showed that three organic acids were predominantly secreted by jasmine and glutinous rice subjected to various Cd concentrations, in the following order: oxalic > citric > maleic acids. The amounts of Cd and Zn released in the paddy soils were dependent on soil pH, as well as the types and concentrations of LMWOAs. Citric acid showed the greatest effectiveness to extract Cd and Zn from the paddy soil at Mae Sot, while oxalic acid showed the lowest ability to desorb these metals from the soils. The amounts of Cd and Zn released from the acid soil were higher than those released from the alkaline soil for all extracting agents. This suggested that the ability of LMWOAs to desorb Cd and Zn from soil components depends on soil pH. Our study demonstrated that very low concentrations of organic acids secreted from rice roots can potentially enhance Cd and Zn bioavailability to rice, especially when the soil pH is low. Due to the potential health threat that Cd poses to the local population in the Mae Sot district, this study demonstrated the necessity of removing Cd from the soil solution and the fraction of Cd that can potentially be desorbed from soil components, before the paddy soil is used for rice production.

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Chapter 6

CONCLUSIONS AND IMPLICATIONS

Based on the findings discussed in the previous five chapters, the following conclusions can be made:

i) In both alkaline and acidified soils, at all flooding periods and draining conditions, Cd and Zn had different geochemical forms, were distributed separately, and sorbed to different soil components.

ii) Soil pH, flooding periods, and draining conditions had a greater impact on the speciation and solubility of Cd than Zn.

iii) The kinetics of Cd and Zn released in both the alkaline and acidified soils, at all flooding periods and draining conditions, were the same and characterized by two steps -an initial rapid release, followed by a slower release.

iv) In the alkaline soil, Cd carbonate species, i.e. $CdCaCO_3$ and $CdCO_3$, were the primary phases that retained Cd in the alkaline paddy soil at Mae Sot.

v) Cadmium sulfide was not an important species in controlling Cd solubility in the alkaline soil. Only a small amount of CdS was found during 150 days of flooding. This species became dominant after the soil was acidified, but was not detected after the acidified soil was flooded.

vi) Treating the soil with HNO_3 and K_2SO_4 can change the nature of the mineral phases and the desorption capacity of Cd in the paddy soil of Mae Sot, but does not alter Zn speciation. Cadmium sorbed to kaolinite was the most dominant species in the flooded acidic soil. The amount of this species increased with increasing flooding periods.

vii) The stability of Zn was very high, and greater than Cd, especially in the acidified soil.

viii) In both alkaline and acidified soils, at all flooding periods and draining conditions, the same primary phases containing Zn were found, i.e. a mixture of Zn-LDH (Zn-Al-LDH, and/or Zn/Mg-hydrotalcite-like) and Zn-phyllosilicates (Zn-kerolite). Our results from desorption experiments suggested that Zn is stable in these phases, since less than 25% of this metal was released in all samples.

ix) In most samples, Cd was mainly associated with Ca, while Zn was mainly associated with Cu.

x) Three predominant organic acids were secreted by jasmine and glutinous rice subjected to various Cd concentrations, in the following order: oxalic > citric > maleic acids. The amounts of Cd and Zn released in the paddy soils were dependent on soil pH, as well as the types and concentrations of LMWOAs. Citric acid showed the greatest effectiveness to extract Cd and Zn from the paddy soil at Mae Sot.

The identification of the phases in which Cd is distributed in the soil of Mae Sot, using synchrotron-based techniques, provided a new understanding on Cd speciation in paddy soils. It was thought that CdS was the primary species controlling Cd solubility in the paddy soil, especially during flooding period. Based on this information, farmers were recommend not to drain water out from paddy fields before harvesting rice, which is impractical in field settings. Our study suggests that draining before harvesting rice should not play a crucial role in enhancing Cd bioavailability in the paddy soils of Mae Sot, because CdS is not a primary species. This study also shows that the period of flooding can greatly alter Cd speciation. Additionally, we found that roots of two rice cultivars, cultivated at a contaminated paddy soil in Mae Sot, can secrete important LMWOAs that can enhance Cd and Zn desorption in paddy soils. Therefore, this study demonstrates the need to remediate Cd from the contaminated paddy soils, before cultivating rice at these sites, due to the serious health threat that Cd poses to the local population. Remedial actions in these soils must be addressed using a multi-discipline approach. Knowledge from soil science and chemistry will help us find the proper chemical agents to promote Cd release in the soil. Well-engineered techniques will enable us to remove Cd from the contaminated site or minimize its transport into the soil. Further research is needed to determine and optimize the procedures that could be employed to remediate Cd from the contaminated paddy soils of Mae Sot.