POTENTIAL UTILIZATION OF COFFEE GROUND MATERIAL AS CADMIUM AND ZINC STABILIZING AMENDMENTS : A CASE STUDY OF MAE TAO, TAK, THAILAND

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Environmental Management
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2015

ความเป็นไปได้ในการใช้กากกาแฟเพื่อเป็นตัวปรับปรุงดินและตรึงแคดเมียมและสังกะสีในดินพื้นที่แม่ตาว จังหวัดตาก

นางสาวมณฑาทิพย์ ใจสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการจัดการสิ่งแวดล้อม (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

Thesis Title	POTENTIAL UTILIZATION OF COFFEE GROUND MATERIAL AS CADMIUM AND ZINC STABILIZING AMENDMENTS : A CASE STUDY OF MAE TAO, TAK, THAILAND
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มณฑาทิพย์ ใจสุข : ความเป็นไปได้ในการใช้กากกาแฟเพื่อเป็นตัวปรับปรุงดินและตรึงแคดเมียม และสังกะสีในดินพื้นที่แม่ตาว จังหวัดตาก (POTENTIAL UTILIZATION OF COFFEE GROUND MATERIAL AS CADMIUM AND ZINC STABILIZING AMENDMENTS : A CASE STUDY OF MAE TAO, TAK, THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. เพ็ญรดี จันทร์ภิวัฒน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. ศีลาวุธ ดำรงศิริ, 90 หน้า.

งานวิจัยนี้ทำการศึกษาความเป็นไปได้ในการใช้กากกาแฟ และถ่านกากกาแฟ เป็นสารปรับปรุงดินเพื่อปรับเสถียรแคดเมียมและสังกะสี สำหรับดินปนเปื้อนแคดเมียมซึ่งรวบรวมจากแม่สอด จังหวัดตาก ความเข้มข้นของแคดเมียและสังกะสีในดินถูกตรวจพบว่ามีค่าเฉลี่ยเท่ากับ 47.2 และ1,660 มก. / กก. ตามลำดับ หลังการจัดเตรียม กากกาแฟและถ่านกากกาแฟ ถูกนำไปผสมลงในดินปนเปื้อนในปริมาณร้อยละ 2.5 5 10 และ 20 โดยน้ำหนัก และ ถูกบ่มเป็นระยะเวลาทั้งสิ้น 8 สัปดาห์ เมื่อสิ้นสุดการบ่ม จึงทดสอบด้วยการสกัดลำดับส่วนด้วยวิธีของ BCR การสกัดด้วย DTPA และการทดสอบความเป็นพิษต่อพืช เพื่อประเมินการเคลื่อนที่ได้ง่าย ความพร้อมต่อการดูดดึงของพืช และ ความเป็นพิษของแคดเมียมและสังกะสีในดินที่ปรับปรุงแล้ว

ผลการทดลองพบว่าการเติมกากกาแฟและถ่านกากกาแฟลงในดินปนเปื้อนทำให้สารอาหารและวัตถุอินทรีย์ในดินเพิ่มขึ้น ในด้านของรูปแบบของแคดเมียมและสังกะสี การศึกษาพบว่าแคดเมียมในรูปที่แลกเปลี่ยนได้ และรูปที่ถูกรีดิวซ์ได้มีค่าลดลงเมื่อสัดส่วนของกากกาแฟเพิ่มขึ้น การกระจายตัวของแคดเมียมและสังกะสีในรูปต่าง ๆ ในทุกสัดส่วนของการผสมกากกาแฟและถ่านกากกาแฟพบว่าคล้ายกันทั้งหมด นอกจากนี้ยังพบว่าแคดเมียมส่วนมากอยู่ในรูปที่แลกเปลี่ยนได้ และรูปที่ถูกรีดิวซ์ได้ ในขณะที่สังกะสีส่วนใหญ่อยู่ในรูปที่ถูกรีดิวซ์ได้ และรูปที่ถูกออกซิไดซ์ได้ ในด้านของความพร้อมต่อการดูดดึงของพืช พบว่า แคดเมียมที่พร้อมต่อการดูดดึงโดยพืชมีค่าลดลงเฉลี่ยร้อยละ 1.98 และ 2.80 ในดินที่ปรับปรุงด้วยกากกาแฟและถ่านกากกาแฟ ตามลำดับ ในขณะเดียวกัน สังกะสีในรูปที่พร้อมต่อการดูดดึงโดยพืชกลับมีค่าเพิ่มขึ้น ผลทดสอบความเป็นพิษต่อพืชโดยการงอกของเมล็ดข้าว พบว่า การเพิ่มสัดส่วนของถ่านกากกาแฟสามารถเพิ่มการงอกและความยาวของรากได้ ผลการศึกษาโดยรวมชี้ให้เห็นว่า สามารถพิจารณาใช้ถ่านกากกาแฟเป็นวัสดุปรับปรุงดินเพื่อตรึงแคดเมียมในดินปนเปื้อนได้

สาขาวิชา การจัดการสิ่งแวดล้อม

ปีการศึกษา 2558

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5587594720 : MAJOR ENVIRONMENTAL MANAGEMENT

KEYWORDS: COFFEE GROUNDS / SOIL AMENDMENT / WASTE UTILIZATION / HEAVY METALS / TOXICITY

MONTATIP JAISOOK: POTENTIAL UTILIZATION OF COFFEE GROUND MATERIAL AS CADMIUM AND ZINC STABILIZING AMENDMENTS : A CASE STUDY OF MAE TAO, TAK, THAILAND. ADVISOR: PENRADEE CHANPIWAT, Ph.D., CO-ADVISOR: SEELAWUT DAMRONGSIRI, Ph.D., 90 pp.

The present research studied the potential utilization of untreated coffee ground (UCG) and carbonized coffee ground (CCG) as Cd and Zn stabilizing amendments in Cd contaminated soil collected from Mae Sot, Tak. Soil was determined with the average Cd and Zn concentrations of 47.2 and 1,660 mg kg^{-1}, respectively. After preparation, UCG and CCG was separately mixed into the contaminated soil with the mixing ratio of 2.5%, 5%, 10% and 20% (w/w). All amended soils were incubated for a total of eight weeks. At the end of incubation period, BCR sequential extraction, DTPA extraction, and plant toxicity test were conducted in order to assess the mobility, plant availability, and toxicity of Cd and Zn in amended soils.

The results revealed that the addition of UCG and CCG to contaminated soil resulted in the increase in soil nutrients and OM concentrations. In terms of Cd and Zn distribution, the exchangeable and reducible Cd were found to be decreased with the increasing mixing ratio of UCG. Similar results of Cd and Zn fractionations were observed in all mixing ratio of both UCG and CCG amended soils. In addition, the major Cd fractions during the whole aging process were exchangeable and reducible fractions. Whereas, Zn were mainly found in the forms of reducible and oxidizable fractions. In term of plant availability, Cd was found to be decreased by an average of 1.98% and 2.80% in UCG and CCG amended soil, respectively. At the same time, an increase in Zn plant availability was found in both types of amended soils. The results of phytotoxicity on rice germination, an increase in CCG mixing ratio could improve the possibilities of seed germination as well as root growth. The overall findings indicated that CCG can be considered as Cd immobilizing amendment to the contaminated soil.

Field of Study: Environmental Management

Academic Year: 2015

Student's Signature ....

Advisor's Signature ....

Co-Advisor's Signature ....

ACKNOWLEDGEMENTS

First of all, I would like to acknowledge my advisor Dr. Penradee Chanpiwat and my co-advisor Dr. Seelawut Damromgsiri for sparking my curiosity in heavy metal contaminated soil science. I would also like to thank them for giving me this great opportunity to pursue my research under their supervision. This thesis could not have been completed without the assistance of my advisor and co-advisor.

Besides, I would like to express my sincere gratitude to all thesis committee members, Dr. Srilert Chotpantarat, Dr. Chantra Tongcumpou, and Dr. Pensiri Akkajit for their valuable comments and suggestions.

I would like to acknowledge the financial supports from the International Program in Hazardous Substance and Environmental Management (HSM), Graduate School, Chulalongkorn University and the Risk Assessment and Site Remediation (RAR) Research Cluster of the Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University. I would like to appreciate both HSM and ERIC laboratory staffs for their great supports throughout the experiment and laboratory works.

Finally, I truly appreciate my parents for their love, support, and encouragement that continues to keep me inspired to accomplish my goals.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The hazards of heavy metal contamination to the environment and all living organisms, especially to humans, have become serious concerns globally. It is widely known that residual heavy metals from human activities such as those from factories, transportation, power stations, and mining may leak and persist in the environment (Banerjee, 2003; Bergback et al., 1994; Norgate et al., 2007; Orisakwe et al., 2004; Swaddiwudhipong et al., 2007). Soil is one of the important environmental and living media for plants, microorganisms, animals, and humans. In particular, soil is crucial in the field of agriculture. However, it also bears a large amount of pollutants, including toxic heavy metals (Bian et al., 2014; Clemente et al., 2006). It was found that soil near a metal-liferous mine had 100-1,000 times of heavy metals concentrations higher than the background or uncontaminated soil (Adriano, 1986). The contamination of soil with heavy metals not only leads to losses of agricultural areas but also issues concerning the presence of toxic metals in the food chain (Chaney et al., 2004; Simmons et al., 2005; Sriprachote et al., 2012; Wuana and Okieimen, 2011). Cadmium (Cd) accumulated in crops, especially rice, which consequently caused an outbreak of Itai-itai disease in Japan at the beginning of the twentieth century, is one of the most classic examples of metal toxicity on human health through the food chain.

Thailand is known as one of the world's leading producers of agricultural products, especially rice (Sriprachote et al., 2012; Swaddiwudhipong et al., 2007; Zarcinas et al., 2004). It was reported that 50% of agricultural areas which equal to 114,644 km^2 are rice fields (Sriprachote et al., 2012). However, there have been reports on serious Cd contamination of paddy soil and rice grown in the vicinity of a large zinc (Zn) mine located in Mae Sot, Tak (Simmons et al., 2005). In addition, several hundred local people were found with elevated Cd concentrations in their blood about 16.4 times higher than national average of Cd in blood (0.5 $\mu g\ g^{-1}$ creatinine), which was assumed to be related to their consumption of Cd contaminated rice grown in that area (Akkajit, 2015; Simmons et al., 2005; Sriprachote et al., 2012; Unhalekhaka and Kositanont, 2008). Therefore, remediation of Cd contaminated paddy soil is essential for the prevention of Cd contamination in both rice grain and environment (Chantana et al., 2007).

Various processes such as phytoremediation, ion exchange, cementation, and precipitation have been widely used to treat heavy metal contaminated soil. However, in the last decade, the utilization of waste such as, bark, lignin, seaweed chitin, zeolite, clay, fly ash, as well as bone to stabilize heavy metal in soil has been in the spotlight because of its cost effectiveness (Dermatas and Meng, 2003; Guo et al., 2006). Recently, studies of coffee by-products on heavy metal removal have shown its relatively applicable removal efficiency, especially in contaminated wastewater (Namane et al., 2005; Tokimoto et al., 2005; Utomo and Hunter, 2006). In addition, previous research has shown the possibility of direct coffee ground application to the cultivation of mushroom and lettuce (Cruz et al., 2012; Zhang and Sun, 2014). The increase in soil nutrient content, particularly in potassium was found after coffee grounds addition (Cruz et al., 2014; Kasongo et al., 2011; Liu and Price, 2011). However, there is still limited information on the potential of coffee by-products as soil amendments to stabilize available forms of heavy metals in contaminated soil.

1.2 OBJECTIVES

The overall objective of this study was to study the potential utilization of coffee grounds as Cd and Zn stabilizing agent. The main objectives were;

1. To investigate the feasibility of using untreated coffee grounds and carbonized coffee grounds as stabilizing amendments to immobilize exchangeable and plant available Cd and Zn in contaminated soil.
2. To determine the eight-week aging effect on the distribution of exchangeable and plant available forms of Cd and Zn in untreated coffee ground and coffee ground amended soil.
3. To determine the phytotoxicity of amended soil to rice (*Oryza sativa L.*) germination.

1.3 HYPOTHESES

1. Carbonized coffee grounds can immobilize higher concentrations of exchangeable and plant available Cd and Zn in contaminated soil than untreated coffee grounds.
2. Both untreated coffee ground and carbonized coffee ground amended soil with aging process can immobilize exchangeable and plant available Cd and Zn in soil.
3. The phytotoxicity of amended soil on rice germination can be decreased after coffee ground addition.

1.4 SCOPE OF THE STUDY

There are three main parts of the experiments conducted in this study (Figure 1.1) including;

First, both contaminated soil and coffee ground materials were air dried, homogenized and sieved through a 2 mm sieve. Coffee ground materials were then incubated at 105 °C for 24 hour and 500 °C for 2 hour to obtain untreated coffee ground (UCG) and carbonized coffee ground (CCG), respectively. Afterward, contaminated soil, UCG and CCG were characterized for their characteristics as shown in Figure 1.1.

Secondly, different ratio of UCG and CCG were separately mixed to the contaminated soil and incubated in a pot under room temperature for 8 weeks to study the aging effects of UCG and CCG amended soil on exchangeable and plant available Cd and Zn distribution. Throughout aging process, amended soils were collected and analyses for their chemical characteristics (Figure 1.1).

Finally, germination tests of both UCG and CCG amended soil were conducted to determine the toxicity of both amended soils on rice germination through the percentages of relative seed germination, relative root growth and germination index (Figure 1.1).

1.5 EXPECTED OUTCOMES

1. Untreated coffee grounds and carbonized coffee grounds can reduce exchangeable and plant available Cd and Zn in contaminated soil.
2. After eight-week pot experiment, concentrations of exchangeable and plant available Cd and Zn will be reduced.

3. Both untreated coffee grounds and carbonized coffee grounds can increase root elongation and rice germination.

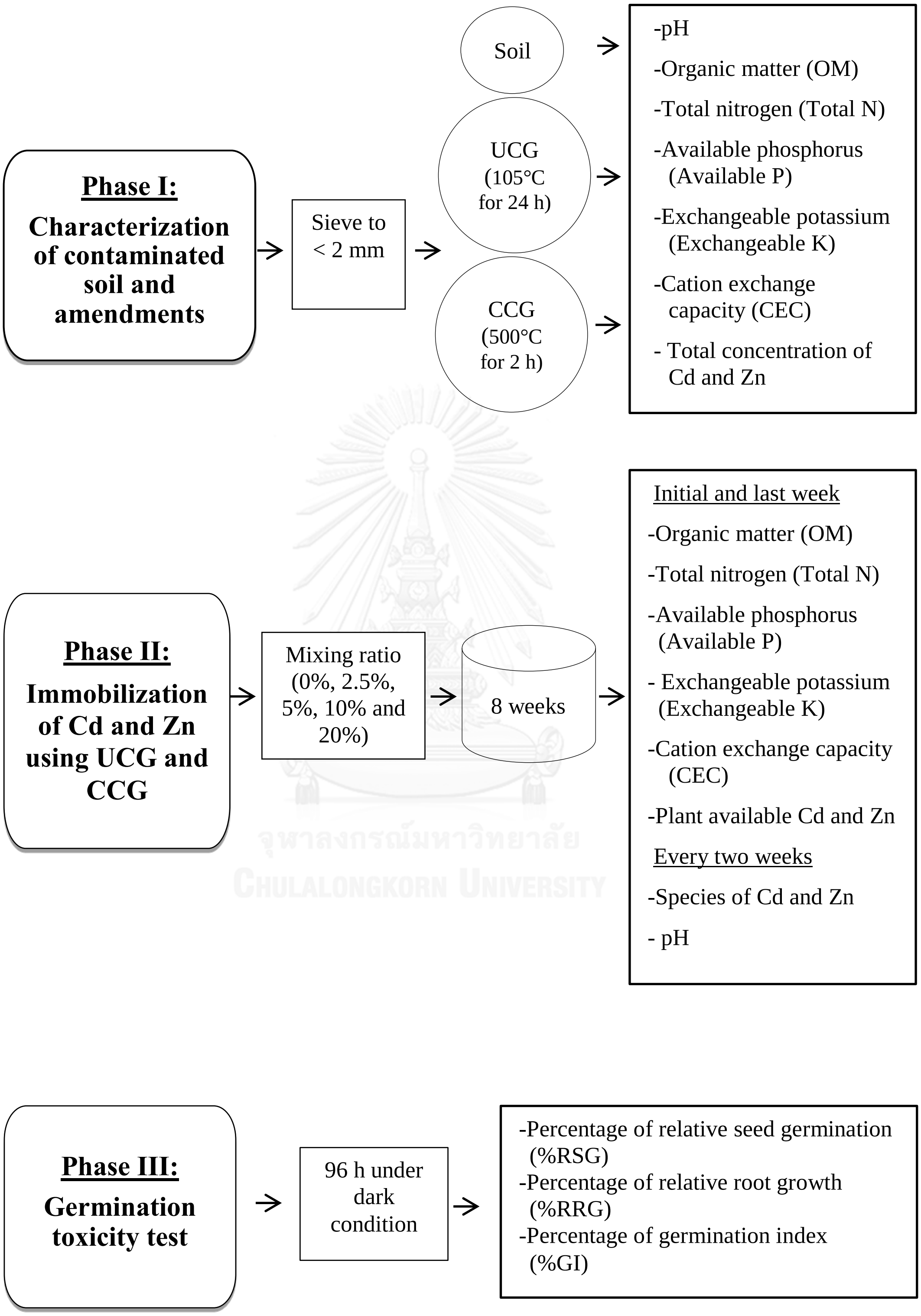

Figure 1.1 Schematic diagram of the study

CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 HEAVY METAL CONTAMINATION IN SOIL

Generally, Cd, Zn, lead (Pb), chromium (Cr), arsenic (As), copper (Cu), and mercury (Hg) are easily observed in contaminated soil (Wuana and Okieimen, 2011). Those heavy metals are persistent and very toxic. In general, heavy metals are available for the uptake of plants and living organisms (Chaney et al., 2004) while their bioavailability does not decrease in the long term. In general, heavy metals are usually available for the uptake of plants and living organisms (Chaney et al., 2004). Thus, long term contamination of heavy metal in the environment could cause the bioaccumulation and biomagnification in organisms along the food chain. Main causes of the dispersion of heavy metal in environment happening widely and seriously in the world are anthropogenic activities such as industrial, mining and agricultural activities as well as disposal of municipal sewage. The distribution and transport of heavy metals in the environment was depicted in Figure 2.1 showing that both anthropogenic and natural activities are the sources contributing heavy metals to atmosphere, lithosphere and hydrosphere (Mulligan et al., 2001). Soil as an example, usually contains miscellaneous and plentiful heavy metals such as Cd, Cr, Cu, Ni, Pb, Zn (Mulligan et al., 2001; Sutherland, 2010). There are many reports on adverse impacts of heavy metal contaminated soil in many countries (Chantana et al., 2007; Simmons et al., 2005; WHO, 2010; Wuana and Okieimen, 2011). Therefore, public concerns have been raised in order to protect the potential risks of heavy metal soil pollution to the environment especially human.

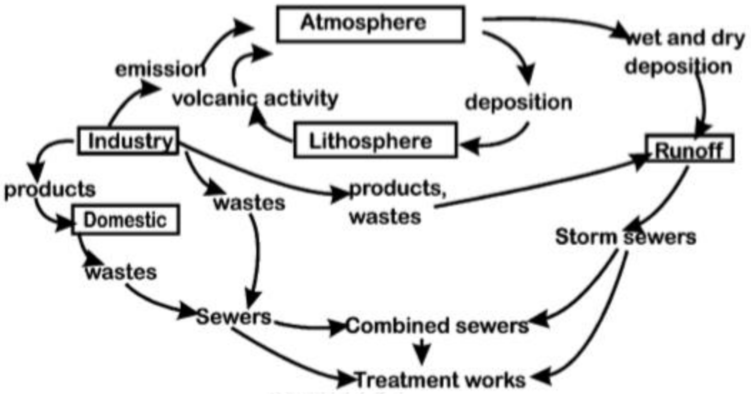

Figure 2.1 Dispersion of heavy metal in the environment

(Source: Mulligan et al., 2001)

2.1.1 Background concentration of Cd and Zn in soil

Table 2.1 summarizes concentrations of Cd and Zn in world natural soil as well as USA and Thailand. As can be seen in the table, wide variation in both Cd and Zn concentrations were found. Background concentrations of any elements in soil are closely related to geology in each continent and country (Wuana and Okieimen, 2011). In addition, factors that affects to the distribution of elements are including groundwater chemistry, speciation and mechanisms of elements (Buekers, 2007; Wuana and Okieimen, 2011).

Table 2.1 Concentrations of Cd and Zn in natural soils (mg kg^{-1})

	World[1]	USA[2]	Thailand[3]
Cd	0.01-2.0	0.50-1.7	0.01-1.3
Zn	10-300	88-236	0.1-140

Note: [1] Adriano (1986); [2] Bradford et al. (1996); [3] Zarcinas et al. (2004)

2.1.2 Concentrations of Cd and Zn in contaminated soil

Figure 2.2 shows the incidences of Cd and Zn contamination in soil caused by smelting and ores processing around the globe. Concentrations of Cd and Zn in the contaminated soil were higher than their background concentrations (Table 2.1) up to 425 and 122 times, respectively (Chlopecka et al., 1994; Chopin and Alloway, 2007; Hanl et al., 2009; Hooda, 2010; Horckmans et al., 2007; Limei et al., 2008; Sivry et al., 2010; Spence et al., 2014; Taylor et al., 2010; U.S. EPA, 1992). These incidences of Cd and Zn have caused adverse effects to the surrounding environment and many organisms. Sometimes the contamination eventually causes death (Duruibe et al., 2007; Jarup, 2003; Simmons et al., 2005; Sriprachote et al., 2012).

Figure 2.2 Worldwide Cd and Zn contamination in soil

In the case of Thailand, Cd and Zn contamination in soil especially in Mae Tao, Tak, has been reported since 1947 (Thamjedsada, 2011). Total Cd concentration in contaminated paddy soils were very high (up to 72 times greater than the EU soil standard of 3 mg kg^{-1} for agricultural soil (Unhalekhaka and Kositanont, 2008) or 5.8 times higher than the Thailand soil quality standard for habitat and agriculture of 37 mg kg^{-1} (PCD, 2004). The mobile and bioavailable fraction of Cd and Zn in contaminated soils were 61% and 25%, respectively (Sriprachote et al., 2012). Sriprachote et al. (2012) found that total concentrations of Cd and Zn in agricultural soil collected from Pha Te village, Mae Tao were about 0.63 to 30.4 mg kg^{-1} and 14.40 to 594 mg kg^{-1}, respectively. In addition, Cd contents in sediment and water nearby Mae Sot district were also exceeded the European maximum permissible level for agricultural soil (Phaenark et al., 2009; Weeraprapan et al., 2015). Accordingly, all literatures indicated that exposure to Cd contamination in soil can be considered as a source of public health's Cd exposure especially through the ingestion of contaminated food and soil. There are many reports about the close relationship of negative health impacts and Cd exposure on a daily basis ingestion (Dudka et al., 1994; Kosolsaksakul et al., 2014; Lu et al., 2015; Simmons et al., 2005; Sriprachote et al., 2012; Zarcinas et al., 2004).

2.2 IMPACTS OF HEAVY METAL CONTAMINATED SOIL

Once contaminated to the soil, heavy metal usually accumulate and cause negative toxicological effects to living organisms in surrounding environment (Beesley et al., 2010; Houben et al., 2013; Hu et al., 2014; Simmons et al., 2005). Many of the serious health concerns in millions of European and Asian have been observed. As and Cd can cause cancer. Pb and As can cause neurological damage and lower IQ. Hg, Pb, and Cd can cause kidney disease. Pb and Cd can cause skeletal and bone diseases (Chantana et al., 2007; Jarup, 2003; Swaddiwudhipong et al., 2007).

The agency for Toxic Substances Management Committee has listed Cd as the sixth most toxic substance that damages human health (Su et al., 2014). In addition, the U.S. Department of Health and Human Services found that cadmium and certain cadmium compounds are probable or suspected carcinogens (U.S. EPA, 1999). Cd is not only causing negative impacts to human but also cause chlorosis, necrosis, wilting, red-orange coloration of leaves, reduction in growth plant (Adriano, 1986). Toxicity of Cd related to uptake Cd in the rhizophere in plant root zone. There are several reports on elevated Cd concentrations in vegetables and animals growing in the Cd contaminated areas. For example, a survey in Japan in 2004 revealed the elevated Cd concentrations in rice, vegetables, seaweeds and several cereal grains on an average of 10% to 44% (Clemens et al., 2013). Heavy metal accumulation in food chains is specifically considered important with respect to Cd (Sandstead and Au, 2007; Vries et al., 2007). The development of high blood pressure, iron-poor blood, liver disease, nerve damage and brain damage were found in the animals given Cd contaminated food and water (U.S. EPA, 1999).

Adverse effect of Cd exposure through food chain became an issue after the occurrence of the Itai-itai syndrome in Japan (Adriano, 1986; Chantana et al., 2007). Dietary intake with high levels of Cd can severely irritate or bother stomach and cause vomiting and diarrhea. Long term Cd consumption could cause kidney damage, lung damage, liver damage, and osteoporosis (Chantana et al., 2007; Sriprachote et al., 2012; Swaddiwudhipong et al., 2007).

In the case of Thailand, contamination of Cd in both soil and foods has been found in a Zn potential area in Mae Tao, Tak. Simmons et al. (2005) found that more than 95% of rice samples were contaminated with about 300 times of Cd exceeding the Codex standard regulation. Many research confirmed the relationship between Cd intake and negative health impacts on local people (Simmons et al., 2005; Swaddiwudhipong et al., 2007; Unhalekhaka and Kositanont, 2008).

In contrast to Cd, Zn is an essential element for the normal growth and reproduction of plants, animals and humans (Adriano, 1986; Alloway, 2008; Phaenark et al., 2009). However, only low concentrations are necessary, on the other hand, high Zn concentrations could cause yield reduction, Fe chlorosis-alike symptoms, as well as root elongation inhibition (Adriano, 1986). The adverse effects of excess dietary Zn in animal were lethargy, nausea, vomiting, diarrhea and other signs of gastrointestinal distress. Potential Zn toxicity in various organ of human are well documented (Sandstead and Au, 2007; Vries et al., 2007). For example long term Zn exposure can lead to the disturbance of copper (Cu) metabolism, reduction in iron function and immune system, microcytosis of the red blood cell, neutropenia as well as liver and heart damage.

2.3 SPECIATION AND FRACTIONATION OF HEAVY METAL IN SOIL

Total concentration of heavy metal can be used to estimate only the degree of soil contamination; however, it may not an appropriate choice of the environment impact assessment for soil pollution (Fernandez et al., 2004; Margui et al., 2004; Sutherland, 2010). Cd is generally more mobile and more soluble than other metals (Tapia et al., 2010). The determination of specific chemical species using chemical reagents can be used to assess the mobility and partitioning of elements in soils. Different chemical species can be obtained depending on the reagents and extraction methods used. In general, either single or sequential extraction methods are usually applied to the soil study (Hooda, 2010).

2.3.1. Single extraction

Theoretically, single extraction was developed as the extraction method for cationic trace metals (Hooda, 2010) and their exchangeable concentration in soil (Alvarenga et al., 2009). Each extraction condition usually applies different extractant, liquid: solid ratio, and as well as extraction time. Table 2.2 shows several single chemical extraction methods which were previously applied to the soil study. As can be seen from Table 2.2, different extractants are used to extract different species of metals of interests. These methods are still widely used since there is no risk for sample losses as compared to the sequential extraction. In addition, results can be obtained faster than the results of sequential extraction.

Table 2.2 Single chemical extraction used for soil study

Type of chemical	Chemical extractant	Binding form of metal
Strong chelating solution	Ethylenediamine tetraacetic acid (EDTA), Diethylenetriamine pentaacetic acid (DTPA)	Available
Unbuffered neutral salt solutions	$CaCl_2$, $MgCl_2$, NH_4NO_3, $NaNO_3$, KNO_3	Available and mobile
Organic (weak) acids or combined acid salt solutions	CH_3COONH_4, CH_3COONa	Available, exchangeable, carbonate
Diluted mineral acid solutions	HCl, HNO_3, HCl + HNO_3 or HCl + H_2SO_4	Residual and silicate

The single extraction method using DTPA as an extractant is, of particular, method of interest in this study. This method was applied to determine heavy metal plant availability in many research and furthermore recommended by Department of Agriculture (DOA) (DOA, 2010).

2.3.2 Sequential extraction

Sequential extraction is a crucial and extensive analytical method which can provide the information on heavy metals dynamic and mobility (Pueyo et al., 2008; Sutherland, 2010; Tessier et al., 1979). It is usually used to assess potential environmental risks and hazards (Fernandez et al., 2004; Zimmerman and Weindorf, 2010). Several methods such as Tessier's method (Tessier et al., 1979), BCR method (Sutherland, 2010), short extraction procedure (Maiz et al., 2000), Geological Society of Canada (GCS) procedure (Zimmerman and Weindorf, 2010), and Gal´an Procedure

(Zimmerman and Weindorf, 2010) were introduced as the appropriate sequential extraction methods. However, some methods such as Gal´an Procedure and GCS method are not applicable to the solid samples (Gleyzes et al., 2002; Margui et al., 2004; Quevauviller, 2002; Sahuquillo, 2003; Sutherland, 2010). Thus, BCR sequential extraction, one of the most popular sequential extraction methods, have been recommended for the study of heavy metals fractionation in soils.

BCR method is a modified Tessier's sequential extraction proposed by the Standards, Measurement, and Testing Programme (SMT). BCR sequential extraction has been applied to a wide type of solids media including crop soils, forest soils, roadside soils, smelter-contaminated soils, marine sediments, lake sediments, river mining-impacted sediments, urban river sediments, road-deposited sediments, atmospheric particulate matter, ferromagnesian clay minerals, mining waste, and sewage sludge (Alvarenga et al., 2009; Fernandez et al., 2004; Pueyo et al., 2008; Sutherland, 2010; Zimmerman and Weindorf, 2010). This sequential extraction appears to be more operationally effective, faster and easier than Tessier's method because it combines the exchangeable and carbonate fractions into one blinding form (Fernandez et al., 2004). In addition, Alvarenga et al. (2009) found the correlation between metal concentrations in plant with their concentrations obtained from the first BCR extraction step. Chemical fractions obtained after each BCR sequential extraction step are including;

Exchangeable fraction (S1): The exchangeable fraction is readily released under acidic condition. Metal species included in this fraction are weakly adsorbed, easily exchangeable, and bond to carbonate species. Re-mobilization of metals can occur in this fraction due to the low pH environment and adsorption and desorption reactions. This fraction is believed to be the most available form of metal for plant uptake (Gleyzes et al., 2002; Ngiam and Lim, 2001).

Reducible fraction (S2): The reducible fraction usually indicates potential and ability of metals in the forms of Fe and Mn oxyhydroxides which could be released under anoxic and reducing condition. Re-mobilization can occur under redox conditions (Ngiam and Lim, 2001).

Oxidizable fraction (S3): The oxidizable fraction is considered to be less bioavailable due to the fact that it is generally associated with stable high molecular weight humic substances that release small amount of metals in a slow manner. This fraction can probably form organometallic complexes (Fernandez et al., 2004). Oxidation of this fraction under oxidizing conditions in natural water can cause remobilization.

Table 2.3 summarized three fractions of metal species which can be obtained from the BCR sequential extraction. Mobility and bioavailability of metals usually decreases as fractional sequence increases. Thus, the exchangeable and weak acid soluble fractions are the most bioavailable fractions. They are also very toxic to plant and can be accumulated along the food chain. Meanwhile, reducible and oxidizable fractions are relatively stable under normal soil condition (Gleyzes et al., 2002; Peng et al., 2009; Sutherland, 2010).

Table 2.3 Summary of releasing conditions, forms, mobility, and bioavailability of metal species obtained from BCR sequential extraction

Fraction	Releasing condition	Binding form	Mobility and bioavailability
Exchangeable and weak acid soluble	Low pH, changes in the ionic composition of water	Carbonate	Higher
Reducible	Anoxic	Iron and manganese oxides	High
Oxidizable	Oxidation	Natural organic substances, sulfide	Moderate

2.4 FACTORS AFFECTING HEAVY METAL AVAILABILITY AND MOBILITY IN SOIL

2.4.1 Acidity and basicity

Acidity and basicity (pH) is a master driving factor on heavy metal availability and mobility in soil (Meers et al., 2007). It usually affects the coprecipitation and the formation of surface complexes of soil layer. In addition, pH can also influence the precipitation-dissolution reactions, the redox reactions, the mobility and leaching processes, the colloidal dispersion as well as the bioavailability of metal ions (Adriano, 1986; John and Leventhal, 1995). In general, the solubility and availability of metallic species such as Cd^{2+} and Zn^{2+} decreases to a greater extent as the pH of the soil increases. For example, Adriano (1986) found the remarkable decreases in mobility and plant toxicity of both Cd and Zn at pH 6.6 and 6.5, respectively.

2.4.2 Cation exchange capacity

In principal, cation exchange capacity (CEC) is a factor used to determine the total capacity of a soil to hold any exchangeable cations. Briefly, the negative soil particles generally attract the positive metal cations (NMSP, 2007). This means that the higher the CEC values, the greater amounts of heavy metal cations absorbed onto the soil. As a consequence, the potential hazards of toxic cations are reduced (Adriano, 1986; Lucchini et al., 2014a; Mcbride et al., 1997). It was confirmed by the result reported by Kashem et al. (2011) who found a strong negative correlation between CEC and the concentrations of plant available toxic metals. This might be caused by the surface complexation, ion exchange, and surface precipitation between heavy metals and soil particles (Bradl, 2004). Furthermore, soil with high CEC values could also strongly resist the changes in soil pH due to its large buffering capacity. However, it should be noted that the CEC of soil is largely depends on the amount and type of clay, organic matter (OM), and Fe-, Al-, and Mn-oxides (Adriano, 1986; Mcbride et al., 1997).

2.4.3 Aging

Normally, an increase in aging time resulted in a reduction of mobile and bioavailable heavy metal concentration. This is due to the increase in sorption of metal cations onto the soil particles through various mechanisms such as the diffusion into micropores, surface nucleation and precipitation (Houben et al., 2013). Therefore, it can be concluded that aging of contaminated soils could, in principle, decrease the bioavailability of the pollutants (Bolan et al., 2014).

2.4.4 Organic matter

The high content of organic matter (OM) can also reduce heavy metal mobility. As OM is consisting of a highly complex mixture of humic and fulvic acid, the stabilization of heavy metal and humic complexes can be formed and finally, decrease in its mobility. Adriano (1986) and Hooda (2010) found that the carboxyl and phenolic groups which can disengage their H^+ ions could reduce Cd and Zn mobility especially through ion exchange, complex formation, adsorption, and chelation reactions. Three main possible mechanisms of heavy metal ions binding to humic acid are including 1) chelation between carboxyl and phenolic hydroxyl groups, 2) chelation between two carboxyl groups, and 3) complexation with carboxyl group (Baker and Khalili, 2005). The affinity of metal cations complexed with OM generally decease in the following orders; $Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$ (Adriano, 1986; Cooperband, 2002; Hooda, 2010).

Thus, the more OM concentrations, the greater ability of soil to bind cations of heavy metal (Fellet et al., 2011; Tapia et al., 2010). In addition, high OM also leads to an increase in the CEC content in soil. Therefore, soil with high OM and CEC values might be able to bind more cations in heavy metal contaminated soil.

2.4.5 Other factors

Soil containing high oxides of Fe, Mn and Al can also be considered to play an important role in heavy metal binding especially by those mineral oxide phases. Generally, hydrous oxides of Fe, Mn and Al oxides could contribute to the formation of the coating on crystalline particles in the soil solid phase (Adriano, 1986; Fijalkowski et al., 2012). Once the binding and formation are formed, the lower in metal availability and mobility is then expected.

2.5 REMEDIATION TECHNIQUES OF HEAVY METAL CONTAMINATED SOIL

There are various methods, presently, to remediate heavy metal contaminated soil. In general, soil remediation techniques can be classified into three types including physical, chemical, and biological remediation (U.S. EPA, 1997). However, no specific method can be recommended as the most appropriate remediation technique for heavy metal contaminated soil. Table 2.4 compares three types of remediation techniques with special concerns on the suitability for land treatment, cost effectiveness, and remediation time required (Alloway, 2008; Dube et al., 2001; Hu et al., 2014; Limei et al., 2008; Madejon et al., 2009; Orisakwe et al., 2004). Though, soil replacement, one example of physical remediation, is a rapid cleanup method, there are several constraints such as, high remediation cost (250-500$) and environmental problems caused by the spreading of contaminated soil and dust particle during the removal of contaminated soil. For phytoremediation, this technique is cost effective (15-40$) but it usually takes longer remediation time (18-60 months) than other method (Guo et al., 2006; Lambert et al., 2003).

In contrast to amendment utilization, one of the chemical remediation techniques, is recommended as the appropriate remediation technique for heavy metal contaminated soil. This technique is the cost effective (90-200$), environmental friendly, and timely effective (6-9 months) remediation method (Bian et al., 2014; Biederman and Harpole, 2013; Clemente et al., 2015; Fellet et al., 2011; Guo et al., 2006; Lambert et al., 2003; Madejon et al., 2009; U.S. EPA, 2007). It is believed that this method can sustainably remediate the heavy metal contaminated sites. This is because, in the last decades, some researchers have been using organic wastes such as bark, lignin, chitin, seaweed, zeolite, clay, fly ash, sewage sludge, composted agricultural byproducts and bone etc. as heavy metal stabilizing amendments (Beesley et al., 2010; Dermatas and Meng, 2003; Guo et al., 2006). Moreover, results reported by Alvarenga et al. (2009) also shown that those organic wastes can also improve soil properties and characteristics such as water holding capacity, pH buffering capacity, and levels of OM and nutrients.

Table 2.4 Comparison of different remediation techniques

Criteria / Remediation technique	Area size	Cost effectiveness	Time required
Physical (Soil replacement, soil spading)	1	1	3
Chemical (Chemical leaching, chemical fixation, electrokinetic remediation)	3	2	2
Biological remediation (Phytoremediation, bioaugmentation, biosparging)	2	3	1

Note: 3= very good, 2= good, 1= poor

2.6 REMEDIATION OF HEAVY METAL CONTAMINATED SOIL USING AMENDMENT

2.6.1 Typical characteristics of coffee ground

Untreated coffee ground mainly contains organic compounds (96%). Other small compositions (1.96% to 4.6%) are mineral compounds including sodium, magnesium, calcium, and sulfur. Functional groups in coffee ground such as carboxylic, lactonic, and phenolic have shown their ability to retain heavy metals. In addition, coffee ground components such as pectin, cellulose and lignin can also adsorb Cd (II) and Zn (II) ions by 65 to 85% and 48 to 79%, respectively (Azouaou et al., 2010; Oliveira et al., 2008; Utomo and Hunter, 2006). Furthermore, Yen and Lin (2015) and Utomo and Hunter (2006) have found that good adsorption capacity of spent coffee ground over a fairly wide pH range. While, Azouaou et al. (2010) reported the optimal condition of Cd^{2+} adsorption on spent coffee ground at adsorbent dosage of 9 g with pH 7 and temperature at 20 °C. However, different adsorption capacities of Cd (II) and Zn (II) can be found in the coffee ground prepared using various preparation processes as summarized in Table 2.5. It is obviously found that different preparation methods could also resulted in different maximum adsorption capacities (q_m).

Figure 2.3 shows the topography of the coffee ground surface determined using the scanning electron microscopy (SEM) (Azouaou et al., 2010; Farah, 2012; Plaza et al., 2012). The transformation of coffee ground characteristics can be achieved by using a carbonization process with different temperatures (Plaza et al., 2012). The specific surface area and pore of carbonized coffee ground were found to be increased with the increasing carbonization temperature. This is due to the volatilization of organic compound containing in the coffee ground during the carbonization process (Plaza et al., 2012).

Table 2.5 Adsorption capacity of coffee ground for aqueous solution Cd and Zn removal

Preparation	Adsorbate	q_m (mg g^{-1})	References
Leached by NaOH solution	Cd(II)	3.2	Utomo and Hunter (2006)
	Zn(II)	6.1	
Heated	Cd(II)	5.46	Yen and Lin (2015)
Dried at the ambient air and sieved	Cd(II)	15.65	Azouaou et al. (2010)

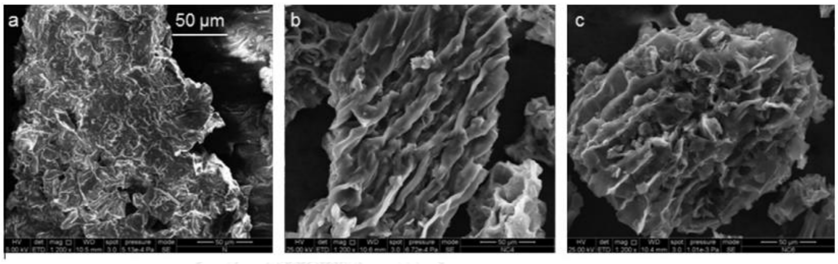

Figure 2.3 SEM pictures of (a) dried spent coffee grounds, (b) char carbonized at 400 °C, and (c) char carbonized at 600 °C

(Source: Plaza et al., 2012)

2.6.2 Typical characteristics of biochar

It was reported that the carbonization process could increase pH, CEC, and surface area of biochar while the carbon recovery is decreased (Lehmann, 2007). In addition, Lehmann (2007) and Plaza et al. (2012) found the increases in pore size and specific surface area of carbonized coffee ground with the increasing carbonization temperature. Figure 2.4 shows that 500 °C is an optimum temperature for biochar production. Jiang and Xu (2013) also reported the increase in heavy metal fixation with the increase in acidic groups (carboxyl, lactones, lactols) and soil negative charge. The functional group of biochar for example, COOH and OH can increase the specific adsorption of metals in soil amendment (Beesley et al., 2010; Cui et al., 2011; Jiang and Xu, 2013).

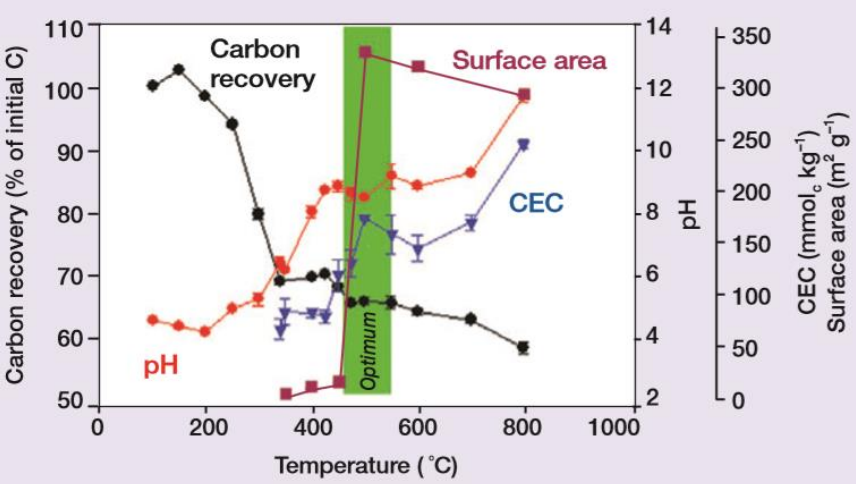

Figure 2.4 Properties of biochar regarding different production temperature

(Source: Lehmann, 2007)

2.6.3 Heavy metal immobilization by amendment

Various types of amendments for example, municipal and domestic wastes such as bark, straw, hull, coir, poultry manure, sewage sludge, and compost have shown their high potential to immobilize heavy metal in soil (Demirbas, 2008; Guo et al., 2006). Among those amendments, green wastes such as organic wastes from orchard, poultry farm, industrial and agricultural operations as well as household organic wastes are known as good heavy metal immobilizing amendments (Bailey et al., 2014; Bian et al., 2014).

These green wastes have received increasing attentions as promising materials to stabilize soil contaminants. Table 2.6 summarizes results of organic amendments used to remediate Cd and Zn contaminated soil. Based on Table 2.6, heavy metal immobilization depends not only on experimental condition and extraction method of metals but it is also depending on factors such as pH, OM, CEC, nutrient contents, metal concentrations, particle size of soil and amendment, etc.

Table 2.6 Immobilization of Cd and Zn in soil using organic waste and biochar

Amendment	Mixing Ratio	Scale	Extraction of metal	Aging Duration	Results	Reference
Pine bark (PB), spent mushroom (SM), and pruning waste + biosolids (BS)	-	Lab	DTPA-$CaCl_2$	4 weeks	BS decreased Cd by 38.15%	Tapia et al. (2010)
Sugarcane filter cake, vermin-compost, palm kernel pie, lime, phosphate rock	0.89 to 10.86 g kg^{-1}	Lab	$Ca(NO_3)_2$	16 hours	Maximum specific Cd sorption capacity ranged from 0.89 to 10.86 g kg^{-1}	Zenteno et al. (2013)
Wheat straw (350 to 550 ^oC)	0, 10, 20, 40 t ha^{-1}	Field	$CaCl_2$	2 years	In two-year experiment, Cd content was reduced by about 52.5%	Cui et al. (2011)
Wheat straw biochar (350 to 550 ^oC)	0, 10, 20, 40 t ha^{-1}	Field	DTPA	3 years	In three-year experiment, Cd content was reduced by about 24%	Bian et al. (2014)
Sewage sludge (SS), unsorted municipal solid waste (MSWC) and garden waste compost (GWC)	25, 50 and 100 Mg ha^{-1}	Lab	BCR sequential extraction	28 days	MSWC reduced Zn by 37.2 %	Azouaou et al. (2010)

Table 2.6 Immobilization of Cd and Zn in soil using organic waste and biochar *(cont.)*

Amendment	Mixing Ratio	Scale	Extraction of metal	Aging Duration	Results	Reference
Biosolid compost (BC), sugar beet lime (SL), and combination of leonardite plus sugar beet lime (LESL)	30 Mg ha^{-1} of BC, 25 Mg ha^{-1} of LE mixed with 10 Mg ha^{-1} of SL and 30 Mg ha^{-1} of SL	Field	$CaCl_2$	4 years	At 0-15 cm soil layer, SL reduced Cd and Zn by 73% and 88%, respectively	Madejon et al. (2009)
Coal fly ash (CFA) (5%), straw (ST) (2%) and CFA +ST	ST (2 % w/w), CFA + ST (5+2) (% w/w)	Lab	$CaCl_2$	6 weeks	CFA + ST effectively reduced the mobility and availability of Cd and Zn by 67.1% and 73.3%, respectively	Hu et al. (2014)
Boiler ash, filter cake, and vinasse	3 % (w/w)	Lab	BCR sequential extraction	84 days	Filter cake (30:0:0) reduced Cd and Zn by 4.7% and 39.9 %, respectively	Akkajit et al. (2013)
Prune residues (500 °C)	0%, 1%, 5% and 10%	Lab	DTPA-extractable	15 days	Biochar decreased about 10 times of Cd bioavailability, but no significant differences in Zn bioavailability between before and after aging	Fellet et al. (2011)

Table 2.6 Immobilization of Cd and Zn in soil using organic waste and biochar *(cont.)*

Amendment	Mixing Ratio	Scale	Extraction of metal	Aging Duration	Results	Reference
Compost (C), hardwood biochar (B)	Soil (S): 600 (mL) S + C: 400+200 S + B: 400+200 S + C + B: 200 + 200 +200	Lab	Water-extractable trace metal	60 days	Significantly reduction in Cd and Zn concentration in the pore water after BC application	Beesley et al. (2010)
Wood (450 °C)	25 + 25 and 50 + 50 t ha^{-1}	Field	Sequential extractions	4 years	The repeated biochar treatments increased residual Cd and Zn by an average of 22% and 59%, respectively	Lucchini et al. (2014a)
Straw (600 °C)	1%, 5%, and 10%	Lab	$CaCl_2$	56 days	About 5% to 35% of mobile Cd was reduced in 5% and 10% of biochar. About 5% to 48% of mobile Zn was reduced in 5% and 10% of biochar.	Houben et al. (2013)

2.6.4 Mechanisms of heavy metals remediation by amendment

Amendment could stabilizes heavy metal in soil by precipitation, adsorption and complexation processes. These stabilization and immobilization reactions may retard heavy metal mobility or in other words, reduce soluble, very active and bioavailable metal fractions which are presented in the soil (Alvarenga et al., 2009; Hu et al., 2014). The mechanisms of heavy metal immobilization with amendment might be attributed to electrostatic interactions (interaction between electrically charged in amendment and mobile fraction of heavy metal), precipitation (mobile fraction of heavy metal that fix and setting down in amendment) and some other reactions as shown in Figure 2.5. Buekers (2007) have classified interactions of heavy metal immobilization by amendment can be classified into two types including fast reactions (within minutes or hours) and slow reactions (within days or years). In relation to immobilization, the remarkably increased in soil pH, CEC and OM in amendment may lead to the decrease in heavy metal mobilization (Pardo et al., 2011; Sherene, 2010).

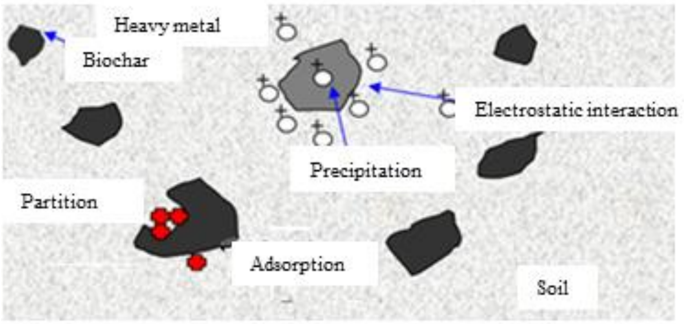

Figure 2.5 Mechanisms of heavy metal remediation

(Source: Tang et al., 2013)

2.7 SEED GERMINATION TEST

Seed germination test is one of the most common techniques used to determine the maximum germination potential and root growth. It is a simple and very sensitive test method for heavy metal toxicity (Miaomiao et al., 2009; Pardo et al., 2011; Prechthai et al., 2008). Furthermore, it is fast and easy to conduct the experiment.

There are three equations included in the seed germination test. They are; 1) relative seed germination (%RSG), 2) relative root growth (%RRG), and 3) germination index (%GI). The GI has been used and proven to be a very sensitive parameter, since it combines germination and root growth (Teaca and Bodirlau, 2008). RSG (%) can be computed as number of seeds germinated in the extract divided by number of seeds germinated in the control multiply by 100. RRG (%) can be calculated as mean root length in the extract divided by mean root length in control multiply by 100. While, GI (%) is RSG multiply by RRG and then, divided by 100.

These three percentages could finally indicate phytotoxicity. Hui and Hang (2009) summarized that 50% to 85% of either one of these three indexes could indicate low germination. The low percentages strongly confirmed the effects of heavy metal and other chemical on plant. In contrast, the percentages higher than 100 would indicate beneficial effects on plant, especially on the root elongation (Miaomiao et al., 2009).

Plants can develop complex mechanisms to control the access of heavy metals to their tissue. However, heavy metal toxicity primarily depends on plant species and the concentration of specific heavy metals (Jamal et al., 2006; Pardo et al., 2011).

CHAPTER III

RESEARCH METHODOLOGY

3.1 LABORATORY

All experiments except CCG preparation and germination test were conducted in the laboratory of the Environmental Research Institute, Chulalongkorn University (ERIC). Whereas, CCG preparation and germination test were conducted in the laboratory of the Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University.

3.2 MATERIALS AND METHODS

3.2.1. Sample collection

Soil

Surface agricultural soil (0-20 cm) contaminated with Cd and Zn used in the study was collected from Mae Tao, Mae Sot District, Tak Province, Thailand (N 16.673861, E 98.626007).

Coffee ground

Spent coffee ground materials were collected from the Black Canyon coffee shop located in the Siam Square area in Bangkok.

3.2.2 Sample preparation

Soil

Soil sample was air-dried, homogenized, sieved through a 2-mm sieve and kept in clean plastic boxes prior to further characterization and experiments.

Coffee ground

Spent coffee ground materials were prepared regarding to two different treatment conditions. First, the untreated coffee ground (UCG) was dried in an oven at 105 °C for 24 h and sieved through a 2-mm sieve. Second, coffee ground was carbonized (CCG) in a muffin furnace to remove cellulose and color at 500 °C. The final temperature of 500 °C was chosen in this study according to the higher percentage of remained fixed carbon was found at 500 °C (51.4%) than 300 °C (37.7%) (ASTM, 1993). After treatments, both UCG and CCG were kept in clean plastic boxes for further study.

3.2.3 Chemical solution

Analytical grade of all chemicals were used in this present study. All chemical solution was prepared with 18 MΩ deionized water. Laboratory glasswares and plasticwares were firstly cleaned with DI water and then, soaked with 10% nitric acid for 24 h prior to use.

BCR solution

- Exchangeable extraction solution was prepared from 17.43 mL of acetic acid and adjusted to 1,000 mL with DI water.

- Reducible extraction solution was prepared from 3.62 g of hydroxylamine hydrochloride in 400 mL of DI water. This solution was transferred to a volumetric flask, and adjusted to 1,000 mL with DI water. This solution was freshly prepared prior to use.

- Oxidizable extraction solution was prepared from hydrogen peroxide and ammonium acetate. Hydrogen peroxide was used as supplied by the manufacture. Ammonium acetate was prepared from 77.08 g of ammonium acetate in DI water.

Then, this solution was adjusted the pH to 2.0±0.1 with 2 N HNO_3. Finally, solution was made up to 1,000 mL with DI water.

DTPA solution

The DTPA solution was prepared from 1.975 g of diethylenetriamine pentaacetic acid (0.005M $C_{14}H_{23}N_3O_{10}$), 14.92 g of triethanolamine (0.01M $HOCH_2CH_2)_3N$ and 1.47 g of calcium chloride (0.001M $CaCl_2$) in 250 mL of DI water. Then, DTPA solution was adjusted pH to 7.3 with 2 N HCl, stirred and adjusted to 1,000 mL with DI water. This solution was kept in dark and cool condition.

3.3 POT EXPERIMENT

The experiment was conducted in a clean plastic box. UCG and CCG were separately mixed with soil sample at 0%, 2.5%, 5%, 10%, and 20 % (w/w) mixing ratio. Weight of all pot experiments were approximately 1.5 kg. These amended soil samples were incubated under room temperature for 8 weeks. Moisture content of all pots was maintained at about 25%. All experiments conducted in this study were replicated for 5 times in order to reduce the experimental and environmental errors.

3.4 SAMPLE ANALYSES

Soil, UCG and CCG were analyzed for physical and chemical characteristics as summarized in Table 3.1. All analyses were conducted in triplicates to assure the accuracy of the result. Concentrations of Cd and Zn in soil, UCG and CCG were determined by flame atomic absorption spectrometry (FAAS). Instrumental detection limits were 0.01 ppm for Cd and 0.1 ppm for Zn. Cd and Zn were analyzed at the wavelengths of 228.8 and 213.9 nm for Cd and Zn, respectively.

Table 3.1 Analytical methods for physical and chemical characteristics of soil, UCG and CCG

Parameter	Method of analysis	Reference
Soil type	Soil suspension	DOA, 2010
pH	1:2 (soil: water)	DOA, 2010
	1:10 (amendment: water)	Fang and Wong, 1999
OM	Walkley-Black method	DOA, 2010
CEC	Leaching with 1M NH_4-acetate	DOA, 2010
Total Cd and Zn	Aqua regia digestion	DOA, 2010
Total nitrogen (Total N)	Estimation from OM content multiply by 0.05	DOA, 2010
Available phosphorus (Available P)	Bray II	DOA, 2010
Exchangeable potassium (Exchangeable K)	Ammonium acetate 1N, pH 7	DOA, 2010

In the case of pot experiments, amended soil and control soil samples were analyzed for their pH and metal speciation for every two weeks. However, OM, CEC, total N, available P, exchangeable K and plant available concentrations of Cd and Zn were analyzed only at the initial and after aging experiments.

BCR sequential extraction recommended by Akkajit et al. (2013) as shown in Figure 3.1 was conducted in order to determine Cd and Zn speciation. After each extraction step, the extracted solution was separated by centrifugation at 3000 g for 20 min. Sample solutions were poured into clean polyethylene containers. Then, residue was washed with 20 mL of DI water, shaken for 30 min and centrifuged for 20 min at 3000 g. The supernatant was then decanted and completely removed without any loss of residue. It should be noted that the residual metal fraction is not a specie of concerned for BCR sequential extraction. Thus, this residual metal fraction extraction was not further conducted after the oxidizable fraction was completely extracted.

The plant available Cd and Zn in amended and control soils were extracted by DTPA solution following the method recommended by Lindsay and Norvell (1978). Briefly, 10 g of soil were transferred to an Erlenmeyer flask. Then, 20 mL of DTPA solution was added. The mixture was shaken in an end-over-end shaker at 120 rpm for 2 h. After that, samples were filtered through a GF/C filter paper using a Buchner funnel and a vacuum pump. Finally, the samples were analyzed for Cd and Zn concentrations by means of FAAS.

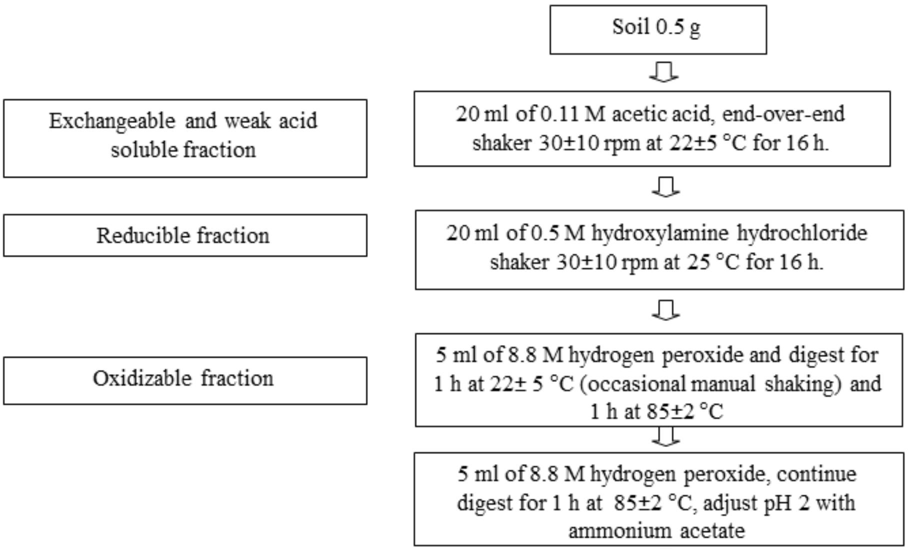

Figure 3.1 Schematic diagram of BCR sequential extraction

3.5 GERMINATION TEST

The toxicity of amended soil was evaluated through the germination of Khao Dok Mali 105 (KDML 105) rice grain (*Oryza sativa L.*). The tests were only conducted with the amended soil the minimum (2.5%) and maximum (20%) of amendment mixing ratios.

To conduct the germination test, 10 g of dried soil sample was extracted by 100 mL of distilled water for 15 h under room temperature. After extraction, extractant was centrifuged at 10,000 g for 20 min. Then, 5 mL of supernatant was soaked into a petri dish containing Whatman No.1 filter paper. Afterward, a total of 15 rice seeds were placed on the filter paper. The petri dishes were, then, incubated at 25 °C in dark condition for 96 h (U.S. EPA, 1996; Walter et al., 2006). For quality control, DI water (control test) and four replicates of toxicity test were conducted. The germination rate and root elongation rate of rice seed were finally, measured at the end of the incubation.

The percentages of relative seed germination (%RSG), relative root growth (%RRG), and germination index (%GI) as shown in Eq.1 to Eq.3, respectively, were calculated according to Walter et al. (2006).

$$\text{RSG (\%)} = \frac{\text{number of seeds germinated in soil extract}}{\text{number of seed germination in control}} \times 100 \qquad \text{(Eq.1)}$$

$$\text{RRG (\%)} = \frac{\text{mean root length in soil extract}}{\text{mean root length in control}} \times 100 \qquad \text{(Eq.2)}$$

$$\text{GI (\%)} = \frac{\text{RSG} \times \text{RRG}}{100} \qquad \text{(Eq.3)}$$

3.6 STATISTICAL ANALYSES

All analyses were done using the Statistical Package for Social Science (SPSS) version 17.0 software. ANOVA was used to determine the differences in concentrations of exchangeable, reducible, oxidizable and residual Cd and Zn in amended soil at different mixing ratios. Pearson’s correlation was used to determine the relationship between Cd and Zn concentrations, chemical characteristic and levels of toxicity. P value of less than 0.05 was used to determine the significance level.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 CHARACTERISTICS OF SOIL AND AMENDMENT

Characteristics of contaminated soil and two soil amendments (UCG and CCG) are summarized in Table 4.1.

Contaminated soil contained 53% of sand, 24% of slit, and 22.4% of clay and can be classified as sandy clay loam. According to the soil quality standard regulated by the DOA (DOA, 2010), the contaminated soil was slightly alkaline and contained moderate levels of CEC and OM. Considering nutrient levels, this contaminated soil had low total nitrogen, very high available phosphorus and adequate exchangeable potassium. Total Cd and Zn concentrations ranged from 44.7 to 52.0 mg kg^{-1} and 1,550 to 1,719 mg kg^{-1}, respectively. Concentrations of Cd were 1.2 to 1.4 times higher than the soil quality standard for habitat and agriculture of 37 mg kg^{-1} (PCD, 2004). In general, agricultural soil usually contains low concentration of Zn ranging from 4 to 73% (Zarcinas et al., 2004). Due to the study area, Mae Sot district, Tak is a zinc-rich area thus, about 1.7 to 1.9 times of Zn concentrations higher than soil and sediment quality standard for Zn for areas outside mining of 914.3 mg kg^{-1} were determined (DPIM, 2006).

As clearly shown in Table 4.1, UCG and CCG had different chemical characteristics. According to the DOA soil quality standard (Appendix A), UCG and CCG can be categorized as a strong acid and a very strong alkaline material, respectively. This due to the production of biochar from organic material generally resulted in the increasing pH of the product (DOA, 2010; Tang et al., 2013). In addition, UCG had very high OM and CEC. While, CCG was high in OM content and comparatively medium in CEC level. Comparing this two amendments, UCG had higher OM and CEC levels than CCG because OM was carbonized under high temperature during the CCG production. Previous researchers reported that biochar produced from woody material intended to contain low CEC values (Gaskin et al., 2008; Kookana et al., 2011). Thus, CEC which is originated from the functional group of organic material can also be diminished at the same time of OM carbonization. Levels of OM and CEC in the UCG agree well with the results reported by Venegas et al. (2015) in which high OM usually resulted in high CEC. Furthermore, UCG had very high total nitrogen, available phosphorus and exchangeable potassium contents. CCG had medium total nitrogen, high available phosphorus and exchangeable potassium according to the soil quality standard regulated by the DOA (2010) shown in Appendix A.

As CCG was produced at the temperature of 500 °C, total nitrogen was found to be lower comparing to UCG. This due to the fact that nitrogen usually volatilize when the temperature is higher than 200 °C (Kookana et al., 2011). While, phosphorus and potassium can be volatiled at about 700 to 800 °C. Therefore, total nitrogen in UCG was higher than CCG. On the other hand, CCG had higher available P and exchangeable K than UCG. The results reported above were in accordance with Beesley et al. (2011) who found the increasing of P, Mg and Ca contents with increasing temperature of biochar production. For Cd and Zn levels, higher concentrations of both elements were determined in CCG. This can be explained by the loss of organic matter and other vaporizable components from raw coffee ground during the CCG production. The results obtained were similar to the results reported by Yachigo and Sato (2013) and Hossain et al. (2010). They found the increases in total concentrations of heavy metals such as Cd, Cr, Ni, and Pb in the biochar. In addition, both researchers found the reduction of trace element bioavailability in the carbonized product, comparing to the raw feedstock.

Table 4.1 Chemical characteristic of soil and coffee ground materials [mean ± SD, n=3]

Parameter	Units	Soil	UCG	CCG
pH		7.52±0.64	5.17±0.01	9.53±0.20
OM	%	2.63±0.03	62.2±0.2	4.06±0.15
CEC	$\text{cmol}_c\ \text{kg}^{-1}$	13.0±2.4	22.8±0.5	12.0±0.49
Total N	%	0.13±0.00	5.32±0.02	0.33±0.15
Available P	mg kg^{-1}	56±10	288±13	798±35
Exchangeable K	mg kg^{-1}	130±7	4,775±66	6,750±50
Total Cd	mg kg^{-1}	47.2±2.1	0.004±0.01	0.017±0.01
Total Zn	mg kg^{-1}	1,660±50	4.55±0.44	25.1±1.8

4.2 EFFECTS OF COFFEE GROUND AMENDMENT ON CHEMICAL CHARACTERISTICS OF SOIL

4.2.1 Effect of amendments on soil characteristics

Soil pH, OM, CEC and nutrient (N, P, K) contents are the keys for the characterization of soil quality and the understanding of behavior of heavy metals in soil (Abdelhafez et al., 2014; Adriano, 1986). Table 4.2 and Table 4.3 summarize chemical characteristics of soil amended with UCG and CCG at the beginning and the end of aging process. Effects of amendments to the chemical characteristics can be described as follow.

As shown in Table 4.2, the addition of UCG of all mixing ratio to contaminated soil led to a significant decrease in pH value compared to the pH of control soil ($p<0.05$). Soil pH was found to be decreased when mixing ratio of UCG amended soil increased. At the end of aging experiment, the increase in pH value of amended soil was found. However, significant increase in pH can be observed only at 20% mixing ratio ($p<0.05$). Liu and Price (2011) found that spent coffee grounds can help maintaining a neutral pH in the composting systems. The results of this study were in accordance with the increase of soil pH after spent coffee ground addition which was reported by Kasongo et al. (2011), who explained that the increase of amended soil pH after aging was mainly due to the increase of anion concentration and decarboxylation effect of coffee ground (Yan et al., 1996).

In contrast to UCG addition, the addition of CCG has brought to the significant increase in soil pH comparing to the pH of contaminated soil ($p<0.05$). This might be caused by the increase in negative charge (OH^-) which is formed during the carbonization process. Adriano (1986) also stated that the negative charge is strongly related to the increase in soil pH. Table 4.2 shows that soil pH tends to increase when mixing ratio of CCG amended soil increased. After aging experiment, significant decrease in pH values of all mixing ratio was found ($p<0.05$). This can be explained by the decrease of basic species such as carbonates or oxy/hydroxides in biochar (Yao et al., 2010).

At initial aging experiment, when compared to the OM of control soil, OM of UCG amended soil was found to be significantly increased when mixing ratio of UCG amended soils were increased ($p<0.05$). UCG amendment soil was found with higher OM content than the control soil. At the end of aging experiment, the increase of soil

OM in high mixing ratio of UCG amended soil was found. This is due to the reason that coffee pulp which consists of organic components such as cellulose and lignin can be decomposed slowly (Adi and Noor, 2009).

The addition of CCG has brought to increase in soil OM comparing to the OM of contaminated soil. Comparing to the levels of OM in UCG amended soil, CCG amended soil had lower OM content. It is mainly caused by low OM in CCG at the beginning of aging process. Thus, all mixing ratio of CCG amended soil also had lower OM content than UCG amended soil. At the end of aging experiment, the increase in OM content of CCG amended soil was found. However, significant increase in OM can be observed only at 2.5% and 10% mixing ratio ($p<0.05$). This can be explained by the partial decomposition of OM by soil microbial community.

At initial aging experiment, increasing mixing ratio of UCG amended soils can increase the CEC content in amended soil as compared to control soil. The increase in CEC was mainly due to application of organic amendment. These results have been explained by Wang et al. (2014) who found that organic amendments can promote the flocculation of clay minerals. In principle, clay and OM in the soil are the major soil components controlling soil negative charge and could finally lead to the potential increase of soil CEC.

On the contrary, increase in mixing ratio of CCG amended soils was found to cause the lower in CEC content in amended soil. Due to CCG amendment had lower CEC value than control soil. After aging experiment, decrease in CEC content in UCG and CCG amended soils were observed. While all mixing ratios of UCG amended soils as well as 10% and 20% of CCG amended soils had significant higher CEC content than control soil. This result can be explained by the decrease in pH value of both amended soil and control soil which consequently caused the reduction in CEC value (Hochman et al., 1992).

At initial aging experiment, the increase in mixing ratio of UCG amendment to the contaminated soil tend to significantly enhance the concentrations of total N, available P and exchangeable K as compared to control soil ($p<0.05$). As UCG is a nutrient-rich amendment, the addition of UCG to soil could then resulted in nutrient levels. However, fluctuations of total N in UCG amended soils were found. Higher mixing ratio of UCG tends to increase total N due to the mineralization of nitrogen. Furthermore, available P tends to be increased after aging experiment. Particularly, 20% of UCG amended soil was found with significant increase in available P concentration when compare to the level of available at the initial aging experiment ($p<0.05$). Whereas, exchangeable K tends to decrease after aging experiment. However, significant differences in exchangeable K content could not be observed between samples collected at the initial and end of aging experiment.

In case of CCG amended soils, the increase in mixing ratio of CCG amendment to contaminated soil tends to elevate total N, available P and exchangeable K content compared to control soil at initial aging experiment. Significant increase of available P and exchangeable K in CCG amended soil were found ($p<0.05$) due to the reason that CCG amendment had high available P and exchangeable K contents. After aging experiment, higher concentrations of total N and exchangeable K were observed in CCG amended soil. Anderson et al. (2011) found that biochar could induce soil microbial community and thus, enhance the decomposition of total N. Similarly, Rondon et al. (2006) found the positive effect of elevated K availability through biochar additions after aging experiment. While, reduction in available P was found in all CCG amended soil. Nelson et al. (2011) reported that phosphorus in biochar could be decreased after 56 days of incubation.

Table 4.2 Chemical properties of UCG and CCG amended soil at different mixing ratio [mean ± SD, n=3]

Treatment	Mixing ratio	Duration	pH	OM (%)	CEC (cmol$_c$ kg^{-1})
UCG	2.5%	Initial	7.01 ± 0.22^{c}	5.65 ± 2.27^{b}	13.1 ± 1.7^{ns}
		Week 8	$7.17\pm0.10^{b,c,d}$	4.28 ± 0.13^{c}	$11.0\pm0.2^{a,b,c}$
	5%	Initial	$7.13\pm0.35^{c,d}$	6.68 ± 0.37^{b}	13.6 ± 1.4^{ns}
		Week 8	$7.09\pm0.41^{b,c,d}$	5.49 ± 0.11^{d}	$11.5\pm0.5^{c,d}$
	10%	Initial	6.72 ± 0.11^{b}	8.44 ± 0.53^{c}	16.4 ± 2.3^{ns}
		Week 8	6.88 ± 0.07^{b}	9.15 ± 0.60^{e}	13.4 ± 0.1^{e}
	20%	Initial	5.90 ± 0.04^{a}	14.3 ± 1.40^{d}	17.3 ± 3.6^{ns}
		Week 8	6.19 ± 0.68^{a}	16.7 ± 0.70^{f}	17.0 ± 0.7^{f}
CCG	2.5%	Initial	7.27 ± 0.15^{d}	2.63 ± 0.10^{a}	12.5 ± 1.9^{ns}
		Week 8	$6.96\pm1.00^{b,c}$	2.83 ± 0.03^{a}	10.5 ± 0.3^{a}
	5%	Initial	7.51 ± 0.36^{e}	2.77 ± 0.17^{a}	12.7 ± 2.1^{ns}
		Week 8	$7.09\pm0.21^{b,c,d}$	2.98 ± 0.03^{a}	10.5 ± 0.3^{a}
	10%	Initial	7.62 ± 0.02^{e}	3.01 ± 0.18^{a}	12.6 ± 1.9^{ns}
		Week 8	$7.25\pm0.12^{c,d}$	3.63 ± 0.16^{b}	$11.2\pm0.5^{b,c}$
	20%	Initial	7.90 ± 0.18^{f}	3.34 ± 0.53^{a}	12.6 ± 1.6^{ns}
		Week 8	7.30 ± 0.37^{d}	$3.96\pm0.21^{b,c}$	12.0 ± 0.5^{d}
Control	-	Initial	7.52 ± 0.06^{e}	2.63 ± 0.03^{a}	13.0 ± 2.4^{ns}
		Week 8	$7.08\pm0.96^{b,c,d}$	2.67 ± 0.25^{a}	$10.6\pm0.2^{a,b}$

Table 4.3 Nutrient concentrations in UCG and CCG amended soil at different mixing ratio [mean ± SD, n=3]

Treatment	Mixing ratio	Duration	Total N (%)	Available P (mg kg-^{1})	Exchangeable K (mg kg^{-1})
UCG	2.5%	Initial	0.28±0.11^{b}	41±6^{a}	207±13^{b}
		Week 8	0.22±0.01^{a,b}	43±0^{a}	185±12^{b}
	5%	Initial	0.33±0.02^{b}	52±3^{a}	250±14^{b,c}
		Week 8	0.28±0.01^{b}	43±0^{a}	259±11^{c}
	10%	Initial	0.42±0.03^{c}	44±5^{a}	517±30^{d}
		Week 8	0.59±0.21^{c}	51±2^{b}	471±1^{e}
	20%	Initial	0.72±0.07^{d}	56±6^{a}	977±23^{e}
		Week 8	0.83±0.04^{d}	78±0^{d}	939±17^{g}
CCG	2.5%	Initial	0.13±0.01^{a}	54±8^{a}	304±33^{c}
		Week 8	0.14±0.00^{a}	53±5^{b}	284±9^{d}
	5%	Initial	0.14±0.01^{a}	63±5^{a}	518±25^{d}
		Week 8	0.15±0.00^{a,b}	63±2^{c}	543±4^{f}
	10%	Initial	0.15±0.01^{a}	133±39^{b}	941±33^{e}
		Week 8	0.19±0.01^{a,b}	83±9^{e}	986±14^{h}
	20%	Initial	0.17±0.03^{a}	189±45^{c}	1,820±95^{f}
		Week 8	0.20±0.10^{a,b}	136±5^{f}	1,946±15^{i}
Control	-	Initial	0.13±0.00^{a}	56±10^{a}	130±7^{a}
		Week 8	0.13±0.00^{a}	42±2^{a}	123±3^{a}

4.2.2 Effect of amendments on Cd and Zn fractionation

The distribution of Cd and Zn fractions in this study were determined using the BCR sequential extraction and aqua regia digestion to achieve four fractions in amended soil and control soil during 8 weeks (Initial, week 2, week 4, week 6, week 8) of aging. Percentages of Cd and Zn distribution in UCG and CCG amended soil (with 0, 2.5%, 5%, 10% and 20% mixing ratio) and control soil are shown in Figure 4.1 to Figure 4.4.

4.2.2.1 Cd fractionation in amended and control soils

Percentages of Cd distribution are depicted in Figure 4.1 and Figure 4.2. At initial aging experiment, exchangeable and weak acid soluble, in other word is the mobile fractions were found to be decreased, increase in reducible residual fractions were increased in response to the increase in mixing ratio of UCG amended soil. However, significant changes in the distribution of all fractions as compared to control soil could not be observed. The reduction in mobile Cd found in the present study was similar to results reported by Azouaou et al. (2010) in which a rapid initial uptake rate of Cd was found after 1 h. Consequently, the extent of exchangeable fraction decreases with the increase of contact time since the numbers of vacant sites on the surface of coffee grounds are decreased.

Whereas, the increase in exchangeable fraction and the reduction in reducible and oxidizable fractions were observed as the mixing ratio of CCG amended soil increased. However, no significant differences could not be observed in the distribution of all fractions as compared to control soil. These findings can be referred to the functional groups (carboxylic and phenolic) on the surface of biochar particles, which could attract Cd (Houben et al., 2013).

Consequently, the initial aging experiment resulted in the moderately significant negative correlation between CEC and exchangeable Cd in the UCG and CCG amended soil ($r=-0.652$, $p<0.05$). In addition, the significant negative correlation were determined between OM and exchangeable Cd ($r=-0.678$, $p<0.05$) as well. This result strongly confirmed that both CEC and OM are one the factors controlling Cd mobility and bioavailability. These results are in good agreement with the results reported by Jiang et al. (2012) and Spence et al. (2014) in which soils with higher CEC values could reduce the translocation of Cd from soil to plant. In addition, it was also confirmed by Benedetti et al. (1996) that natural organic matters such as humic and fulvic acids with the natural organic electrolytes (lignin, cellulose) and their functional groups have the capability to bind exchangeable Cd.

During aging experiment, though fluctuations of Cd fractions were determined, the major species were exchangeable and reducible fractions. These results are in accordance with Bian et al. (2014) who reported the fluctuation of heavy metal species in organic waste amended soils. Moreover, about 80% of Cd was found in the form of exchangeable and reducible fractions (Arain et al., 2008). Akkajit et al. (2013) found that soil sample collected from Mae Sot, Tak was mainly contaminated with Cd in the form of exchangeable and reducible fraction. In this study, percentage distribution of exchangeable, reducible, oxidizable, and residual fractions in UCG amended soil were in the ranges of 24% to 57%, 41% to 70%, up to 7%, and up to 2%, respectively. Compared to UCG amended soil, the percentage distribution of exchangeable, reducible, oxidizable, and residual fractions in CCG amended soil were in the range of 31% to 58%, 38% to 62%, up to 6%, and up to 4%, respectively. While the exchangeable, reducible, oxidizable, and residual fractions in control soil were in the ranges of 37% to 61%, 39% to 57%, up to 5%, and up to 3%, respectively. Clemente et al. (2006) found that properties of soil after the addition of organic materials will be mainly depended on the degradation process of organic materials. As a result, the degradation process could affect heavy metal solubility. Organic amendments which contain a high proportion of OM and CEC thus, can decrease the bioavailability of heavy metals in soil by adsorption and by forming stable complexes with humic substances. Houben et al. (2013) also summarized that OM can redistribute heavy metals from exchangeable form into the more stable forms such as oxidizable and residual fractions. Shuman et al. (2007) confirmed that Cd in the exchangeable form can be decreased by the organic amendments. The formation of stable complexes between Cd and OM-rich material such as composted biosolid has also been reported by other groups of researchers including Evangelou et al. (2004); Halim et al. (2003); Tapia et al. (2010).

Percentage distributions of Cd in exchangeable and reducible forms tend to be decrease in response to the UCG amended soil after aging experiment. As compared to the control soil, an increase mixing ratio of UCG amended soil could decrease exchangeable and residual fractions on the other hand, reduce the reducible fraction at the end of aging process. In particular, UCG amended soil was found with significant reduction in exchangeable Cd compared to the initial aging experiment ($p<0.05$). Additionally, it was found that the 10% of UCG amended soil can significantly reduced exchangeable Cd compared to the initial aging experiment. While, the 20% of UCG amended soil significantly increased residual Cd compared to the initial aging experiment ($p<0.05$). Consequently, compared to initial and after aging experiment, the percentage of Cd in exchangeable and reducible fractions were about to decreased by about 4% and 6%, respectively. On the other hand, the oxidizable and residual fractions were found to be increased by 3%.

Compared to the control soil, an increase in mixing ratio of CCG amended soil caused the decrease in exchangeable fraction but increase in reducible fraction. In addition, the 10% of CCG amended soil significantly reduced exchangeable Cd as compared to the initial aging experiment ($p<0.05$). These results indicated that the exchangeable and reducible Cd were decreased by about 5% and 2%, respectively. However, oxidizable and residual fractions were increased by 4% and 1%, respectively. In case of control soil, no significant decrease in exchangeable fraction could not be determined. Oxidizable and residual fractions were observed after aging experiment. While an increase in reducible fraction was observed. Based on the results obtained, control soil was found with the reduction in exchangeable fraction by 2% and an increase in reducible fraction by 2%. After aging experiment, strong significant negative correlations were found between CEC and exchangeable Cd ($r=-0.849$, $p<0.05$), OM and exchangeable Cd ($r=-0.833$, $p<0.05$) and total N and exchangeable Cd ($r=-0.867$, $p<0.05$) in both UCG and CCG amended soil. It could be concluded that the addition of either UCG or CCG amendments could increase the concentrations of OM, CEC and total N. The rises in soil OM, CEC and total N then, induce the immobilization of exchangeable Cd due to Cd precipitation and Cd adsorption (Houben et al., 2013; Lucchini et al., 2014b).

The order of Cd distribution in both UCG and CCG amended soils and control soil were mainly found with reducible > exchangeable and weak acid soluble > oxidizable ≥ residual fractions. The reduction in the percentage distribution of exchangeable Cd fraction was observed in both UCG and CCG amended soils. In the case of oxidizable fraction, the increase in its concentration was analyzed in both amended soils. This finding confirmed that both UCG and CCG can effectively immobilize Cd by organic bonding.

This study found that Cd was predominantly presented in the reducible and exchangeable fractions. The results of the percentage distribution of all fractions were then interpreted in term of the environmental risks. According to Jain (2004), the percentage distribution of 1% to 10%, 11% to 30%, 31% to 50%, and more than 50% indicate the low, medium, high, and very high risks to the environment, respectively. In particular, soil or sediment with more than 50% potential risk can be considered as hazardous substance and metals containing in the media can be easily transferred to the food chain. Therefore, it can be assumed that both UCG and CCG amended soil in this study may pose high risk to the environment because they were mostly found with 25.54% to 33.25% and 31.62 to 35.54%, respectively.

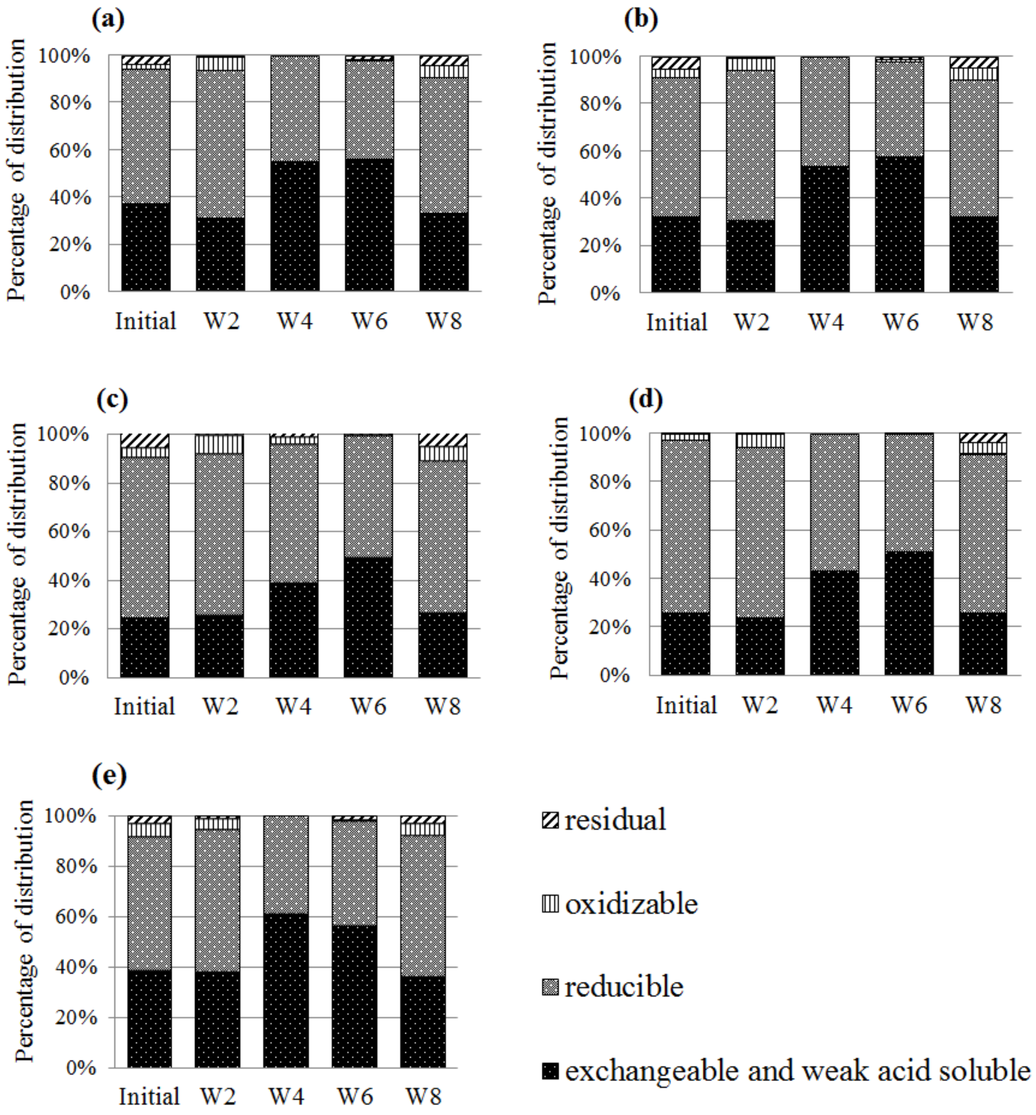

Figure 4.1 Percentage distribution of Cd fractions during aging process with different mixing ratio of UCG (a) 2.5% (b) 5% (c) 10% (d) 20% and (e) control soil

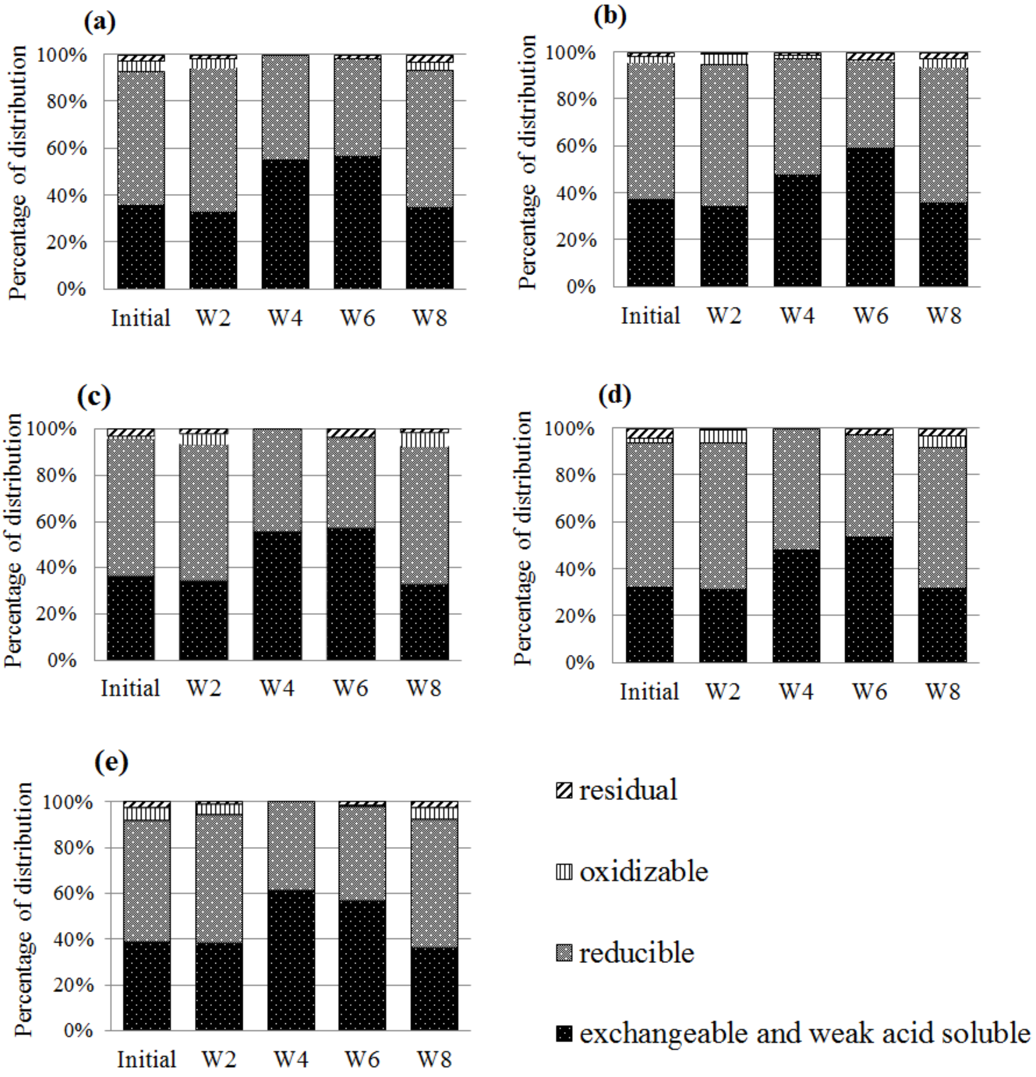

Figure 4.2 Percentage distribution of Cd fractions during aging process with different mixing ratio of CCG (a) 2.5% (b) 5% (c) 10% (d) 20% and (e) control soil

4.2.2.2 Zn fractionation in amended and control soils

The percentage distribution of Zn fractions was shown in Figure 4.3 and Figure 4.4. At initial of aging experiment with UCG, decrease in exchangeable fraction and increase in oxidizable fraction was observed, while percentages of reducible and residual fractions were fluctuated. Meanwhile, for experiment with CCG, increase in reducible fraction and decrease in residual fraction was observed, while the fluctuated results were found in exchangeable and oxidizable fractions. According to the initial aging experiment, the significant negative with moderate correlation between CEC and exchangeable Zn ($r=-0.585$, $p<0.05$), OM and exchangeable Zn ($r=-0.427$, $p<0.05$) and total N and exchangeable Zn ($r=-0.422$, $p<0.05$) in the UCG and CCG amended soil were observed. According to interpretation of the correlation coefficient by Taylor (1990). These results supported that OM, CEC and total N can be considered as factors affecting Zn mobility and bioavailability. However, comparing to the control soil, significant change of Zn fractionation could not be observed in both UCG and CCG amended soil. Normally, Zn is considered as the most mobile heavy metal and usually exist as inorganically complexes and exchangeable forms due to stronger affinity to non-residual fraction (Alvarenga et al., 2009).

Fluctuations of Zn fractionation during aging experiment were observed. The reducible and oxidizable fractions were the major observed Zn species. Distribution percentage of Zn in UCG amended soil in the form of exchangeable, reducible, oxidizable, and residual fractions were in the range of 12 to 25, 29 to 44, 21 to 43, and 12 to 21, respectively. In the case of CCG amended soil, percentage of Zn in exchangeable, reducible, oxidizable, and residual fractions were found in the range of

13 to 25, 32 to 50, 18 to 44, and 7 to 22, respectively. While, percentage of Zn in control soil were in exchangeable, reducible, oxidizable, and residual in the range of 15 to 23, 30 to 40, 23 to 36, and 14 to 19, respectively.

After aging experiment, exchangeable fraction of Zn tended to be increased in UCG amended soil. Exchangeable Zn in UCG amended soil at 10% mixing rate was increased significantly during aging experiment ($p<0.05$). In addition, significant increased ($p<0.05$) of reducible Zn fraction was exhibited in UCG amended soil (except 10% of UCG) while oxidizable fraction of Zn decreased. Significant decrease ($p<0.05$) of oxidizable fraction after aging was observed in 10% and 20% mixing rate of UCG amended soil. On the contrary, significantly increase of residual fraction ($p<0.05$) was observed in 10% and 20% mixing rate after aging experiment. Therefore, at the end of aging process, Zn in exchangeable and oxidizable fraction tended to decrease while Zn in reducible and residual fraction tended to increase. Kim et al. (2014) stated that spent coffee ground contain numerous anionic organic functional groups such as O-H, C-H, C=O, C-O that promoted formation of Zn complexes. In case of CCG amended soil, decrease in exchangeable fraction was observed for all mixing rate. The significantly decreased of exchangeable fraction ($p<0.05$) was observed in 10% CCG amended soil. Increase of residual fraction was found. However, significant differences in reducible, oxidizable and residual fractions between initial and after aging experiment were not found. Thus, at the end of aging process, exchangeable and reducible Zn fractions decreased by up to 2 and 4%, respectively. While, oxidizable and residual Zn fractions increased up to 3 and 2%, respectively. In control soil, decrease in exchangeable and reducible fractions and increase in oxidizable and residual fractions were found.

No significant correlation between exchangeable Zn and other chemical characteristics was found. On the other hand, the literatures revealed the relation between some soil chemical characteristics and Zn immobilization. Addition of phosphate fertilizers increase sorption of Zn onto soil (Yan et al., 2015). As reviewed by Gonzalez et al. (2006); Lucchini et al. (2014a) alterations in CEC and pH by amendment application are the main parameters controlling heavy metal mobility and sorption.

Order of Zn fractionation in UCG and CCG amended soil were reducible > oxidizable > residual > exchangeable fraction. High reducible and oxidizable fractions of Zn was observed by Nwoko et al. (2012) during aging experiment of amended soil. The study of Alvarenga et al. (2009) found that reducible and oxidizable fraction of Zn were increase with the increase of garden residue addition into soil. Zn can be immobilized by hydroxides, carbonates, sulfides, phosphates, and several other anions as well as form complexes with organic ligands (Kumpiene et al., 2008).

The distribution pattern of Zn fractionation in control soil was found in oxidizable > reducible > residual > exchangeable fraction. This finding confirmed that Zn was predominantly in the reducible and oxidizable fractions. Nevertheless, exchangeable Zn in UCG and CCG amended soil were 14.51 to 15.86% and 14.71 to 15.10%, respectively, therefore, classified in medium risk, as described by Jain (2004).

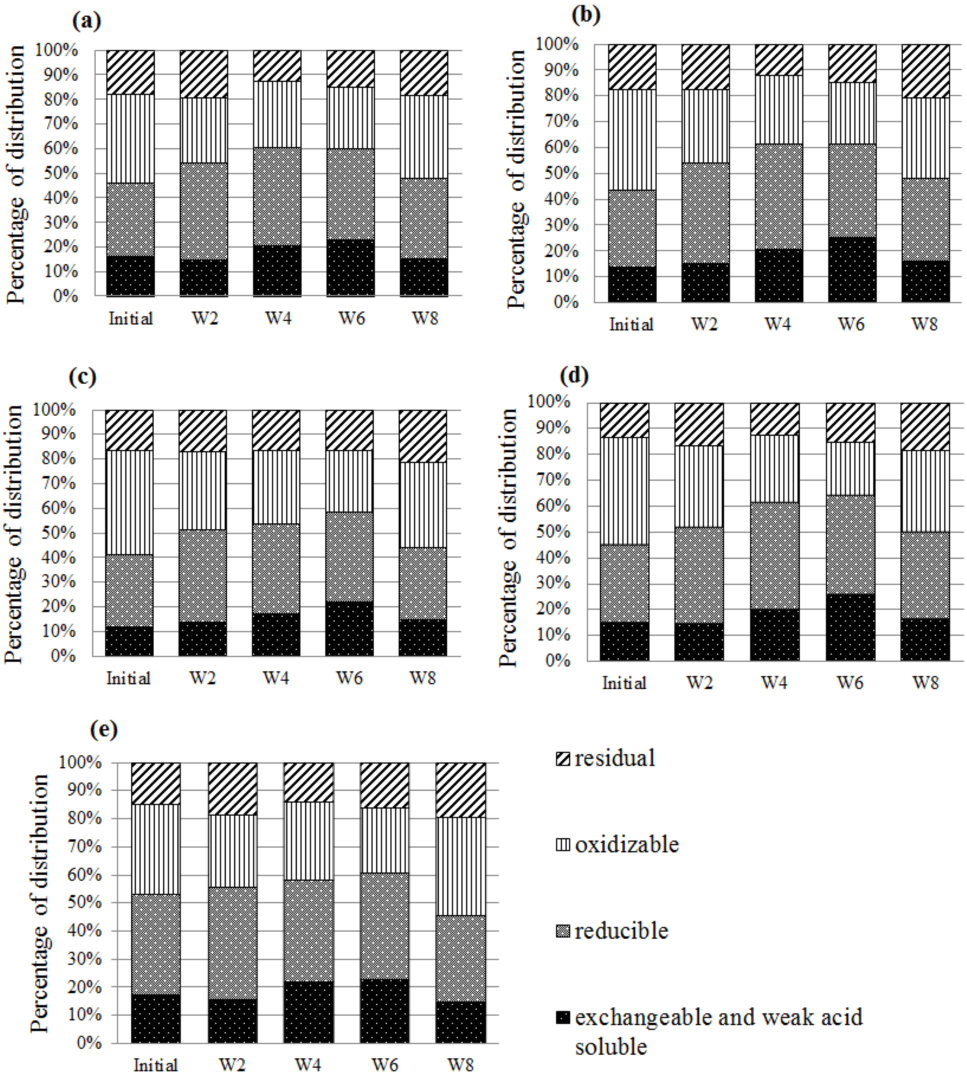

Figure 4.3 Percentage distribution of Zn fractions during aging process with different mixing ratio of UCG (a) 2.5% (b) 5% (c) 10% (d) 20% and (e) control soil

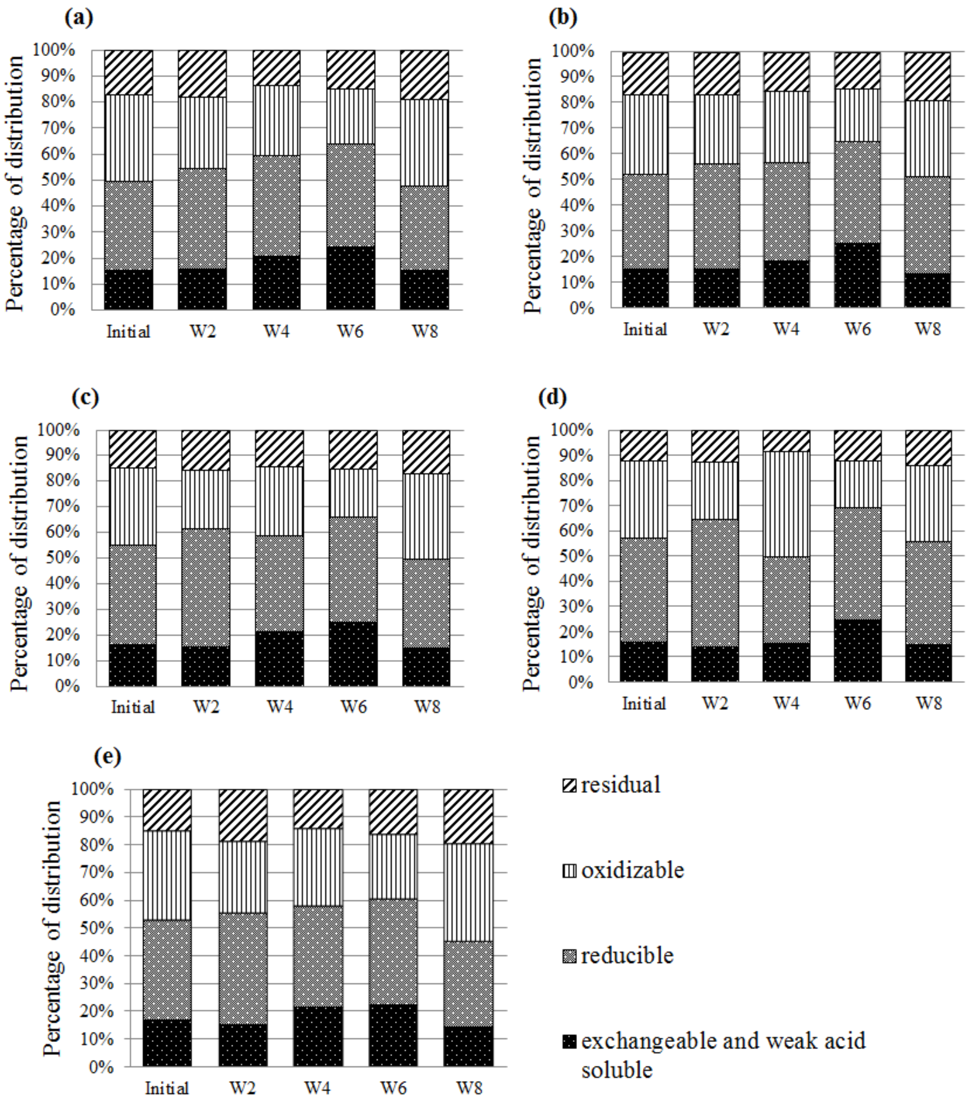

Figure 4.4 Percentage distribution of Zn fractions during aging process with different mixing ratio of CCG (a) 2.5% (b) 5% (c) 10% (d) 20% and (e) control soil

4.2.3 Effect of UCG and CCG amendments on plant available Cd and Zn

Concentrations of plant available Cd and Zn were obtained from DTPA extraction is summarized in Table 4.4.

4.2.3.1 Plant available Cd concentration in amended and control soils

At initial aging experiment, trend of plant available Cd concentrations was not found in UCG mixing ratio. While, plant available Cd concentrations was found significantly decreased when mixing ratio of CCG amended soil increased ($p<0.05$). Fellet et al. (2011) observed reduction in the plant available Cd concentration after addition of biochar into heavy metal contaminated soil, which may be the result of the dilution effect of amendment after the biochar application. At initial of aging experiment, there was significant negative correlation between plant available Cd concentrations in amendment soil and pH ($r=-0.681$, $p<0.05$), between plant available Cd concentrations in amendment soil and available P ($r=-0.801$, $p< 0.01$), and between plant available Cd concentrations in amendment soil and exchangeable K ($r=-0.701$, $p<0.05$).

Reduction of plant available Cd concentrations were found at the end of aging experiment for both UCG and CCG amended soils, especially for 5% UCG, 10% UCG and 10% CCG amended soils ($p<0.05$). The reduction of plant available Cd was up to 6.9% in 5% of UCG amended soil and 7.51% in 10% of CCG amended soil. Apparently, plant available Cd in 20% of UCG and 2.5% of CCG was increased. Furthermore, there was found a significant negative correlation between plant available Cd concentrations in amendment soil and pH ($r=-0.695$, $p<0.05$), between plant available Cd concentrations in amendment soil and available P ($r=-0.667$, $p< 0.01$), and between plant available Cd concentrations in amendment soil and exchangeable K ($r=-0.594$, $p<0.05$). This result confirmed the strong influence pH, available P, exchangeable K on Cd plant availability. It is well documented that Cd plant availability depends on soil pH (Su and Wong, 2004; Tapia et al., 2010).

4.2.3.2 Plant available Zn concentration in amended and control soils

At initial of aging experiment, plant available Zn concentration tended to be increased when mixing ratio of UCG in amended soil increased. While, plant available Zn concentration was significantly decreased when mixing ratio of CCG in amended soil increased as opposed to control soil ($p<0.05$), which may be the result of the dilution effect from biochar application (Fellet et al., 2011). There were significant negative correlation between plant available Zn concentrations in amendment soil and pH ($r=-0.889$, $p<0.05$), and between plant available Zn concentrations in amendment soil and available P ($r=-0.582$, $p< 0.05$).

At the end of aging process, both UCG and CCG amended soil exhibited the increase of plant available Zn concentration. Especially, significant increase ($p<0.05$) of plant available Zn concentrations were found in experiments at 5% UCG, 20% of UCG, and 10% of CCG amended soils. Morikawa and Saigusa (2011) found that application of coffee ground in paddy field increased plant available Zn concentration. The significant negative correlation between plant available Zn concentrations in amendment soil and pH ($r=-0.863$, $p<0.05$) was observed. Thus, the increased in Zn plant availability in contaminated soil related to increase of mixing ratio of UCG and CCG to amended soil. The decline of pH by the addition of amendments affected to Zn plant availability of this metal in soil (Mandal and Hazra, 1997; Pardo et al., 2011).

Table 4.4 Plant available concentrations of Cd and Zn in both amended and control soils at initial and after aging [mean ± SD, n=3]

Treatment	Mixing ratio	Cd (mg kg^{-1})		Zn (mg kg^{-1})	
		Initial	Week 8	Initial	Week 8
Control	-	10.2 ± 0.8^{d}	9.82 ± 0.62^{d}	95.2 ± 10.2^{c}	90.1 ± 7.0^{bc}
UCG	2.5%	11.2 ± 0.3^{ef}	10.4 ± 0.9^{de}	94.1 ± 3.5^{c}	92.8 ± 10.6^{c}
	5%	11.6 ± 0.4^{ef}	10.8 ± 0.2^{de}	98.2 ± 5.0^{c}	108 ± 2^{d}
	10%	11.9 ± 0.4^{f}	11.1 ± 0.2^{e}	112 ± 3^{d}	116 ± 2^{d}
	20%	10.9 ± 0.6^{de}	12.3 ± 1.2^{f}	126 ± 7^{e}	150 ± 5^{e}
CCG	2.5%	9.25 ± 0.42^{c}	9.75 ± 0.51^{d}	80.4 ± 4.2^{b}	90.9 ± 6.4^{bc}
	5%	9.21 ± 0.13^{c}	8.68 ± 0.33^{c}	82.7 ± 2.1^{b}	85.7 ± 4.0^{abc}
	10%	8.26 ± 0.04^{b}	7.64 ± 0.19^{b}	77.9 ± 1.2^{b}	82.0 ± 0.6^{ab}
	20%	6.57 ± 0.42^{a}	6.35 ± 0.17^{a}	67.4 ± 5.2^{a}	77.1 ± 3.5^{a}

Means with different letters within the column of the same species indicate significant differences between values; Duncan test ($p<0.05$)

4.3 EFFECT OF DIFFERENT AMENDMENT ON SEED GERMINATION

Seed germination results are shown in mean percentages of RSG, RRG, and GI in Table 4.5. All value of %RSG, %RRG and %GI of all treatments were significantly different ($p<0.05$). The %RSG, %RRG and %GI form the test with UCG was very low reflecting its toxic effect which may cause by strong acid nature of UCG (Table 4.1). Result exhibited the decrease of %RSG, %RRG, and %GI when mixing ratio of UCG in amended soil increased. In contrast, mean of %RSG, %RRG, and %GI were increased when mixing ratio of CCG in amended soil increased. The significant negative moderate correlation (Pearson) between plant available Cd and %RRG ($r=-0.581$, $p<0.05$) and plant available Zn and %RRG ($r=-0.540$, $p<0.05$) was observed in seed germination tests. According to interpretation of the correlation coefficient by Taylor (1990), the concentration of plant available Cd and Zn showed such a clear relationship to %RRG. In contrast, the significant positive correlation with moderate coefficient between pH and %RRG was found (r=0.566, p<0.05). Therefore, the highest mean of %RSG and %GI were noted in 20% of CCG corresponding to the low plant available Cd and Zn concentration. Soil pH showed strong affect in seed germination test. The pH of 20% CCG amended soil was neutral. These results are similar to the previous findings from study of biochar on plant growth promoting (Biederman and Harpole, 2013; Park et al., 2011). As seen in Table 4.5, the %RSG, %RRG, and %GI deriving from experiment by UCG and 20% of UCG in amended soil were significantly lower than control soil. This suggests that the germination of seed was inhibited by low soil pH providing by UCG.

The %GI of UCG and CCG amended soil were higher than 85% indicated appropriate condition for seed germination, as described by Hui and Hang (2009).

Table 4.5 Percentage of %RSG, %RRG and %GI in both amended and control soils at after aging experiment [mean ± SD, n=4]

Treatment	Mixing ratio	RSG (%)	RRG (%)	GI (%)
Control soil	-	96.4 ± 3.6^{b}	133 ± 12^{cd}	128 ± 14^{cd}
UCG	-	12.1 ± 8.8^{a}	5.23 ± 2.65^{a}	1.00 ± 0.76^{a}
CCG	-	92.1 ± 6.4^{b}	90.5 ± 20.8^{b}	84.9 ± 21.9^{b}
UCG	2.5%	98.2 ± 1.8^{b}	143 ± 8^{bcd}	140 ± 9^{cd}
	20%	93.9 ± 1.6^{b}	110 ± 16^{bc}	103 ± 11^{bc}
CCG	2.5%	98.2 ± 1.0^{b}	114 ± 14^{bc}	112 ± 9^{bc}
	20%	98.2 ± 1.8^{b}	157 ± 14^{d}	155 ± 11^{d}

Means with different letters within the column of the same species indicate significant differences between values; Duncan test ($p<0.05$)

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Based on this study, the following major conclusions can be summarized as follow.

- The addition of UCG and CCG to contaminated soil resulted in the increase in soil nutrients and OM concentrations. In terms of Cd and Zn distribution, the exchangeable and reducible Cd were found to be decreased with the increasing mixing ratio of UCG.
- Similar results of Cd and Zn fractionations were observed in all mixing ratio of both UCG and CCG amended soils.
- Aging process could reduce the Cd plant availability and finally, reduce plant toxicity. However, plant availability of Zn were found in both UCG and CCG amended soils.
- For phytotoxicity of Cd and Zn on rice seed, an increase in CCG mixing ratio could improve the possibilities of seed germination as well as root growth.
- Overall findings indicated that CCG can be considered as Cd immobilizing amendment to the contaminated soil.

5.2 RECOMMENDATIONS FOR FUTURE WORK

- The single and combined effects of heavy metal on the immobilization process in the contaminated soils should be studied.

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APPENDICES

APPENDIX A

STANDARDS OF SOIL CHEMICAL PROPERTIES

Table A-1 Classification of soil pH (DOA, 2010)

pH range	Degree of acidity or acid
< 3.5	Ultra acid
3.5 -4.4	Extreamly acid
4.5-5.0	Very strong acid
5.1-5.5	strong acid
5.6-6.0	Moderately acid
6.1-6.5	Slightly acid
6.6-7.3	Netural
7.4-7.8	Slightly alkaline
7.9-8.4	Moderately alkaline
8.5-9.0	strong alkaline
>9.0	Very strong alkaline

Table A-2 Classification of soil organic matter (OM) (DOA, 2010)

OM content (%)	Degree of OM
0.5-1.0	Very low
1.0-2.0	low
2.0-3.0	Moderate
3.0-5.0	High
>5.0	Very high

Table A-3 Classification of phosphorus (P) in soil (DOA, 2010)

P content (mg/kg)	Degree of P
<5	Very low
5-10	low
10-20	Moderate
20-25	High
>25	Very high

Table A-4 Classification of potassium (K) in soil (DOA, 2010)

K content (mg/kg)	Degree of K
<120	low
120-190	Moderate
191-300	High
>300	Very high

APPENDIX B

CONCENTRATIONS OF Cd and Zn OBTAINED FROM BCR SEQUENTIAL EXTRACTION

Table B-1 Concentrations of Cd obtained from BCR sequential extraction at different mixing ratio of UCG amendment

2.5% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	18.63	17.63	22.59	28.88	18.51
Reducible	28.30	35.16	18.51	21.47	32.04
Oxidizable	1.06	3.33	0.00	0.17	2.63
Residual	1.97	0.39	0.00	0.95	2.48
Total	49.96	56.51	41.10	51.48	55.66
CV.	12.08				

5% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	16.31	14.71	20.49	26.68	17.10
Reducible	30.19	30.60	17.84	18.73	31.16
Oxidizable	1.66	2.60	0.00	0.59	2.75
Residual	2.76	0.24	0.00	0.50	2.50
Total	50.92	48.14	38.33	46.49	53.51
CV.	12.16				

10% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	11.44	12.11	15.39	21.36	13.70
Reducible	30.68	31.50	22.30	21.46	32.15
Oxidizable	1.77	3.65	1.18	0.21	2.96
Residual	2.61	0.25	0.50	0.07	2.74
Total	46.50	47.52	39.37	43.09	51.56
CV.	10.11				

20% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	9.63	10.07	12.77	20.52	11.43
Reducible	26.51	29.45	16.91	19.66	29.54
Oxidizable	0.83	2.30	0.00	0.05	1.95
Residual	0.21	0.16	0.00	0.07	1.84
Total	37.17	41.99	29.68	40.30	44.77
CV.	14.92				

Table B-2 Concentrations of Cd obtained from BCR sequential extraction at different mixing ratio of CCG amendment

2.5% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	18.13	17.35	22.52	28.57	19.17
Reducible	28.97	32.49	18.37	21.26	32.16
Oxidizable	2.27	2.07	0.00	0.08	1.79
Residual	1.29	1.04	0.00	0.79	1.82
Total	50.66	52.96	40.89	50.70	54.95
CV.	10.82				

5% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	17.13	17.36	20.68	28.40	18.15
Reducible	27.00	30.62	21.59	17.97	29.71
Oxidizable	1.12	2.15	0.71	0.27	1.79
Residual	0.84	0.48	0.46	1.56	1.40
Total	46.10	50.61	43.44	48.21	51.05
CV.	6.65				

10% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	15.74	16.70	20.15	25.56	15.75
Reducible	25.85	28.73	16.10	17.63	28.85
Oxidizable	0.65	2.30	0.00	0.09	2.85
Residual	1.33	1.14	0.00	1.69	0.93
Total	43.57	48.88	36.24	44.96	48.37
CV.	11.45				

20% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	12.08	12.64	13.77	20.12	12.83
Reducible	23.35	25.23	14.71	16.39	24.47
Oxidizable	0.66	2.40	0.00	0.03	2.07
Residual	1.61	0.21	0.00	0.97	1.21
Total	37.70	40.48	28.48	37.52	40.58
CV.	13.41				

Table B-3 Concentrations of Cd obtained from BCR sequential extraction in control experiment

Control Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	20.05	20.08	23.33	28.19	19.58
Reducible	27.22	29.35	14.78	20.74	30.24
Oxidizable	2.80	2.27	0.00	0.19	2.77
Residual	1.53	0.66	0.00	0.89	1.58
Total	51.60	52.37	38.11	50.01	54.17
CV.	13.01				

Table B-4 Concentrations of Zn obtained from BCR sequential extraction at different mixing ratio of UCG amendment

2.5% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	296.2	266.1	421.9	454.4	297.8
Reducible	555.1	723.5	826.9	735.0	630.3
Oxidizable	669.5	482.2	554.0	491.8	660.5
Residual	336.1	356.0	266.9	303.5	361.7
Total	1,857.0	1,827.8	2,069.7	1,984.6	1,950.4
CV.	5.05				

5% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	240.4	266.0	401.7	471.0	304.7
Reducible	537.4	699.7	787.0	683.5	624.0
Oxidizable	693.6	504.3	521.6	453.7	604.6
Residual	311.1	312.4	235.5	276.2	398.5
Total	1,782.5	1,782.4	1,945.8	1,884.4	1,931.8
CV.	4.24				

10% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	200.3	232.2	324.9	388.0	265.5
Reducible	485.8	625.8	684.2	646.6	538.0
Oxidizable	710.5	532.0	561.7	451.6	634.0
Residual	277.2	287.3	307.5	290.5	392.5
Total	1,673.8	1,677.2	1,878.3	1,776.8	1,830.0
CV.	5.16				

20% UCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	211.9	208.0	328.6	402.5	254.0
Reducible	439.4	550.4	700.4	609.3	547.5
Oxidizable	601.3	466.6	431.9	329.6	506.6
Residual	197.0	245.7	212.2	238.8	293.2
Total	1,449.7	1,470.6	1,673.1	1,580.2	1,601.3
CV.	6.01				

Table B-5 Concentrations of Zn obtained from BCR sequential extraction at different mixing ratio of CCG amendment

2.5% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	283.0	284.7	438.8	471.0	298.0
Reducible	636.0	707.8	810.7	781.8	642.1
Oxidizable	617.5	504.2	569.0	412.3	657.0
Residual	324.8	335.7	284.8	291.8	377.0
Total	1,861.4	1,832.3	2,103.3	1,956.9	1,974.1
CV.	5.50				

5% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	281.5	279.7	377.9	475.2	246.5
Reducible	660.8	754.0	767.6	745.5	683.8
Oxidizable	560.7	500.2	558.5	386.6	543.6
Residual	297.9	302.8	312.0	268.3	339.4
Total	1,800.9	1,836.7	2,016.0	1,875.5	1,813.2
CV.	4.67				

10% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	272.4	255.7	404.9	450.9	263.7
Reducible	656.1	762.6	712.4	741.1	617.6
Oxidizable	508.8	382.4	517.3	336.5	595.4
Residual	253.5	262.8	272.6	275.0	303.3
Total	1,690.8	1,663.5	1,907.3	1,803.6	1,780.0
CV.	5.49				

20% CCG Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	238.8	211.8	340.0	386.3	230.4
Reducible	611.5	760.7	767.9	697.0	641.1
Oxidizable	462.3	339.4	940.7	289.1	475.3
Residual	181.2	195.4	192.6	195.0	218.7
Total	1,493.8	1,507.2	2,241.2	1,567.5	1,565.5
CV.	19.00				

Table B-6 Concentrations of Zn obtained from BCR sequential extraction in control experiment

Control Experiment

BCR Fraction	Initial	W2	W4	W6	W8
Exchangeable	327.6	302.6	482.3	472.4	299.4
Reducible	673.4	781.7	802.9	787.1	613.6
Oxidizable	602.1	498.8	613.3	481.0	708.0
Residual	281.6	370.0	308.5	335.2	393.5
Total	1,884.6	1,953.2	2,207.1	2,075.8	2,014.5
CV.	6.08				

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