BIOAVAILABILITY AND FRACTIONATION OF Cd AND Zn IN CONTAMINATED SOILS AMENDED BY FILTER CAKE, VINASSE AND BOILER ASH FROM ETHANOL PRODUCTION PLANT

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for the Degree of Doctor of Philosophy Program in Environmental Management
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ปริมาณที่นำไปใช้ได้และการแยกลำดับส่วนของแคดเมียมและสังกะสีในดินปนเปื้อน โดยการเติมกากหม้อกรองน้ำกากส่าและขี้เถ้าหม้อไอน้ำจากโรงงานผลิตเอทานอล

นางสาวเพ็ญศิริ เอกจิตต์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title BIOAVAILABILITY AND FRACTIONATION OF Cd AND Zn IN CONTAMINATED SOILS AMENDED BY FILTER CAKE, VINASSE AND BOILER ASH FROM ETHANOL PRODUCTION PLANT

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เพ็ญศิริ เอกจิตต์ : ปริมาณที่นำไปใช้ได้และการแยกลำดับส่วนของแคดเมียมและสังกะสีในดินปนเปื้อนโดยการเติมกากหม้อกรองน้ำกากส่าและขี้เถ้าหม้อไอน้ำจากโรงงานผลิตเอทานอล (BIOAVAILABILITY AND FRACTIONATION OF Cd AND Zn IN CONTAMINATED SOILS AMENDED BY FILTER CAKE, VINASSE AND BOILER ASH FROM ETHANOL PRODUCTION PLANT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. จันทรา ทองคำเภา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: Asst. Prof. Thomas DeSutter, 148 หน้า.

ขี้เถ้าหม้อไอน้ำ (Boiler ash) กากหม้อกรอง (Filter cake) และน้ำกากส่า (Vinasse) เป็นของเสียจากกระบวนการผลิตเอทานอล การนำของเสียกลับมาใช้ประโยชน์ใหม่เป็นสารปรับปรุงดินและตัวยับยั้งการเคลื่อนตัวของโลหะ เป็นอีกทางเลือกหนึ่งที่ใช้ประโยชน์จากวัตถุดิบได้อย่างมีประสิทธิภาพ และเป็นการเพิ่มมูลค่าของของเหลือ ดังนั้นงานวิจัยนี้จึงเกิดขึ้นโดยการเติมขี้เถ้าหม้อไอน้ำ กากหม้อกรองและน้ำกากส่าในอัตราส่วนที่แตกต่างกันปริมาณ 3 เปอร์เซ็นต์ (น้ำหนักสารปรับปรุงดินต่อน้ำหนักดิน) ในดินปนเปื้อนแคดเมียมและสังกะสีที่ความเข้มข้นต่างระดับ ในระดับต่ำ (LCdS) และสูง (HCdS) เป็นเวลา 3 เดือน ผลการทดลองพบว่าการเติมขี้เถ้าหม้อไอน้ำและกากหม้อกรองตัวเดียวที่ปริมาณ 3 เปอร์เซ็นต์และการผสมระหว่างขี้เถ้าหม้อไอน้ำกับน้ำกากส่าอย่างละ 1.5 เปอร์เซ็นต์ ทำให้แคดเมียมในรูปที่นำไปใช้ได้ (BCR1+2) ลดลงอย่างมีนัยสำคัญในช่วง 2-4 สัปดาห์ ของการทดลอง จากผลการทดลองนี้จึงนำไปสู่การทดลองปลูกอ้อย (*Saccharum officinarum* L.) เป็นเวลา 4 เดือน เพื่อศึกษาผลของของเสียต่อการเจริญเติบโตของอ้อย การเก็บสะสมของโลหะหนักในต้นอ้อยและการแยกลำดับส่วนของแคดเมียมและสังกะสี โดยวิธีการสกัดลำดับส่วนด้วยวิธี BCR จากผลการทดลองพบว่าดินทั้ง 2 ความเข้มข้น แสดงผลที่แตกต่างกัน ในดินที่มีความเข้มข้นของแคดเมียมต่ำผลผลิตทางการเกษตรของต้นอ้อยเพิ่มขึ้นเป็น 6 และ 3 เท่าของน้ำหนักแห้งในส่วนเหนือพื้นดินของต้นอ้อย (เพิ่มจาก 8.46 กรัมต่อต้น เป็น 57.6 กรัมต่อต้น) และของราก (จาก 2.10 กรัมต่อต้นเป็น 6.59 กรัมต่อต้น) ตามลำดับ โดยเปรียบเทียบจากตัวตั้งต้นที่ไม่ได้เติมสารปรับปรุงดิน ในขณะที่ดินที่มีความเข้มข้นของแคดเมียมสูง (HCdS) เจริญเติบโตของอ้อยไม่ต่างกันทางสถิติ ดินที่มีความเข้มข้นของแคดเมียมต่ำ (LCdS) ไม่พบความแตกต่างอย่างมีนัยสำคัญในการดูดซึมของโลหะหนักโดยอ้อย (0.44-0.52 มิลลิกรัมแคดเมียมต่อกิโลกรัมของดิน และ 39.9-48.1 มิลลิกรัมสังกะสีต่อกิโลกรัมของดิน) แต่อย่างไรก็ตามการลดลงของแคดเมียมในดิน (BCR1+2) (35.4%-54.5%) และการเปลี่ยนแคดเมียมไปอยู่ในอินทรียวัตถุฟอร์มได้แสดงให้เห็นถึงประโยชน์ของของเสียต่อการส่งเสริมการเจริญเติบโตของพืชและการยับยั้งแคดเมียมในดินปนเปื้อนที่มีความเข้มข้นของแคดเมียมต่ำ (LCdS) ในทางกลับกันการเติมขี้เถ้าหม้อไอน้ำและกากหม้อกรอง ที่ปริมาณ 3 เปอร์เซ็นต์ในดินปนเปื้อนที่มีความเข้มข้นของแคดเมียมสูง (HCdS) ทำให้เกิดการลดลงของการดูดซึมของแคดเมียมในส่วนเหนือพื้นดินของต้นอ้อย (61.9% และ 48.9% ตามลำดับ) และปริมาณที่นำไปใช้ได้ของแคดเมียมในดิน (BCR1+2) (6.8% และ 4.0% ตามลำดับ) ไม่มีลักษณะความเป็นพิษของโลหะหนักในอ้อยที่ปลูกและการเคลื่อนตัวของโลหะหนักไปที่อ้อยต่ำ แสดงโดยค่าบ่งชี้การเคลื่อนตัวโยกย้ายของโลหะหนัก ($TF<1$) ผลการทดลองนี้จึงเป็นประโยชน์ต่อการคาดคะเนการเคลื่อนตัวของโลหะหนักและการวิเคราะห์เพื่อนำของเสียจากโรงงานผลิตเอทานอลกลับไปใช้ใหม่ในอุตสาหกรรมการเกษตร

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต ....
ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ....

5287849520: MAJOR ENVIRONMENTAL MANAGEMENT
KEYWORDS: BCR/ BIOAVAILABILITY/ BOILER ASH/ CADMIUM/ FILTER CAKE/ SUGARCANE WASTE-PRODUCTS/ VINASSE

PENSIRI AKKAJIT: BIOAVAILABILITY AND FRACTIONATION OF Cd AND Zn IN CONTAMINATED SOILS AMENDED BY FILTER CAKE, VINASSE AND BOILER ASH FROM ETHANOL PRODUCTION PLANT. ADVISOR: ASST. PROF. CHANTRA TONGCUMPOU, Ph.D., CO-ADVISOR: ASST. PROF. THOMAS DESUTTER., Ph.D., 148 pp.

Boiler ash (BA), filter cake (FC), and vinasse (VN) are three major wastes produced from ethanol production processes. Waste utilization from ethanol production as soil amendments and metal immobilizing agents is one of a promising and sustainable options to help utilize materials effectively, reduce waste disposal, and adds values to these waste-products. As a consequence, this research was carried out by adding BA, FC, and VN at different ratio (3% w/w) in two different Cd and Zn concentration levels of low Cd (LCdS) and high Cd (HCdS) contaminated soils, respectively for 3 months. The results showed that the addition of single BA and FC (3% w/w), and a combination of 1.5% BA and 1.5% VN (w/w) caused the significant reduction in the bioavaialble Cd concentration (BCR1+2) during the first few week of the soil pot experiment. Based on this, a four-month pot experiment of sugarcane (*Saccharum officinarum* L.) cultivation was further conducted to determine the effect of these waste-products on sugarcane growth, metal accumulation in sugarcane, and fractionation of Cd and Zn in soils by the BCR sequential extraction. Two studied soils (LCdS and HCdS) showed different results. LCdS showed the improved sugarcane biomass production; 6 and 3-fold higher for the aboveground parts (from 8.46 to 57.6 g plant^{-1}) and root (from 2.10 to 6.59 g plant^{-1}), respectively as compared to non-amended soil, while no positive effect was observed in HCdS. There was no significant difference in metal uptake by sugarcane among different treatments in LCdS (0.44 to 0.52 mg Cd kg^{-1} and 39.9 to 48.1 mg Zn kg^{-1}), however, the reduction of the most bioavailable Cd concentration (BCR1+2) in the treated soils (from 35.4% to 54.5%) and the transformation of Cd into organic-matter bound fraction (BCR3) at the end of the pot experiment highlighted the beneficial effects of these waste-products in promoting the sugarcane growth and Cd stabilization in LCdS. Alternatively, the addition of single BA and FC (3% w/w) to HCdS contributed to the reduction of Cd uptake in aboveground part of sugarcane (61.9% and 48.9%, respectively) and bioavailability of Cd (BCR1+2) in the treated soils, but not significantly at $^{*}p<0.05$ (6.8% and 4.0%, respectively). No metal toxicity visually seen in both studied soils and low metal translocation to sugarcanes was observed as indicated by low translocation index (TF<1). The results from this study could provide useful information for the recycling sugarcane waste-products generated from ethanol production plant on agricultural lands for improving sugarcane productivity and predicting metal mobility in the future use.

Field of Study: Environmental Management Student's Signature....................................
Academic Year: 2012 Advisor's Signature....................................
Co-advisor's Signature...............................

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Cd	cadmium
CEC	cation exchange capacity
BCR	sequential extraction proposed by the European Standard, Measurements and Testing (SM&T) program, formerly the Community Bureau of Reference
BCR1	exchangeable metals
BCR2	metals bound to iron and manganese oxides
BCR3	metals bound to organic matter and sulphides
BCR4	metals bound to crystal lattice of minerals
TF	transfer factor
TI%	translocation index
Total.N	total nitrogen concentration
Avail.P	available phosphorus concentration
Exc.K	exchangeable potassium concentration
Zn	zinc
BA	boiler ash
FC	filter cake
VN	vinasse
LCdS	low Cd contaminated soil
HCdS	high Cd contaminated soil
SOM	soil organic matter
OM	organic matter content

CHAPTER I

INTRODUCTION

1.1 BACKGROUND FOR THE STUDY

Cadmium (Cd) and Zinc (Zn) have been contaminated in the agricultural lands at Maesot District, Tak Province, Thailand, emancipated from Zn ore deposits and from mining activities upstream, resulting in above-normal uptake of Cd by rice (*Oryza sativa indica* L.) (Pensiri and Chantra, 2010). This contamination has been an environmental issue since 2003 after the International Water Management Institute revealed their study on Cd contamination in rice and paddy soils at considerable amounts (Simmons et al., 2003). Cd contamination in the rice plants had a direct impact on public health of the local people and there have been many studies reported in the literatures concerning the health issues of the rice consumption (Swaddiwudhipong et al., 2007; Teeyakasem et al., 2007; and Apinan et al., 2009). With the increase in public awareness regarding noxious consequences of contaminated soils in Maesot; various actions included suspended production of rice and promotion of other non-edible crops have been undertaken to reduce and stabilize Cd in soils and its uptake by rice (Phaenark et al., 2009; Akkajit and Tongcumpou, 2010).

According to the Thai energy policy of promoting alternative energy to reduce oil imports and generating the income in the local community accompanied with reducing the risks associated with Cd transfer to food chain, sugarcane plantation (*Saccharum officinarum* L.) for ethanol production had received the attention by the Royal Thai Government (RTG) and promoted in this particular area of Tak province (Silalertruksa and Gheewala, 2009; Silalertruksa and Gheewala, 2010). Sugarcane plantation is widely found in the North, Northeast and Central regions of Thailand (Silalertruksa and Gheewala, 2009). Sugarcane is one of the four major sources of biomass in Thailand other than rice, oil palm (*Elaeis guineensis*) and wood wastes and ranked as the second most economic crop of the country. Expanding agricultural production has resulted in the increased quantities of agricultural residues and agro-industrial byproducts. High tonnage of sugarcane has been projected to be used as feedstock in ethanol production, leaving large quantities of agricultural residues and agro-industrial wastes behind to be managed. Boiler ash (BA), filter cake (FC), and vinasses (VN) are three major wastes produced from ethanol production processes. Waste utilization from ethanol production processes as soil amendments is one of a promising and sustainable options to help utilize materials effectively, reduce waste disposal, and adds values to these byproducts. This approach offers the benefits of recycling residuals to reclaim a damaged or disturbed land rather than disposing of what is generally considered to be waste in landfills or by incineration (EPA, 2007). In addition, these sugarcane waste-products offer beneficial aspects for soil fertility and plant nutrition (N, P and K).

Filter cake, a solid organic residue, and vinasse, a liquid organic residue, are commonly applied to soil as a source of organic matter and soil fertility. Boiler ash, one of the wastes obtained during the process of steam and electrical power generation from ethanol production, is capable of supplying sufficient amounts of plant nutrients (Mg, S, P, K) and has favorable effects on soil water holding capacity and aeration (Jamil and Qasim, 2008). In addition to serving as fertilizer substitute, these waste-products can also be used as mulching material to protect the soil from wind and water erosion, improve soil physical properties and increase yields of various crops such as wheat and sugarcane (Jamil and Qasim, 2008). Soil and fertilizer managements are very important in most of the agricultural soils so that soil quality improvement via soil amendment incorporation is of significance and the recycling of these sugarcane waste-products is highly recommended.

Metal immobilization by addition of soil amendments may represent a potentially rapid and inexpensive approach to prevent metal uptake to the plants. Minimizing solubility and plant bioavailability of heavy metals and metalloids is possible through the use of soil amendments through the sorption on exchange sites of amended materials, absorption, complexation and/or co-precipitation of heavy metals (Simmon et al., 2010;

Burgos et al., 2010). Numerous waste-products have been widely studied and used as soil amendments and immobilizing agents (Gilbert et al., 2008; Lee et al., 2009; Janoš et al., 2010). Nevertheless, the addition of boiler ash, filter cake and vinasse has not been widely studied and thus little is known about sugarcane waste-products generated from ethanol production plants as soil amendments in reducing the bioavailability of heavy metals in contaminated soils. Understanding chemical speciation of metals is therefore necessary to define the metal bioavailability, especially before and after the addition of amendments. Changes in soil composition of amended soils and condition over time can influence the chemical forms of heavy metals in soil. Time-dependent changes in soil characteristics related to the soil pH or mineralization of organic matter may reverse stability of contaminants (Burgos et al., 2010). Understanding the aging can help in predicting the long term change in bioavailability of metals. Assessment of the changes in the metal forms and measurement of soil parameters would allow more insight into mechanisms that might be responsible for metal immobilization (Keller et al., 2005).

For this purpose, various chemical extractions have been developed to determine element behavior including single and sequential extraction. The three-step BCR sequential extraction (proposed by the Standards, Measurements and Testing Programme of the European Union, SM&T) is commonly used to determine the metal fractionation that partitions the metals into different fractions ranging from soluble and/or exchangeable to the residual fraction. The extraction procedures are very useful under defined condition for predicting the metal transformation with respect to their extraction capacity and correlation with plant uptake (Anju and Banerjee, 2010). Based on the above considerations, this research aims to investigate the influence of adding sugarcane waste-products as soil amendments on the bioavailability of Cd and Zn in soil and the influence on plant growth. Aging amended soils was performed in order to evaluate how Cd and Zn fractions are changed with time. Therefore, in order to determine the effectiveness of using sugarcane waste-products in a real scenario as soil conditioner for *in-situ* metal stabilization, an experiment with target plants is of importance to determine the metal accumulation and translocation and its response in plant. Sugarcane was then selected and used as a test plant in this study.

1.2 OBJECTIVES

1. To evaluate the effects of sugarcane waste-products (BA, FC, and VN) from ethanol production plant as soil amendments at different rates on the bioavailability of Cd and Zn in high and low cadmium contaminated agricultural soils,
2. To determine the aging effect on the mobility and bioavailability of Cd and Zn in different amendment treated soils during 3 month-trial period of pot experiment,
3. To investigate the effects of sugarcane waste-products (BA, FC, and VN) as soil amendments on sugarcane growth, and
4. To determine the translocation and distribution of Cd and Zn within sugarcane at different growth stage by time of 4 months.

1.3 HYPOTHESES

Three hypotheses were made about the effects of the sugarcane waste-products (BA, FC, and VN) from the ethanol production plant as soil amendments:

1. Sugarcane waste-products can be used as soil amendments in promoting sugarcane growth and thus subsequently reduce Cd and Zn uptake and/or translocation.
2. Cd and Zn bioavailability in soils can be reduced by the addition of amendments.
3. Aging may have the influence on the reduction of Cd and Zn, with the bioavailability declining with aging.

1.4 SCOPE OF THE STUDY

This study is focused on the use of sugarcane waste-products (BA, FC, and, VN) generated from ethanol production plant as soil amendments for promoting the plant growth and metal *in-situ* stabilization, especially Cd. The application rate was at 3%

(w/w); the three-month soil pot experiment was initially conducted without growing plant under normal day and night condition to avoid all effects of plant variations so that the results could be assigned to the sole soil-amendment interaction, allowing therefore quantification of metal bioavailability affected by the added amendments.

Sugarcane pot experiment was then subsequently performed for determination metal uptake by sugarcane and the effectiveness of waste-products in metal stabilization in soil. The application rates used in this experiment was selected accordingly to the soil pot experimental results reported the reduction in the exchangeable fraction (BCR1) of the BCR sequential extraction as it represents the fraction of metal that are mobile, easily mobilized and have the potential for the plant uptake. A four month growth stage of sugarcane was selected and performed based on the knowledge that the initial stage of plant growth tend to accumulate more Cd than at the mature stage, so that in order to reduce the risk associated with the metal uptake, and the toxicity relating to the metal translocation, initial growth stage of sugarcane is of interest.

Based on the experimental results from sugarcane pot study that highlights the impact of organic matter and pH on metal transformation; the extra soil pot experiment on the effects of different rates of amendment application was then further conducted for four-month incubation in accordance with the time of the sugarcane pot experiment.

1.5 BENEFITS OF THE STUDY

The results from this study can be used in developing a guideline for using sugarcane waste-products as soil amendments in the agricultural soils for improving agricultural productivity, fertilizer management and metal *in-situ* stabilization.

1.6 ORGANIZATION OF THE CONTENT

The content begins with the introduction (Chapter I); theoretical background and literature reviews (Chapter II); and methodology (Chapter III). The following (Chapter IV and V) is focused on the experiment results using sugarcane waste-products generated from ethanol production plant as soil amendments in reducing Cd and Zn bioavailability in soils and metal uptake by sugarcane, respectively. The effects of organic matter in the amendments on metal-organic-bound fraction (BCR3) were further discussed in Chapter VI. Conclusion and recommendations are in Chapter VII.

CHAPTER II

THEORETICAL BACKGROUNDS AND LITERATURE REVIEWS

Industrialization and agricultural activities lead to the production of waste-products that cause the environmental problems, especially heavy metal contamination, if the management is improper. Risk to human health via food chain contamination is very critical particularly in agricultural land contaminated with heavy metals. The reclamation of contaminated lands has been done by many studies through the physical and/or biological technologies (Singh et al., 2009; Wuana and Okieimen, 2011). However, the utilization of waste-products as a soil amendment might be an alternatively suitable option for reclamation of contaminated soils in reducing metal bioavailability by *in-situ* stabilization and improving soil quality due to its availability, high fertility and wastes-disposal associated costs. Therefore, it is necessary to gain a better understanding of chemical characteristics of the soils, especially after the addition of amendments, and its interaction with the amendments. This chapter provides necessary background of soil amendments, metal bioavailability, and includes some related literature and studies of using soil amendments in reducing metal bioavailability which is divided into sections as follows: I. theoretical background; and II literature reviews.

2.1 THEORETICAL BACKGROUNDS

2.1.1 Amendments

Amendments are generally any residuals from other processes and/or any material added to the soil to improve soil properties. Application of soil amendments to agricultural soils can be beneficial by restoring soil quality through balancing pH, adding organic matter, improving soil structure, increasing water holding capacity, re-establishing microbial communities, adding nutrient supply for plant growth, and alleviating compaction (EPA, 2007). There are generally two categories of soil amendments: organic and inorganic. Organic amendments come from living things such as peat, compost, manure, straw, wood chips, and on the other hand, inorganic amendments are man-made and/or from coal-combustion or other types of industries include phosphate fertilizer, sand, pea gravel and etc. (Davis and Wilson, 2005).

After incorporation of soil amendments, the physical, chemical and/or biological condition of soil can be substantially altered due to the characteristics of the added amendments and may include mineralization and degradation of organic matter by microorganisms, and/or physicochemical changes (pH, CEC, etc.). The increase in organic matter content following organic amendment application often results in an improvement in soil physical properties such as increased soil aggregation, soil porosity and water drainage. Improvement in plant productivity has been reported by other due to the improvement of the nutrient content in soils (Pérez-Estebana et al., 2011).

Such changes in soil characteristics after amendment incorporation may have an influence on Cd and Zn bioavailability, both directly (through loads of heavy metal, sorption capacity of inorganic and organic components) and indirectly (through the property changes such as pH and CEC). Soil-based management via addition of amendments to immobilize metals/metalloids offers great opportunity effectively to minimize metal bioavailability in soils. Introduction of organic matter for example, can retain metals in the soil against leaching and/or plant uptake. Metals in soil can bind strongly to the organic matter, thereby reducing metal bioavailability and plant toxicity. The increment of soil organic matter content encourages metal redistribution from soluble/exchangeable form to the formation of stable metal-organic complexation which could diminish the solubility of the metal ions in the soils. The effect of organic matter on metal mobility has been reported by others (Janoš et al., 2010; Pérez-Estebana et al., 2011; Hernandez-Soriano and Jimenez-Lopez, 2012). Insoluble complexation of metals/metalloids, chemical precipitation and sorption mechanism are the possible mechanisms that retain metals in the soil and thus reducing metal accumulation by plants.

In addition to the increase in organic matter, the change of soil pH after amendment application is one of the significant factors that play a role in metal sorption through its effect on surface charge. Raising soil pH using different amendments to reduce plant metal uptake and enhancing metal bioavailability reduction have been reported by others (Beesley et al, 2010; Houben et al, 2012; Hale et al, 2012). Figure 2.1 shows the role of amendment application in reducing metal bioavailability and its uptake by plants.

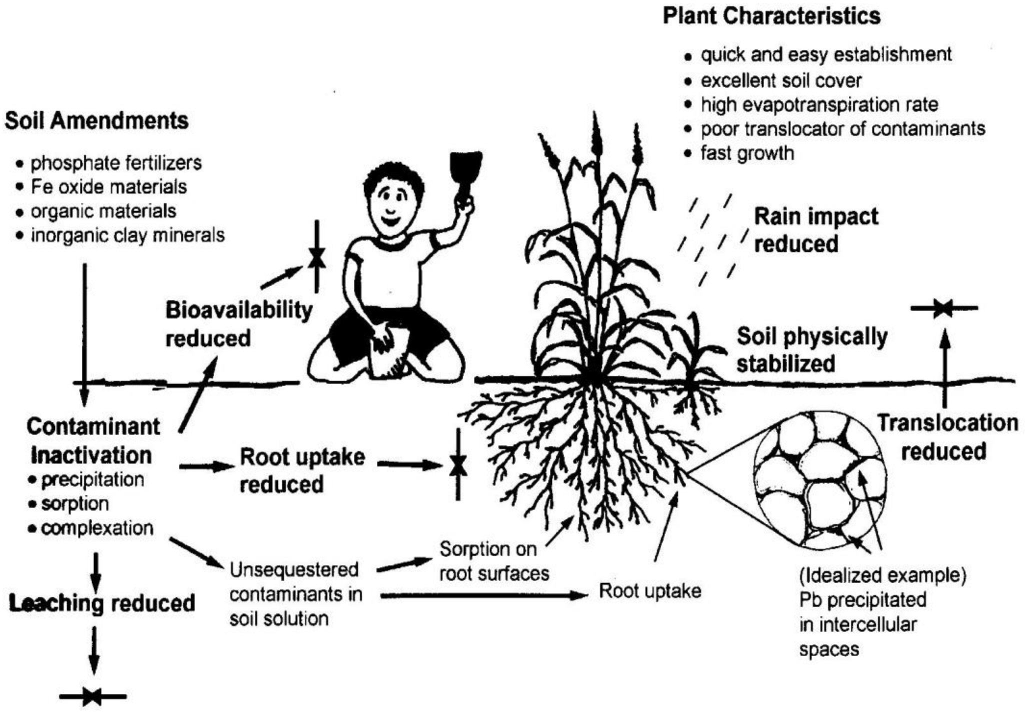


Figure 2.1 Role of soil amendment (source: US EPA 2010)

Metal immobilization by addition of soil amendments appears to be an easy, inexpensive, and flexible approach that may be suitable for application in agricultural soils with low metal concentrations. Numerous waste-products have been widely studied and used as soil amendments and immobilizing agents. Regarding this aspect, there are many studies in the literature assessing the effectiveness of using different amendments (organic, inorganic, waste-product) to immobilize metals in contaminated soils. Table 2.1 shows selected studies using different organic amendments to immobilize Cd and Zn bioavailability in soils.

Table 2.1 Selected literature reviews using different organic amendments to immobilize Cd and Zn bioavailability in soils

Organic amendments	Application rates	References
Biosolid	0, 30 Mg ha^{-1}	Guerra et al. (2007)
	30, 60 Mg ha^{-1}	Pérez-de-Mora et al. (2007)
	20, 200 g kg^{-1}	Sánchez-Martin et al. (2007)
	0, 20000, 80000 kg ha^{-1}	Florido et al. (2011)
	225000 kg ha^{-1}	Siebielec and Chaney (2012)
Farmyard manure	5, 15 g pot^{-1}	Li et al. (2008)
	0, 30, 60, 90 Mg ha^{-1}	Benke et al. (2008)
	350 kg N ha^{-1}	Doelsh et al. (2010)
	40, 80, 120 t ha^{-1}	Achiba et al. (2010)
	30, 60 Mg ha^{-1}	Pérez-Esteban et al. (2011)
	5% (w/w)	Houben et al. (2012)
	1% (w/w)	Hernandez-Soriano and Jimenez-Lopez (2012)
Compost	0, 30, 60, 120 g kg^{-1}	Liu et al. (2009)
	1, 10, 20% (w/w)	Nwachukwa and Pulford (2009)
	9, 10, 90 t ha^{-1}	Santos et al. (2010)
	1:3 (w/w)	Tabia et al. (2010)
	50% (v/v)	Beesley et al. (2010)
	80 g pot^{-1}	Ingelmo et al. (2012)
Industrial byproducts	5, 7.5, 10 Mg ha^{-1}	Tejada et al. (2006)
	224 t ha^{-1}	Gilbert et al. (2008)
	2, 5% (w/w)	Lee et al. (2009)
	5% (w/w)	Houben et al. (2012)
	1% (w/w)	Rodriguez-Jorda et al. (2012)

There are many kinds of materials used as soil amendments, but there have been few studies that have investigated the influence of sugarcane waste-products generated from ethanol plants as soil amendments on the bioavailability Cd and Zn. The following is the detail and characteristics of the ethanol production wastes-products.

2.1.2 Ethanol Process

The simplified overview of ethanol production from sugarcane of Mae Sot Clean Energy Company Limited (Mae Sot district, Tak Province) is illustrated in Figure 2.2. According to the ethanol production process, boiler ash (BA), filter cake (FC), and vinasses (VN) are three major wastes produced. In summary, the shoots of sugarcanes are washed and shredded into smaller pieces. The feedstock is fed to the filter press and extracted into juice (1) and bagasses (2). Bagasses with low moisture content are then sent to the boiler and served as boiler fuel for steam and electricity generation. Boiler ash is generated from this process as waste-products. High Test Molasses (HTM) is the concentrated juice (with 85 brix) will be stored for the off-season use (3). After cane juice is extracted; the juices are clarified by the addition of lime and undergo heating and filtration. The residuals remaining after filtration are called filter cake. The clear juice, the one to produce alcohol in the ethanol production processes, is then fermented by addition of yeast to transform into ethanol. The final process is distillation to produce 99.5% of the ethanol where vinasse is generated as a waste-product. Throughout the process of ethanol production of Mae Sot Clean Energy Company Limited (Mae Sot district, Tak Province) using sugarcanes as a feedstock, each tonne of sugarcane produce 85 liter of ethanol; 10% from the distillation process come out as the ethanol; the remaining 90% is the vinasse, whereas 4% of filter cake came out from the filtration process and 2% of boiler ash generated from the steam and power generation.

2.1.2.1 Boiler ash

Boiler ash is a fine particle with the color of grey to dark black. Boiler ash consists of hollow spheres and contains high concentrations of aluminum oxides and hydroxides which might be used as adsorbing materials for heavy metals. Boiler ash mixture can also be used as a soil additive due to its capacity to supply plant nutrients (Mg, S, K, P, Fe, Mn, Zn and Cu) and improve some chemical and physical properties of soils. When applied to fields as a soil amendment its favorable effects on soil water holding capacity and aeration is very pronounced (Jamil-Khan and Qasim, 2008). The application of boiler ash, like other organic amendments, can also produce good results regarding yields of various crops such as sugarcane (Jamil Khan and Qasim, 2008).

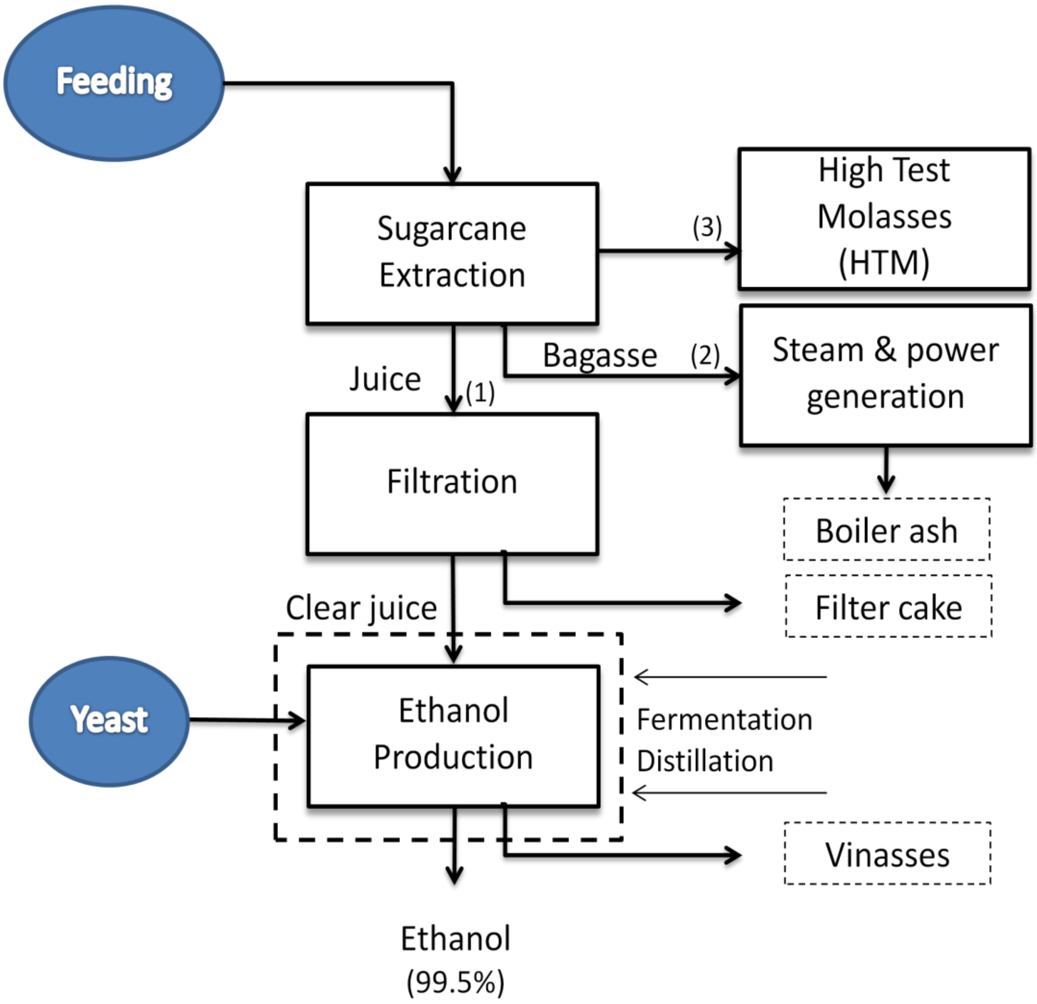


Figure 2.2 Schematic diagram for ethanol productions using sugarcane

2.1.2.2 Filter cake

Filter cake, the precipitated impurities present in the cane juice after removal by filtration, varies in the composition depending on the cane variety, process efficiency and method of filtration and clarification. Filter cake can be a useful fertilizer due to its significant amount of plant nutrients (N, P, and K) and organic matter. Use of filter cake as a soil amendment is the most ideal due to its nutritional value and it is useful as a conserver of moisture and as a soil conditioner. Filter cake contains about 50 to 70% moisture, 5 to 14% crude wax and fat, 15 to 30% fiber, 5 to 15% sugar, and 5 to 15% of crude protein, with appreciable amounts of Si, Ca, P_2O_5, MgO, Fe, and Mn (Yadav and Solomon, 2006).

2.1.2.3 Vinasses

Vinasse or fermentation waste is a valuable liquid organic waste-product generated from distillation after alcohol fermentation. Vinasse is a dark brown, viscous and concentrated liquid, with an unpleasant odor and high turbidity. Vinasse has a high content of inorganic salts, 8 to 10% total solids and contain considerable quantities of other mineral nutrients. Its potassium content is particularly high (8,000 to 10,000 mg L^{-1} effluent) and can be used as fertilizer to supply nutrients into the plant and soil system (Yadav and Solomon, 2006). The physical and chemical characteristics of VN vary widely depending on raw materials and operating conditions of the fermentation and distillation processes. Applying VN to soils of sugarcane fields is currently the most common practice and is part of an integrated irrigation and fertilization system. Recycling VN onto the land by dumping can lead to changes or alterations in soil minerals (Bueno et al., 2009) and crop yields may suffer due to the anoxic conditions created in the soil. Therefore, certain precautions must be taken when applying VN to soil and treatment of VN before its application may be necessary. However, there is still debate about whether the nutrients in the vinasse would eventually compensate for the initial, apparent detrimental effect (Resende et al., 2006).

2.1.3 Bioavailability

Metal speciation is of major importance to provide the qualitative information on their reactivity under changing conditions and crucial information about ecotoxicity characteristics (reactivity, bioavailability, toxicity) of contaminants (Sparks, 1995; Herreweghe et al., 2003). The behavior of the elements in the environment cannot be predicted on the basis of their total concentration because it overlooks the fact that not all of the metal may be labile or available for uptake (Gobran et al., 2001). The total metal content of a soil is distributed among all possible chemical forms (speciation) in the solid, liquid or the biotic phases (Vig et al., 2003). Use of total concentration as a criterion to assess the potential risks may be misleading as the risks may be over-estimated, implies that all forms of a given metal have an equal impact on the environment (Tessier et al., 1979). Despite this, the bioavailability of metals and the toxicity of metals are more closely related to bioavailable metal, rather than total metal concentrations (Bettinelli et al., 2000). Knowing the distribution and transformation of metals under changing conditions of environment is therefore very useful to understanding the processes whereby metals are held in soil and the condition under which they can be released.

In soil, metals exist as a variety of chemical species in a dynamic equilibrium governed by soil physical, chemical, and biological properties (Lasat, 2002). Bioavailability can be defined as the fraction of the total metals that are available for incorporation into receptors through direct absorption or uptake. Metal bioavailability and metal uptake by plants is strongly influenced by the metal in liquid phase rather than in solid phases of the soils. Soil solution is composed of a variety of ion species, either as complex or noncomplex. There are various complex chemical reactions that occur in the soil solution. Figure 2.3 illustrates the possible mechanisms and the pathway of metal ions occurring between the soil solution and the transfer to the plants.

The transfer of metals from soils to the plant is when metal ions present in the soil solution 1) can be taken up by plants and/or 2) in the soil solution as free metal ions; or 3) it can be sorbed on organic and inorganic component of the soil particles. Once the metal ions are taken up by roots 1a); then it move up and transport through the shoot 1b) and later undergo sequestration and/or compartmentation 1c). When metal ions and ligands directly interact where no water molecules are present in between is called the inner-sphere complex. The outer-sphere complex is alternatively formed when water molecule is present in between metal ions and the ligands (3). Precipitation of metals is occurred if the soil solution is super saturated (4). The term sorption is used when the retention mechanism at the soil particles is unknown, in which there can be adsorption, surface precipitation and polymerization.

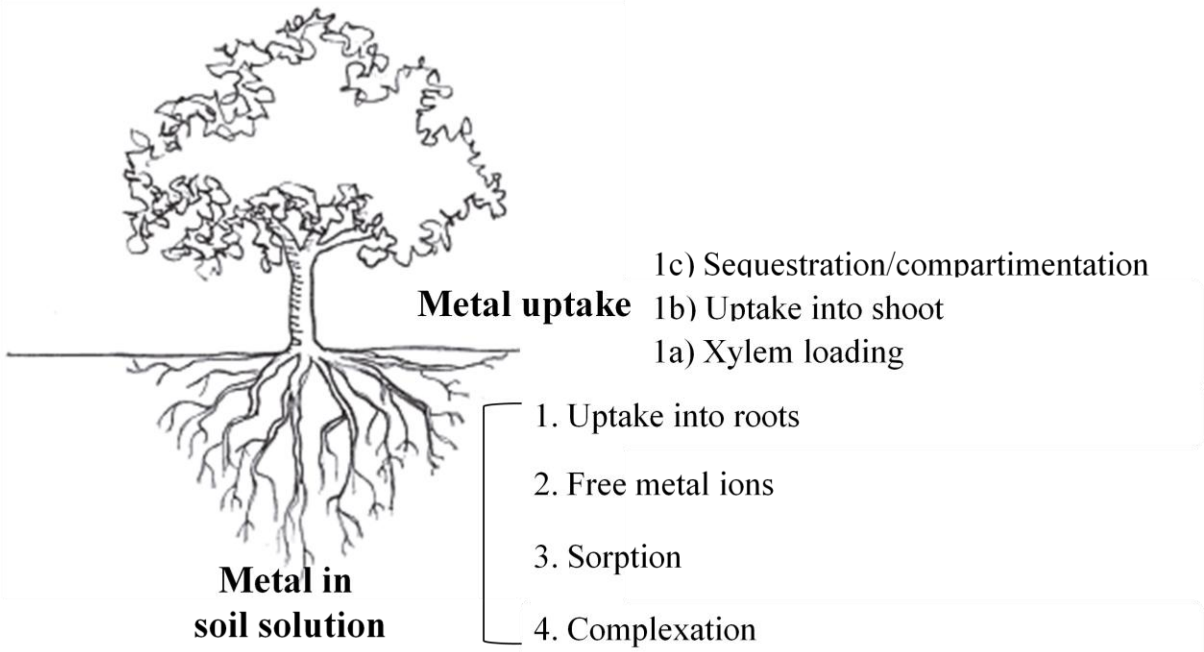


Figure 2.3 possible mechanisms and pathway of metal ions between the soil solution and the plants. (Modified from Singh and Prasad, 2011)

2.1.3.1 Factors effecting metal bioavailability

It can be seen that the metal bioavailability is strongly affected by the adsorption processes. Physical and chemical forces are involved in the adsorption process included physical forces (van der Waals force-partitioning and electrostatic outer-sphere complexes-ion exchange) and chemical forces (inner-sphere complexation such as covalent and hydrogen bonding). Environmental factors have effects on the adsorption process included soil pH, amendment incorporations, the presence of other metal ions, etc. Therefore, because of its effect on metal sorption and plant nutrient availability, soil organic matter content (SOM) and soil pH that has an influence on the fate and transport of metals in soils is covered for a further in-depth discussion as the followings.

2.1.3.1.1 Soil organic matter (SOM)

Soil organic matter (SOM) is a mixture of plant and animal residues in different stages of decomposition, substances synthesized microbiologically and/or chemically from the breakdown products. Soil organic matter content usually ranges from 0.5 to 5% on a weight basis in the surface of mineral soils to 100% in organic soils (Histosols). SOM plays a significant role in affecting the chemistry of soils due to high specific surface, buffering capacity of the soil, and the sources of the nutrients. Even at low levels, the reactivity of SOM is very high. The following is the properties of SOM that affect on chemical and physical properties of soils;

- Improving the moisture retaining properties and water holding capacity of soils (water retention),
- Increasing soil permeability and aeration,
- Enhancing the availability of macronutrients (N, P, K) to plants,
- Maintaining the uniform reaction in the soil via exhibits buffering action in slightly acid, neutral and alkaline ranges, and
- Increasing the CEC of the soils

Soil organic matter consists of nonhumic and humic substances; nonhumic substance consists of low molecular weight acids, carbohydrates, protein, peptides, amino acids, fats, waxes which persists in the soil for a short time. Humic substance alternatively is characterized as high molecular weight and refractory that can be subdivided into humic acid (HA) and fulvic acid (FA), and humin which the classification are based on the solubility in acid and base. SOM has many functional groups; the most common are hydroxyls, carboxyl, phenolic, and amines. The component of SOM constituents has a high affinity for metal cations because of the presence of ligands or functional groups that can chelate metals (Harter and Naidu, 1995). Figure 2.4 shows functional groups in soil organic matter.

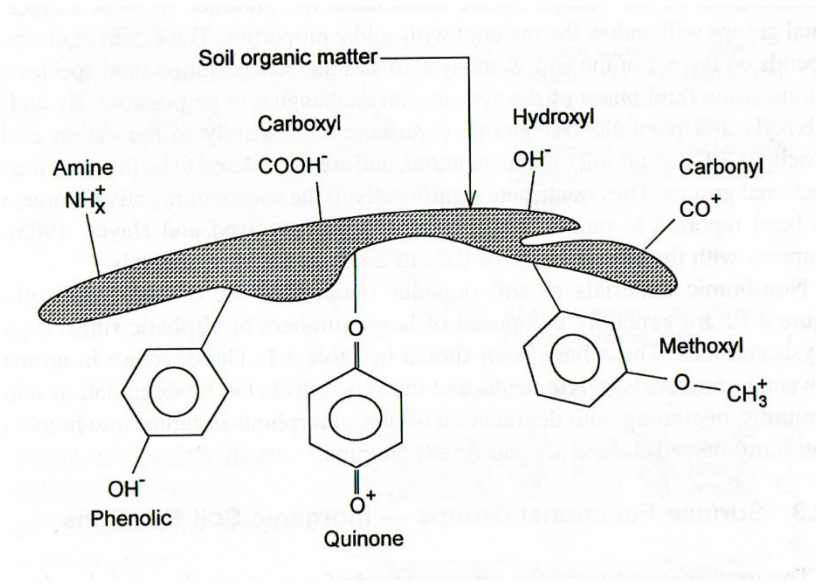


Figure 2.4 Examples of the functional group associated with soil organic matter (SOM) (Source: Yong, 2002).

The SOM-metal reactions include reaction between dissolved organic matter and metal ions; and particulate organic matter and metal ions (complexation). With increasing pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in soil organic matter dissociate, thereby increasing the affinity of ligand ions for metal cations. The general order of affinity for metal cations complexed by organic matter follows (Adriano, 2001): $Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$. Figure show the various types of surface functional groups of SOM.

Based on the above mentioned properties of the soil organic matter, SOM is very important in determining mobility and retention of metals in soil solution through affecting the chemistry of SOM-mineral complexes, stability of organo-mineral aggregates, fate and transport of heavy metals. SOM can reduce metal bioavailability/mobility by forming stable complex with humic substance. Alternatively, SOM can cause an increase in metal bioavailability by forming water soluble chelating complex of organic compounds rich in functional groups with the metals, thus increase metal mobility. The effects on metal bioavailability between soil organic matter and metals depend on different factors, such as soil and amendment pH, and the type of organic matter added.

The addition of sugarcane waste-product as soil amendments provides a significant amount of plant essential nutrients (N, P, K) and high proportion of organic matters. However, soil organic matter (SOM) present in the amended soils can increase the metal concentration in soil, metal bioavailability, and its uptake by plants. The speciation of metals could influence by the nature and transformation of the added sugarcane waste-products through the metal interaction with newly added humic substance. Shortly after the addition of organic material to the soil, the effect on soil properties depend on the degradation process of amended materials, which in turn affects the speciation of metals. Therefore, more attention is given to the soil organic matter and its reaction with soil particles.

2.1.3.1.2 Soil pH

Soil pH is considered as the most important factor affecting metal speciation (Selim and Kingery, 2003) and behavior in soil systems both directly and indirectly. The influence of soil pH on metal availability is due mainly to its effect on the reactions controlling concentrations of metal in the soil solution (Sparks, 1995). Metal speciation changes with pH, affecting metal sorption/desorption on solid surfaces as well as the solubility of metal hydroxide minerals. Metals tend to be adsorbed at different pH values. This results in precipitation of different metals over a large range of pH units. For example, Cd and Zn tend to have adsorption edges at higher pH than Fe and Cu, and consequently they are likely to be more mobile and more widely dispersed (Kabata-Pendias and Pendias, 2001). The most mobile fractions of ions occur at a lower range of pH and at a lower oxidation-reduction potential (Sparks, 1995).

2.1.3.1.3 Aging

Soil properties, including pH, organic matter and clay contents, surface charge, soil solution composition, and contaminant physicochemical properties are responsible for controlling the behavior of metals present in the soil system and affecting the bioavailable fraction in a given soil. These properties are further impacted by the duration of contact (time) between metals and soils with bioavailability declining with aging. A decrease in bioavailability of metals with its contact time with soil is often defined as “aging” (Tang et al., 2006). Aging is expected to have a significant influence on the mobility and bioavailability of trace metals (Lu et al., 2009). The phenomenon of aging (sequestration), in general, results in the apparent decline of bioavailability associated with persistence and increased resistance of the metals as they are transformed into more stable forms with time. Generally, bioavailability of trace metals decreases with increasing time due to the reactions between metal ions and soils; the possible mechanisms included complexation, adsorption, precipitation of metal ions onto particle surfaces, diffusion into the mesopores and micropores of soil (Lu et al., 2005) and/or partition to specific and non-specific sites of organic matter (Naidu and Bolan, 2008). A number of mechanisms may be operational in the retention of heavy metals, and the dominant mechanism may vary with incubation time (Tang et al., 2006).

Aging of contaminated soils and the fate of associated metals can result in sequestration of compounds and is illustrated by Figure 2.5 as 1) once metal is entering into soil solution, 2) it transports into soil macropores, 3) interacts with colloid particles (via either specific or non-specific sorption), 4) binds to the surface of soil colloids and then redistributes among soil solution, solid minerals, and organic phases. With aging, the sorption of metals onto soil particles plays a significant role in determining its mobility and bioavailability. As the initially surface-sorbed metals are slowly redistributed, 5) some move to the interior of soil aggregates (subatomic pores), 6) with a small proportion diffusing into the solid particle from which desorption becomes slow and hence contaminants become to receptors.

In fact, adsorption of trace metals is a rapid process and occurs during the first few hours or days and then slows with time. The initial fast reaction is considered to be metal adsorption on the soil or colloid surface via forming outer sphere complexes driven by the difference in the concentration gradient from the solution phases to the surface of soil minerals and negatively charged organic matter. Following the rapid adsorption, the very much slower continuing sorption reactions can be due to the diffusive penetration or chemisorptions of trace metals into the soil constituents and precipitation (shift from outer sphere to inner sphere metals into Fe-oxides, hydrous oxides of Al and Mn, clay minerals and by diffusion or precipitation in carbonates) (Jalali and Khanlari, 2008).

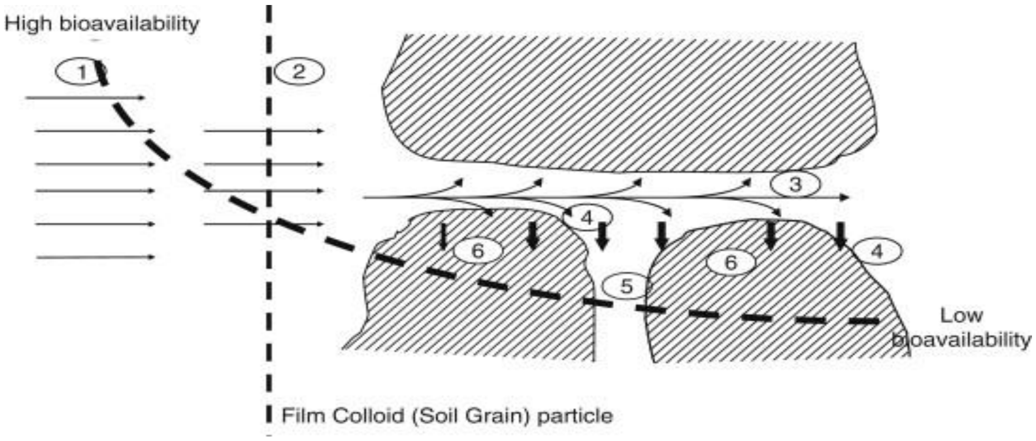


Figure 2.5 Fate of chemicals in soil chemistry (Naidu and Bolan, 2008)

Therefore, in order to understand time effects on metal bioavailability, detailed information on long-term studies is needed on the fractionation of metals after different residence times. The following equation proposed by Naidu and Bolan (2008) shows the relationship of metal bioavailability and other influenced factors (the function of soil properties, environmental conditions, plant characteristics, microorganisms, and the aging time) affecting metal bioavailability.

Bioavailability = f (soil properties · env.conditions · plant characteristics · microorganisms)T

2.1.3.2 Sequential extraction

From the above consideration it can be seen that solid phase metal fraction is very important for metal bioavailability. Therefore, a number of chemical extractions have been developed to determine element behavior including single and sequential extraction in order to fractionate the metals into specific forms/fractions and become a conventional approach. Single and sequential extraction procedures have been commonly applied to assess the mobility and partitioning of elements in sediments, soils and waste materials, enable forms or phases to be measured.

Single extraction uses only one solvent aiming directly for the determination of the available form for the plant uptake. The extractants commonly used include H_2O, KNO_3, EDTA, DTPA and HNO_3. Each extractant is targeted on a single form of each metal depending on the nature of the reactant used, for example H_2O for soluble, KNO_3 for exchangeable, EDTA for carbonate and sulfide precipitation and HNO_3 for residual form of each metal (Iwegbue et al., 2007). Among the various chemical extractant used in the literatures, DTPA (Diethylenetriaminepentaacetic acid), is a chelating agent that transfers metals bound to the solid soil phases into the soil solution by forming water soluble complexes with heavy metals, is selected and used in this study due to the pH of studied soils that are slightly acidic and near neutral.

Sequential extraction, alternatively, is one of the most useful tools to study the fate and transport of metal. Several sequential extractions are commonly used in literature and differ by the number of the steps and reactant used. Among the sequential extraction procedures, the three-step BCR sequential extraction procedure according to the Standards, Measurements and Testing Programme of the European Union (SM&T) is used in this study for determination metal bioavailability. The BCR sequential extraction is an operationally defined sequential extraction procedure. This procedure was selected from several procedures because of its selectivity, reproducibility and repeatability of different step. Table 2.2 shows selected literature reviews using BCR sequential extraction in Cd and Zn bioavailability determination in different solid matrix. There are three successive extraction steps (BCR1, BCR2, and BCR3) with the additional step of the residual extraction (BCR4). Mobility and bioavailability of the metals decrease as to the increasing order of the extraction sequence. Each successive form represents less availability. Table 2.3 summarized the chemical fractions obtained in each step of the BCR sequential extraction and its associated forms of metals. The detail of extraction procedure is given in Appendix A.

The detailed information about origin, mode of occurrence, mobilization and transport of trace metals can be obtained by the operationally defined fractions. The resulting extract is based on the proposed chemical association between the extracted species and solid phases in which it is associated. The principle of this procedure is based on the selective extraction of heavy metals in different physicochemical fractions using a series of extracting reagents of increasing strengths to fractionate elements into several forms with varying mobility. At first, the most mobile fractions will be extracted followed by increasingly absorbed fractions and finally those fractions that are strongly attached to the soil particles. Among the chemical forms, the exchangeable (BCR1) and reducible (BCR2) fractions of the BCR sequential extraction can be used to determine the environmental risk associated with the plant uptake due to it represent the most mobile and bioavailable fraction and high potential for the plant uptake as identified as the direct effect fractions (Filgueiras et al., 2002; Pensiri and Chantra, 2010; Paradelo et al., 2011), while those in the latter stages of the extraction schemes are less available to plants. Therefore, only the first-two step of the BCR sequential extraction was focused throughout in this study with the intension for determination of the bioavailability of metals, particularly Cd.

Table 2.2 Selected literature reviews using BCR sequential extraction in determination Cd and Zn bioavailability in different solid matrix.

Matrix	References
Soils	Favas et al. (2011); Maderova et al. (2011); Waterlot et al. (2011); Bade et al. (2012); Guillén et al. (2012); Wu et al. (2012); Baraud and Leleyter (2012)
Sediments	Janoš et al. (2010); Martínez-Fernández et al. (2011) Nemati et al. (2011); A´ lvarez and Carrillo (2012) Chakraborty et al. (2012); (Qiao et al. 2012); Zhong et al. (2013)
Manures	Achiba et al. (2010)
Wastewater sludge	Fuentes et al. (2004); Kazi et al. (2005); Kazi et al. (2006)
Fly ash	Houben el al. (2012); Qui et al. (2012); Świetlik et al. (2012)
Biofilm	Horváth et al. (2013)

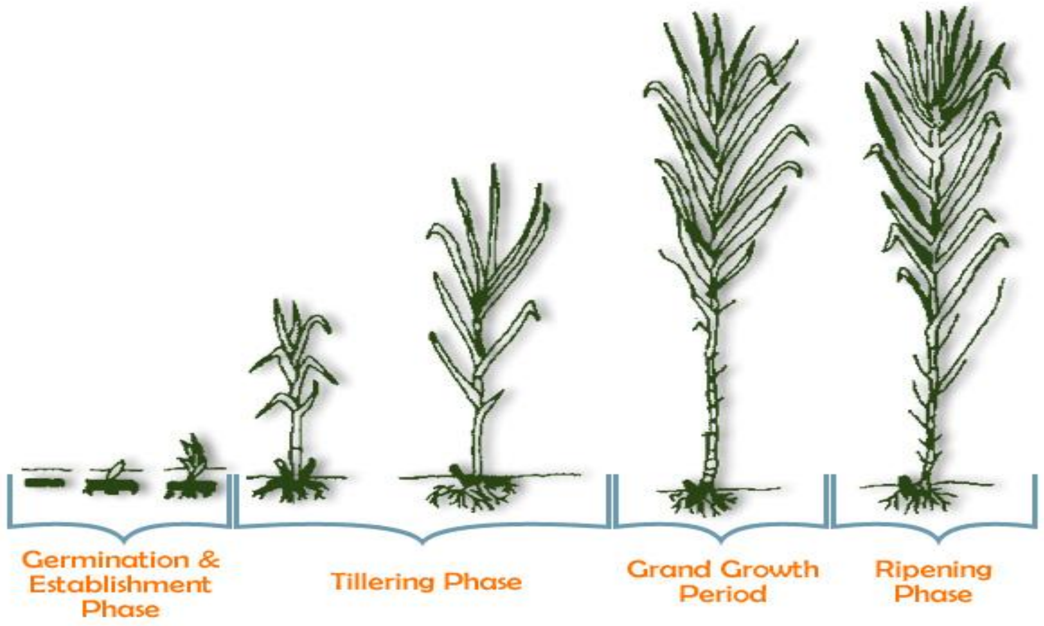
Table 2.3 The three-step BCR sequential extraction and the additional residual fraction (modified from Pérez and Valiente, 2005)

BCR fraction	Reagent used/condition	Associated forms of metals
BCR1: Exchangeable	Acetic acid (CH_3COOH) Shaken for 16 h, 30±10 rpm at 22±5 ºC	Metal in ionic, exchangeable, carbonate, acid soluble forms (lowering pH, change in ionic composition)
BCR2: Reducible	Hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) Shaken for 16 h, 30±10 rpm at 22±5 ºC	Metals associated with hydrous oxides of Fe/Mn, coated on mineral surface by process of co-precipitation, adsorption, surface complex (under anoxic-reducing condition, insoluble sulfide)
BCR3: Oxidizable	Hydrogen peroxide and ammonium acetate solution (H_2O_2+NH_4COOCH_3) 1 h at 22±5 ºC and for 1 h at 85±2 ºC (repeat), and Shaken for 16 h, 30±10 rpm at 22±5 ºC	Metals associated with organic matter, sulfides (decomposition of OM)
BCR4: Residual	*Aqua regia* extraction (HNO_3+HCl) Shaken for 16 h, 30±10 rpm at 22±5 ºC	Metals associated with silicates, crystalline phases (remaining from the previous steps) (weathering process)

2.1.4 Sugarcane

Sugarcane (*Saccharum officinarum* L.) plantation is widely found in the North, Northeast and Central regions of Thailand which ranked as the second most economic crop of the country. The cane planting area in Thailand covers about million hectares and the production is around 55 to 60 million tonnages per year (Prasertsak 2011). The crop can be harvested around 10 to 12 months after cultivation. Sugarcane has essentially four-growth phases which are 1) germination phase, 2) tillering phase, 3) grand growth phase and 4) maturity and ripening phase (Kuyper, 1952). A brief summary of the different stages of sugarcane growth is given in Figure 2.6.

Based on our knowledge and literature reviews, it was found that sugarcane tend to accumulate more Cd at the early stage of growth than at the mature stage. The older plant is likely to concentrate less metal than the younger, 3 to 5 months is the most active phase for sugarcane, so that in order to reduce the risk associated with the metal uptake, and the toxicity relating to the metal translocation, initial growth stage of sugarcane is of interest in this study. Four month growth stage of sugarcane was then selected and performed in this study with the purpose of determining metal uptake at the initial stage of plant growth.



Phases I : **(0 -1 month)**	**Phases II:** **(1-4 months)**	**Phases III:** **(4-8 months)**	**Phases IV:** **(8-12 months)**
The germination phase is the time from planting to the completion of germination of buds	Tillering is a physiological process of repeated underground branching from compact nodal joints of the primary shoot. Early formed tillers give rise to thicker and heavier stalks.	Most important phase of the crop wherein the actual cane formation and elongation and thus yield build up takes place. Leaf production is frequent and rapid during this phase Stalks grow rapidly almost 4-5 internodes per month.	During this phase, sugar synthesis and rapid accumulation of sugar takes plac and vegetative growth is reduced. Cane ripening proceeds from bottom to the top and hence bottom portion contains more sugars than the top portions.

Figure 2.6 The four essential growth phases of sugarcane

(Source: http://www.sugarcanecrops.com/crop_growth_phases/)

2.2 LITERATURE REVIEWS

2.2.1 The uses of various soil amendments in soil quality improvement

Organic, inorganic materials and waste by-products from industrial processes have been widely used as soil amendments. The use of these materials in agriculture offer many benefits including improved soil properties, plant essential nutrients, and metal immobilization in contaminated sites. Different amendments were studied extensively both in greenhouse experiment and in the field sites to evaluate the effectiveness of materials; the following is the literatures of using different materials for improving soil quality.

Tejada et al. (2007) examined two beet vinasse (BV) [fresh BV and composted BV mixed with crushed cotton gin compost (CV)] for their potential in soil restoration. These vinasses were applied annually at 5, 7.5, and 10 t ha^{-1} rates, respectively for a period of four years to a Xerollic Calciorthid soil, Spain. After 4 years of successive soil amendment, the percentage of plant cover decreased 58.3% in BV-amended soils whereas it increased 86% in CV-amended soils, both with respect to the non-amended soil. The application of fresh BV had a detrimental impact on the soil's physical (structural stability decreased 25.2% and bulk density increased 22.9% with respect to the control soil), chemical (exchangeable sodium percentage increased 86.9%), and biological properties (microbial biomass, soil respiration, and dehydrogenase, urease, BBA-protease, aˆ-glucosidase, phosphatase and arylsulfatase activities decreased by 44.9%, 26.2%, 17.6%, 14.8%, 11.1%, 5%, 63% and 59.6%, respectively). This is likely due to the high quantities of monovalent cations, such as Na^+, and fulvic acids were introduced into the soil by the BV, thus destabilizing its structure. However, they observed that when BV was co-composted with a cotton gin crushed compost, the resulting compost had a positive effect on the soil's physical (structural stability increased 26.5% and bulk density decreased 26.3% with respect to the control soil), and biological properties (microbial biomass, soil respiration, and dehydrogenase, urease, BBA-protease, aˆ-glucosidase, phosphatase and arylsulfatase activities increased by 57.1%, 76.4%, 98.4%, 98.2%, 99.8%, 99.4%, 89.8% and 92.3%, respectively, with respect to the control soil).

Ram et al. (2007) studied long term field trials of Lignite Fly Ash (LFA) application for improving soil fertility and crop productivity of groundnut, maize and sun hemp. LFA was applied at various doses with and without press mud (PM) (up to 200 t ha^{-1} of LFA and 10 t ha^{-1} of PM). Chemical fertilizer, along with gypsum, humic acid and biofertilizer was also applied in all treatments as supplementing agents. The result showed increased yield (7 to 89%) of the highest dose of LFA (200 t ha^{-1}) and a greater increase in the content of major and secondary nutrients, and micronutrients, from combination treatments and from LFA treatment alone. The authors found that application of various dosages of LFA up to 200 t ha^{-1}, alone and in combination with press mud, improve the physicochemical properties and fertility of the soil and even though there was a small increase in trace element, these levels were within permissible limits.

Bueno et al. (2009) carried out an experiment to identify the impacts caused by the addition of wine vinasse on some chemical and mineralogical properties of a Luvisol and a Vertisol in La Mancha, Spain. Two hundred grams of agricultural soils were amended with increased amounts of wine vinasse (1, 5, 10, 20, 40, and 70 ml). The results showed a slight variation in pH, rising or falling less than one unit. No significant changes in the clay minerals were observed after amendment with vinasse. The authors concluded that the little variation in acidity is due to the condition of the vinasse and the properties of the soil. The increase in salinity leads to changes in crop productivity and the fluctuation of electrical conductivity with dose and time of application of the vinasse.

2.2.2 The uses of various soil amendments in Cd and Zn immobilization

Li et al. (2008) investigated the effects of both organic and inorganic amendments (limestone, calcium magnesium phosphate, calcium silicate, Chinese milk vetch pig manure, peat, and Zn sulfate) on the bioavailability and the uptake of Cu and Cd by rice in a paddy soil. They found that the concentrations of available Cu and Cd in the soils decreased consistently with increasing rates of amendments application. Compared with the control, the application of 5 g inorganic amendments (limestone, Ca-Mg-P fertilizer, and silicon fertilizer) lowered the concentrations of available Cu by 84.7%, 19.4%, 56.0%, and the available Cd by 29.3%, 9.4%, and 18.8%, respectively. However, only slight decreases in the concentrations of available Cu and Cd (3.1 to 5.2%) were observed in the treatments of Zn fertilizer. Not only inorganic amendment can reduce the bioavailable Cu and Cd concentrations, but the application of organic amendments also reduced the soil available Cu and Cd. Application of 10 g pig manure and Chinese milk vetch and 30 g peat lowered the concentrations of available Cu by 76.1%, 69.3%, 70.8%, and available Cd by 25.7%, 19.9%, 23.0%, respectively. The ranking of available Cu and Cd of different amendments followed the order: limestone < pig manure < peat < Chinese milk vetch < silicon fertilizer < Ca-Mg-P fertilizer < zinc fertilizer < control. The authors also observed that higher rate treatment resulted in a significant lower concentration of available Cu and Cd than the lower rate treatment of the same amendment except for zinc fertilizer.

Tabia et al. (2010) determined the capacity of composted materials [pruning waste and biosolid (BS) (1:3 w/w), spent mushroom compost (SM) and pine bark compost (PB)] as organic amendments to immobilize Cd. They found that the effectiveness of using these materials depends on the soil and material properties and observed that the results may be attributable to differences in pH, the type and amounts of organic matter. Parkpian et al. (2010) also studied different ratios of ash-sludge mixture (1:1, 1:5, 1:10) as soil amendments in pot experiments to determine the optimum condition of ash mixed with sewage sludge that can minimize bioavailable metals. Two soils from Rangsit and Thonburi (Thailand) were used with twelve weeks of incubation. From the experiments, 1:5 (fly ash-mixture) and 1:10 (bottom ash-sludge mixture) were chosen as optimum ash-sludge mixture ratios according to high nutrients and lowest extractable metals. For Rangsit amended soil, the results showed significant increase in metal concentrations with incubation time (from week 4 to week 12) while for Thonburi amended soil, Zn was found to be increased significantly beyond week 4 to 12. It was observed that Cd in the ash-sludge amended soil samples were negligible (0.04 mg kg^{-1}) during the first 8 weeks of incubation but began to increase at week 12, possibly due to the low pH. The authors concluded that ash-sludge application to soil has significantly increase soil nutrients (N and P) and could be used as adsorbing material for minimizing heavy metal toxicity. The improvement in soil quality indicates the potential agricultural application of ash-sludge to soil.

Tica et al. (2011) investigated the impact of 5% (w/w) apatite and Slovakite applications (a commercial mixture of dolomite, diatomite, smectite basaltic tuff, bentonite, alginate and zeolite) on Pb, Zn, Cu and Cd stabilization. The addition of amendments resulted in increased soil pH and decreased DTPA-Cd and Zn bioavailability and water-soluble and exchangeable fractions of Cd and Zn (Tessier's procedure). The significant conversion of the most labile and available metal concentration into less labile fraction was also observed in this study. Guo et al. (2011) investigated the effects of soil amendment (lime, nano-Si foliar solution and used diatomite) on soil pH, heavy metal bioavailability, the growth and metal uptake of three maize (*Zea mays* L.) cultivars grown in a Cd and Zn contaminated acidic soil. The results showed that the addition of lime significantly increased soil pH (0.19 to 0.22 pH units) and reduced NH_4NO_3-extractable Cd and Zn concentrations in soils planted with these three maize cultivars. The maize grain yields had increases and decreased the concentrations of Cd and Zn in the grains and shoots of maize when compared with the control.

Houben et al. (2012) studied the effects of six cost-effective amendments ($CaCO_3$, iron grit, fly ash, manure, bentonite and bone meal) at 5% (w/w) addition on Cd, Zn and Pb leaching and phytoavailability (white lupin, *Lupinus albus* L.) for 14 weeks. It was found that Cd and Zn leaching was reduced by all amendments mainly due to the pH increase. Flow-weighted mean concentrations of Cd and Zn (the total mass of metal leached divided by the total amount of water percolated through the column over the incubation time) was reduced in leachates from 32.9 to 88.3% and 41.2 to 98.5%, respectively. The author concluded that the addition of $CaCO_3$ seem to offered the best compromise as it successfully reduced both the leaching and the phytoavailability of the three considered metals.

Bade et al. (2012) studied the changes in bioavailable concentrations of Pb, Zn, Cu and As in former smelter site soils before and after lime amendment using the the BCR sequential extraction (the sum of the acid-extractable and reducible fractions, BCR1+2). In vitro physiologically based extraction test (PBET) and diffusive gradients in thin-films (DGT) was also investigated and showed consistent results as the BCR procedure. The results showed that the bioavailable concentrations of metals remarkably decreased after lime amendment, except Pb. Zn was reduced from 43.4%

to 6.33% in the first fraction of BCR, while Cu and As reduced from 36.3% to 6.58% and 8.03% to 0.127%, respectively. This indicates that lime is highly effective for the immobilization of Zn, Cu and As, but not for Pb.

Sun et al., 2013 studied the effects of sepiolite (natural hydrated magnesium silicate clay) addition (0.5 to 5% w/w) on Cd bioavailability of soils artificially polluted with Cd (1.25, 2.5, and 5 mg kg^{-1}). The results showed negative correlation between soil pH and extractable Cd concentration. The addition of sepiolite resulted in the reduction of Cd by 9.8-44.6%, 14.0-33.5%, 15.1-22.5%, respectively in accordance with the level of Cd concentration in soils.

2.2.3 The uses of sugarcane mill by-products as soil amendments

Despite many studies conducted on various organic and inorganic amendments, few studies have been carried out to investigate the effectiveness of ethanol waste-products as soil amendments. The following studies are the examples of using sugarcane mill by-products in as soil amendments. Meunchang et al. (2005) to determine how rapidly these materials (filter cake and filter cake mixed with bagasse, at a 2:1 ratio) can be converted to a stable product that may be useful for crop production. The results showed that both mixtures achieved maturity at approximately 90 days as indicated by a stable C/N, low NH_4/NO_3, lack of heat production and a germination index higher than 80%. Mixing filter cake with bagasse helped conserve N during composting, approximately 12 to 15%. The authors suggested that both composts have potential for use in crop production, mixing more bagasse with the filter cake may result in further reduction in N losses. Later, Meunchang et al. (2006) determined the impact of sugar-mill by-products compost (filter cake and bagasse) (at the rate 0, 15, and 45 g per 10 kg^{-1} of soil in each pot) for 50 days on tomato growth (*Lycopersicon esculentum* L.) with and without fertilizer N (at rates of 0, 0.75, and 1.54 g.). The results showed that regarding the addition of fertilizer N, sugar-mill by-products compost enhanced shoot and root growth and nitrogen content. They suggested that sugar mill by-products compost proved to be an effective soil amendment for promoting the growth of tomato plants.

In Thailand, the use of sugarcane waste-products has been studied recently in order to utilize the waste-products for improving the agricultural productivity.

Tangkoonboribun et al. (2006), for example, studied the effect of filter cake and bagasse for improving physical soil properties in Korat soil (Kt, Oxic Paleustults) at Kuchinarai district, Kalasin province. They found no improvement on cane production and no significant effect on bulk density, aggregate stability and particle size distribution by the addition of filter cake at 50 tons ha^{-1} and bagasse at 12.5 tons ha^{-1}. Ingkapradit et al. (2008) studied the effect of filter cake on paddy soil fertility improvement for rice production. The results showed that after filter cake has been incorporated into the soil, pH was increased along with soil fertility improvement. Yield of rice cultivar was increased and was affected by both single filter cake (500, 1,000, 1,500 kg rai^{-1}) application and the combination with the chemical fertilization. However, increase of rice yield was pronounced more for the combination, improved rice yield by 7 to 5% by adding filter cake at 500 kg rai^{-1} along with chemical fertilizer. Soongsud (2008) investigated the effect of spent wash liquor for sugarcane production for 8 and 12 months. The application rate is either single (25 m^3 rai^{-1}), and with composted filter cake (2 ton rai^{-1}) and/or chemical fertilizer (13-13-21 N-P_2O_5-K_2O) (50 and 100 kg rai^{-1}). The author showed that application of spent wash liquor significantly increased growth of sugarcane (diameter and height) as compared to the control, while improved the soil qualities (electrical conductivity, avail.P, exc.K and Mg). Tadsanee (2008) studied the effect of distillery slop (vinasse) (0, 30, 60, 90 m^3 rai^{-1}) and chemical fertilizer (0-0-0, 21-0-0, 20-20-0, 13-13-21 at 60 kg rai^{-1}) on soil fertility, growth and yield of sugarcane (K 88-92 variety) at 8 month growth stage. The results showed that distillery slop significantly increased nutrients in soil, particularly K and Mg. It was found that with the application of distillery slop, chemical fertilizer had no significant effect on plant growth. The rates of distillery slop application at 30 and 60 m^3 rai^{-1} caused the significant increased in plant height and number of shoots as compared to the control. Application of different doses of slop showed a significant difference in terms of sugarcane production (yields) as compared to the control, however it did not make any significant difference on sugar cane production among different treatment (32.5, 31.3, 30.0 ton rai^{-1} for 60, 30, and 90 , respectively).

Ossom (2010) studied the effect of using filter cake as a fertilizer on the cassava *(Manihot esculenta* Crantz) production. Results showed that cassava tuber yields were increased from 400 to 1,090 kg ha^{-1} (without filter cake) to 975 to 3,510 kg ha^{-1}. The author suggested that farmers apply 60 t ha^{-1} of filter cake in cassava production for best crop yield. Abo-Baker Basha, (2011) evaluated the effect of improving locally organic material (filter cake mud by-product of sugar factory) at the rates of 2.5, 5, 7.5, 10 ton/fed which incorporated with biofertilizer (125 kg rock phosphate and with or without addition of nitrogen fixers and phosphate dissolving bacteria) on organic onion bulbs production. The results showed that filter cake mud at 10 ton/fed combined with biofertilizer induced highly increase in all plant growth parameters (foliage dry weight, height, and diameter) and resulted in a significant increase bulb yields. Recently, Utami et al. (2012) studied the effect of sugarcane filter cake at the application rate of 21.37 $Mg.ha^{-1}$on P availability, P uptake and plant growth (Chinese Cabbage, *Brassica sp*.) on the two studied soils of low and high organic carbon content. The results showed the increasing P-uptake and plant growth improvement after 6 weeks of planting, Plant dry weight (g) was significantly increased from 0.12 to 2.44 g and 5.00 to 6.06 g, respectively in the both studied soils. The studies on the use of other sugarcane mill by-products was also studied by Janoš et al. (2010) to determine the fractionation and redistribution of Cd, Cu, Pb and Zn after the addition of coal fly ash (0, 1 and 5%) using BCR sequential extraction. The results showed that Cd is bound mostly in the first two fractions (BCR1and BCR2), while more than 60% of Zn was associated with the residual fraction (BCR4). The addition of fly ash did not significantly affect the acid extractable fraction of Cd in soil (BCR1); however, reduced the mobility and availability of Zn to some extent. Lopareva-Pohu et al. (2011) evaluated the influence of two different coal fly ash (CFA) amendments (silico-aluminous and sulfo-calcic fly ash) on Cd, Pb and Zn contaminated soil. Pot experiments were performed to determine metal mobility and metal uptake by *Lolium perenne* L. and *Trifolium repens* L. The results showed $CaCl_2$ extractable Cd decrease significantly in the treated soils; Cd and Zn concentrations in two plant species were also significantly lower than that of in the control soil. Furthermore, root and shoot biomass productions were significantly higher for the two plant species. Pourrut et al. (2011) evaluated the effect of fly ash amendments (silico-aluminous and sulfo-calcic fly ash) at 23.3 kg m^{-2} on reducing metal phytoavailability of trees (*R. pseudoacacia* L., *A. glutinosa* L., *Quercus robur* L., *A. pseudoplatanus* L., and *Salix alba* L.). The authors found that all tress could grow well on the contaminated site and accumulated low concentrations of metals in their leaves and young twigs. They conclude that these fly ash were effective in decreasing Cd, Pb and Zn concentrations in above-ground parts of trees, especially the sulfo-calcic ash

2.2.4 Fractionation and transformation of Cd and Zn affected by organic amendment application

Changes in chemical fractionation of Cd and Zn as a function of organic amendment application have been widely studied through the use of chemical extractions. Extractable Cd in various amendments [leonardite (LEO), litter (LIT), municipal waste compost (MWC), biosolid compost (BC) and sugar beet lime (SL)] using DTPA-$CaCl_2$ (1:5 v/v) and sequential extraction according to Tessier et al. (1979) was determined by Pérez-de-Mora et al. (2007). The results showed that DTPA is capable of extracting the Cd associated with the exchangeable fraction up to 91%, 61% and 62% from PB, SM, and BS, respectively with respect to the total extraction (using *aqua regia*). Cd distribution among various fractions of Tessier's procedure varied among different composts; the PB showed a high Cd percentage in the soluble fraction (47%), while in the SM and BS was 15% and 20%, respectively. In the organic fraction, the percentage Cd was 8%, 37%, and 35% in the PB, SM, and BS, respectively. Results showed trace element distribution mainly associated with the residual fraction and to a lesser extent with the reducible fraction. The addition of amendments and/or establishment of a plant cover showed no clear effect on Cd and Pb distribution. They found that Zn concentration in the most labile fraction was increased in MWC due to Zn input.

Liu et al. (2009) conducted pot experiments to evaluate the effect of compost application on the immobilization and biotoxicity of Cd in winter wheat (*Triticum aestivum* L.). Soils treated with various levels of Cd (0 to 50 mg Cd kg^{-1} soil) were amended with 0, 30, 60 and 120 g compost kg^{-1} soil. The fractions of Cd in soil were evaluated by a sequential extraction procedure. The results showed that soluble/exchangeable Cd was converted to organic-bound and inorganic precipitates forms, more than 70% reduction in soluble/exchangeable Cd (KNO_3) but increase in the concentration of organic-bound (NaOH) and inorganic precipitates (EDTA) Cd in soils. Fifty percent decrease in Cd uptake by wheat tissue was attributed primarily to the increase of soil pH, complexation of Cd by the organic matter and co-precipitation with P content. The authors concluded that compost amendment of Cd polluted soil not only reduced the uptake of Cd by wheat seeds and stems, but also improved the performance of crops.

Achiba et al. (2010) carried out a field plot experiment to assess the effects of repeated application of municipal solid waste compost in comparison to farmyard manure on the accumulation and distribution of trace metals, as well as organic carbon and nitrogen in a Tunisian calcareous soil. BCR sequential extraction was applied to determine the trace metal concentration. The trace metals showed different fractionation patterns when the BCR sequential extraction scheme is applied on untreated and compost-treated soil. The residual fraction was found to be the major fraction, especially for Cu, Cr, Ni and Zn while Cd was mainly present in the acid-extractable and reducible fraction. Similarly, The BCR sequential extraction was also studied by Janoš et al. (2010) to determine the bioavailability of Cd, Cu, Pb and Zn in the six kinds of amendments (lignite, oxihumolite, potassium humate, iron humate, fly ash and natural zeolite) added to contaminated soil at 1 to 5% (w/w) incorporation. The results showed that Cd was present predominantly in the acid extractable fraction; its mobility was reduced effectively after an addition of potassium humate, while Cu and Pb were bound more strongly to the soil matrix and were reduced after an addition of inorganic amendments of zeolite and fly ash, potassium humate, respectively. Alternatively, more than 60% of Zn was bound strongly in the residual fraction and its mobility was affected slightly by the presence of amendments at the concentration levels used in this work.

Waterlot et al. (2011) studied Cd, Pb and Zn behavior and mobility in highly contaminated soils affected by the past atmospheric emissions of the two smelters using the BCR sequential extraction and various extracting solutions ($CaCl_2$, acetic acid, and citric acid). Hydroxyapatite (HA) and diammonium phosphate (DAP) were used as amendments in this study to evaluate the effects of the amendment on the mobility of Cd, Pb and Zn at 6 months. The results of sequential extraction showed that Cd was mainly bound to the carbonates, soluble and exchangeable fraction (BCR1, 50%), while Pb was strongly bound to oxides and hydroxides of iron and manganese (BCR2, 80%). The mobility of metals in this study was: Cd > Zn > Pb. Alternatively, by means of the various extracting solutions the authors suggested that the extraction capacity of Cd, Pb and Zn depended on the extracting solutions following the decreasing order: citric acid > acetic acid > $CaCl_2$ 1 M > $CaCl_2$.

A field trial experiment was carried out by Abd-Elrahman et al. (2012) to investigate the effect of different amendments (gypsum, citric acid, farmyard manure, compost and the combination) on heavy clay salt-affected soil irrigated with wastewater. Chemically available heavy metals were extracted with DTPA and the results showed that the chemical characteristics of the studied soil included pH, EC_e, soluble ions, SAR and ESP were improved by application of the amendments under irrigation. Regarding the DTPA-extractable heavy metals (Fe, Mn, Zn, Cu, Ni and Cd) in soil, the applied amendments increased the downward movement of heavy metals as indicated by increasing their concentrations with increasing soil depth (0-15, 15-30, 30-60 cm depth). However, DTPA-Cd and Zn concentration of the amended soils was reduced as compared to the non-amended soil at the surface level (0-15 cm depth).

2.2.5 Effect of aging on bioavailability of Cd and Zn

Understanding the aging could help in predicting the changes in metal bioavailability, therefore, experiments conducted on different incubation times (short term and long term incubation period) have been studied extensively in investigation the effect of time on the metal distribution and transformation. Jalali and Khanlari (2008), for example, studied the effect of time on Cd and Zn bioavailability in some calcareous soils of Iran. Five hundred mg kg^{-1} of Zn and 8 mg kg^{-1} of Cd were added as chloride. Samples were incubated for 3 hr, 1, 3, 7, 14, 21 and 28 days at 25 °C. The results showed the changes appeared in the exchangeable fraction after 3 hr and this fraction remained the most dominant fraction after 28 days. The proportion of other fractions also generally increased during the incubation. For Zn, high percentage of exchangeable fraction (7.4%) at the end of incubation was observed; Zn in exchangeable fraction was decreased; where other fractions increased with increasing incubation time. The authors found that metals were adsorbed on the soil surface as the exchangeable form rapidly and then slowly transformed into more stable form.

The effect of accelerated ageing of artificial soils (10, 30, 50% w/w of artificial soil with sewage sludge from urban treatment plant) on metal fractionation (Tessier's sequential extractions and DTPA extraction) was studied by Zapusek and Lestan (2009) by exposing soils to alternate high (105 °C) and low (-25 °C) temperatures to stimulate the environment during long-term exposure to high temperature in the summer and low temperature in winter, respectively. The results showed that accelerated ageing most profoundly affected the fractionation of Zn, which was present in soils in the highest concentrations. Ageing shifted Zn from more labile carbonate and Fe and Mn oxide fractions to non-labile organic matter and the residual. Water soluble Zn increased after ageing, while Zn bound to soil colloids did not change significantly. The authors also found that accelerated ageing significantly decreased heavy metal mobility assessed with phyto-accessibility (DTPA) and thus decreased the potential toxicity of metals in artificial soil mixtures. The authors suggested that temperature might indeed be the single most important factor to foster the kinetics (dynamics) of heavy metal reactions with different soil phases toward final chemical equilibrium under given soil conditions (pH and water saturation).

Aging effect on the Cd and Zn mobility and bioavailability in three typical Chinese soils was investigated by Lu et al. (2009). The experiment was conducted from 3 h to 8 weeks and they found that exchangeable fraction of Zn increased in the first 3 h and then slowly decreased, while Zn in Fe/Mn oxides and organic matter fractions increased consistently in the 8 week incubation, and no changes for residual fraction were observed. In addition, it was found that aging had a significant effect on Cd fraction distribution, of which Cd tended to incorporate from the exchangeable after 3 hr incubation and then changed slightly into more stable fractions in the 8 week incubation. Zhong et al. (2013) investigated the Cd in sediment as influence by sediment composition and time by spiking stable isotope (^{111}Cd) into sediments of different organic content levels for 3 days to 2 months. The authors found the gradual transfer of Cd from the more mobile geochemical phase (carbonate associated phase, BCR1) to more refractory phases (Fe-Mn oxide associated phase, BCR2 and organic associated phase, BCR3) within 2 months and concluded that the distribution of Cd in carbonate or Fe-Mn oxide associated phase could have an influence on the solubilization of sedimentary Cd and its change with contact time.

CHAPTER III

RESEARCH METHODOLOGY

The summary of the experimental framework is illustrated in Figure 3.1 that divided into three different phases as following;

- soil pot experiment,
- sugarcane pot experiment, and
- effect of amendment concentration experiment

The first one soil pot experiment was to study the effects of sugarcane waste-products (BA, FC, and VN) on metal bioavailability in soils, and to find the optimum application rates of these waste-byproducts. The second part, a sugarcane pot experiment which sugarcanes were grown in the pots of the selected application rates from the previous experiment, was to determine sugarcane response in terms of plant growth and metal translocation. The last one, effect of amendment concentration, focused on the impact of organic matter in the amendments on metals fractionation to determine the effects of each waste on metal bioavailability, especially in the organic-bound fraction.

3.1 MATERIALS

3.1.1 Soils

Agricultural soil contaminated with Cd and Zn was collected from Maesot District, Tak Province, Thailand. These soil samples were air-dried and passed through a 2-mm sieve prior to be used in the pot experiment.

3.1.2 Sugarcane waste-products

Sugarcane waste-products (BA, FC, and VN) generated from ethanol production plant of Maesot Clean Energy Company Limited (Tak Province, Thailand) was used in this study as soil amendments (Figure 3.2) and collected twice for soil and sugarcane pot experiment, respectively (April 2011 and 2012). The application was individual, binary and ternary combination at 3% (w/w).

3.1.3 Sugarcane

Sugarcane (*Saccharum officinarum* L.) was obtained from the local sugarcane field at Maesot District, Tak Province. Sugarcane variety of LK92-11 was selected and used in this study due to it is one of the recommendations from Office of the Cane and Sugarboard, Ministry of industry to Thai farmers showing good potential for commercial plantation with high yield production and mostly used for ethanol production.

3.2 METHODOLOGY: SOIL POT EXPERIMENT

Soil pot experiment was focused on the selection of the optimum application rates of sugarcane waste-products as soil amendments in reducing metal bioavailability in soil. The details are as the following;

3.2.1 Experimental setup: soil pot experiment

- Soil samples from Maesot, Tak province were prepared to obtain soils with two different Cd concentrations levels as one at level of normal Cd concentration in soil (designated as LCdS) (< 3 mg kg^{-1}), and two at level of high Cd concentration that occur relatively infrequently under normal field crop production (HCdS) (10-15 mg kg^{-1}).
- The mixing of soil samples was done manually until soil samples are homogenous.
- The boiler ash and filter cake were oven-dried and ground to pass through a 2-mm mesh sieve while the vinasse which was in slurry form was used as it was. All of the three amendments were analyzed for their properties and thoroughly mixed with the soil samples in accordance with design.
- Pot experiment was conducted in 16 plastic pots (14 cm in diameter and 14 cm in height) filling with prepared soil of 2 kg with addition of 3% (w/w) of the amendments.
- The ratios of the amendments were treated in two groups (Table 3.1): one in low Cd contaminated soil for 7 treatments (LCdS) and the other in high Cd contaminated soil for 7 treatments (HCdS). Untreated soils without

amendments were also included in both sets of soils as controls (LC and HC for LCdS and HCdS, respectively).

- The capital L- and H- of the treatment name indicates the treatment in LCdS and HCdS, respectively.
- The ratio of amendments was selected based on the recommended application rate of organic fertilizer in 1,600 m^2 for field crop (1.36-2.27 Mg $year^{-1}$) (Department of Agricultural Extension), ten times of the recommended organic fertilizer rate (3% w/w) were then chosen in order to assess the effect of added soil amendments.
- Eighty-four day soil incubation (from T0 to T84) was performed under normal day-night condition (22 to 35 °C day/night temperature), where T0 and T84 is T = 0 and 84 days incubation, respectively.
- To avoid any input from atmosphere, the experiment was conducted indoor at the 11th floor Petroleum building, Chulalongkorn University.
- All experimental pots were arranged and rotated regularly on the weekly basis. Water losses in the treated soils were compensated by adding deionized water in each experimental pot according to the initial soil moisture content and thoroughly mixed at weekly intervals.

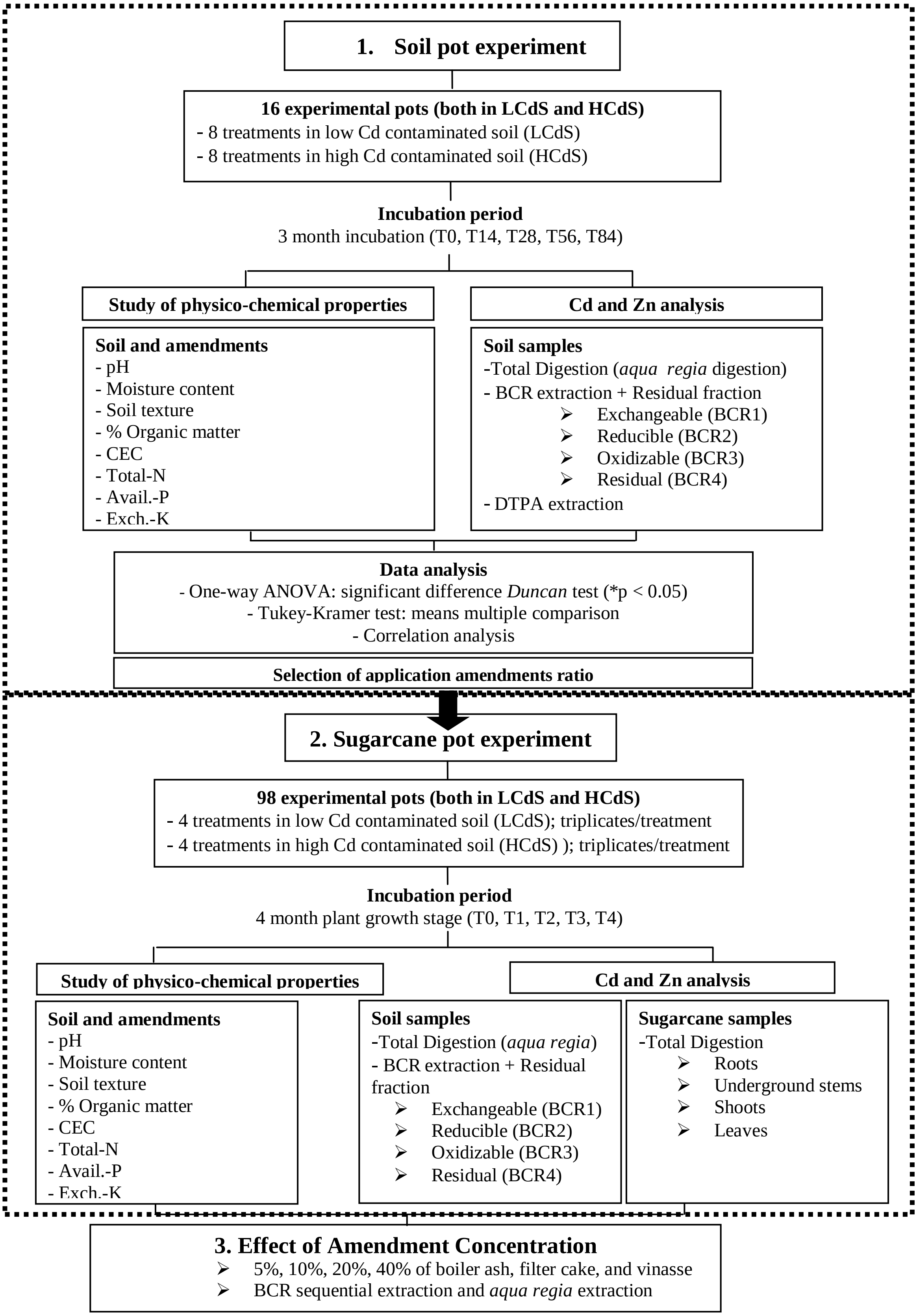


Figure 3.1 Schematic diagram of experimental design

a) boiler ash (BA)

b) filter cake (FC)

c) vinasse (VN)

Figure 3.2 Sugarcane waste-products (BA, FC, and VN) used in this study as soil amendments

3.2.2 Sample collection: soil pot experiment

Sixty-gram composite soil samples (60 g of soil pot^{-1}) of each treatment were sampled on each sampling date at two weeks interval (T = 0, 14, 28, 56, 84 days) designated as T0, T14, T28, T56, T84, respectively. Soil samples were then oven-dried at 60 ºC for 48 hr and dry weight (mg) was recorded. Soil analysis was done after soil samples were ground to pass through a 2-mm mesh. Physico-chemical characteristics of soils and amendments were analyzed including: pH (1:2 and 1:5 solid/water suspensions for soils and amendments, respectively) (Jayasinghe et al. 2010), soil organic matter (SOM) by the Walkley-Black procedure (Nelson and Sommers 1996), cation exchange capacity (CEC) and exchangeable potassium (Exc.K) using 1 N NH_4OAc extraction (pH 7), total nitrogen (Total.N) by the Kjeldahl procedure, available phosphorous (Avail.P) by Bray II method and soil texture analysis using the pipette method.

Table 3.1 Experimental design for the treatments of the soil pot experiment

Treatments (LCdS, HCdS)	The application rates (w/w)
	(filter cake: boiler ash: vinasse) (g ·pot $^{-1}$)
L1*, H1**	(0:0:60)
L2, H2	(0:60:0)
L3, H3	(60:0:0)
L4, H4	(0:30:30)
L5, H5	(30:30:0)
L6, H6	(30:0:30)
L7, H7	(20:20:20)
LC, HC	(0:0:0)

Note:* = treatments in low Cd contaminated soil (LCdS); ** = treatments in high Cd contaminated soil (HCdS)

3.3 METHODOLOGY: SUGARCANE POT EXPERIMENT

A further study of the effects of sugarcane waste-products as soil amendments was conducted with plant to determine the influence of these waste-byproducts on sugarcane growth and metal uptake. The summary of sugarcane pot experiment is explained as following;

3.3.1 Experimental setup: sugarcane pot experiment

- Agricultural soils contaminated with Cd and Zn from Maesot, Tak province were sampled (April, 2011) and prepared to obtain the same Cd concentrations as in the previous study of soil pot experiment.
- Low Cd contaminated soil (LCdS) of 3.0-4.5 mg Cd kg^{-1} and high Cd contaminated soil (HCdS) (15-20 mg Cd kg^{-1}) were used throughout in this experiment.
- Sugarcane pot experiment was conducted for a period of 4 months from April to July 2011 at Institution II, Chulalongkorn University, Thailand.
- The amendments were oven-dried and the soil was air-dried before grounding to pass through a 2-mm mesh sieve and then applied as designed and mixed with the soil samples thoroughly.
- There are four treatments: Cd and Zn contaminated soil without amendment (Ctrl); Cd and Zn contaminated soil with 3% BA (30 g BA kg^{-1} soil), Cd and Zn contaminated soil with 3% FC (30 g FC kg^{-1} soil) and Cd and Zn contaminated soil with combination of 1.5% BA and 1.5% VN (15g BA+15g VN kg^{-1} soil).
- The ratio selection was based on the preliminary study of soil pot experiment reporting the reduction of the exchangeable fraction of Cd (BCR1-Cd). The details of amendment ratios are described in Table 3.2.
- Single-bud setts of sugarcane (LK92-11) with the uniform size (15-cm long) were grown in plastic pot (13-inch diameter, 12-inch height) filled with 10 kilograms of Cd and Zn contaminated soil.
- To prevent soil and leaching losses, all the base of experimental pots were covered with polyethylene plastic bags prior adding soil and amended materials.

- In order to constrain the analytical uncertainty of the results, the plants were grown in three different containers for each treatment during the whole experiment, and individual plant was taken from each container at each harvesting periods. There were 4 batches of experiment in according to the four-month harvesting period. Each batch of plant from each container was analyzed independently and the chemical result of the three plants was averaged for each harvesting times.
- For each treatment, there were three replicates; one sugarcane sett per pot represents one replicate. There were 4 selected treatments (included control), 3 triplicates and 4 collections (4 treatments x 3 triplicates x 4 collection time); resulted in a total of 48 experimental pots.
- Experimental pots were arranged in a randomized complete block design and conducted outdoor under natural sunlight with a transparent roof to prevent the rainfall but allow the air and sunlight pass through.
- No fertilizer addition was used in this study.
- Tap water was watering three times a week during the experiment.

Table 3.2 Experimental design for the treatments of the sugarcane pot experiment

Treatment (LCdS, HCdS)	The application rates (w/w) (FC: BA: VN) (g kg^{-1} soil)
LC*, HC**	(0:0:0)
LA, HA	(0:30:0)
LF, HF	(30:0:0)
LAV, HAV	(0:15:15)

Note:* = treatments in low Cd contaminated soil (LCdS); ** = treatments in high Cd contaminated soil (HCdS)

3.3.2 *Sample collection: sugarcane pot experiment*

Soil and sugarcane samples were sacrificed and collected monthly at the same sampling dates (from T1 to T4 where T1, T2, T3, T4 represent 1, 2, 3 and 4 month growth stage of sugarcane, respectively) and kept in polyethylene plastic bags prior transporting to the laboratory. After collection, the whole sugarcane plants were carefully rinsed using jet sprayed tap water followed by deionized water to clean all the attached particles. Sugarcanes were separated into four different parts included: roots, underground stems, shoots and leaves (Figure 3.3). Shoot height, shoot diameter and number of leaves were measured every harvesting period. Shoot height (cm) was measured from the top of underground stem to the tip of the longest leaf, and the shoot diameter (cm) was measured at sugarcane stalk 15 cm above the underground stem. Roots, underground stems, shoots and leaves were oven-dried at 60 ºC for 72 h., ground in an agate mortar and dry weights (g plant^{-1}) were recorded. Soil and sugarcane samples were then analyzed for Cd and Zn concentration. More detail of analytical procedure for soil and sugarcane analysis is described in Appendix A.

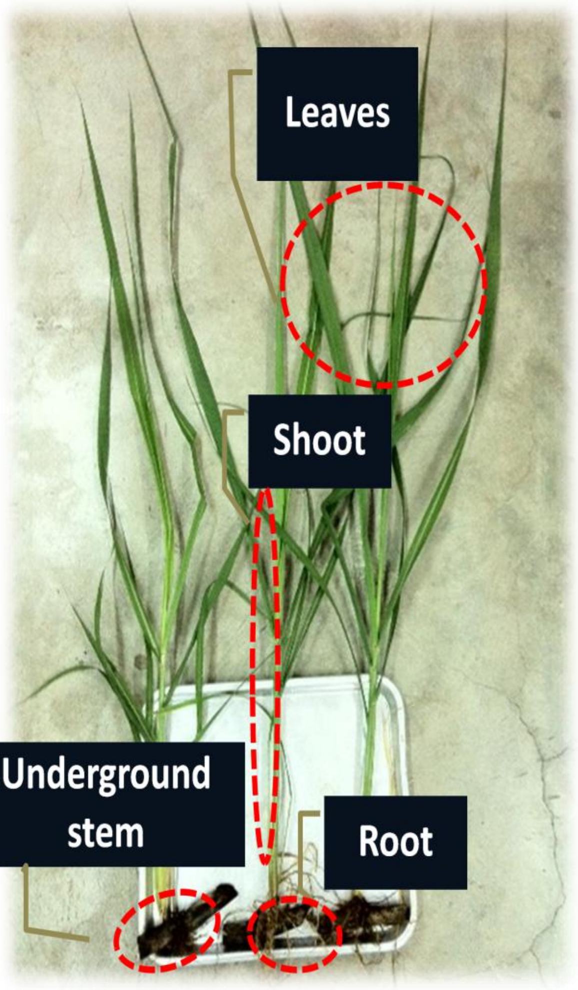
3.4 METHODOLOGY: EFFECTS OF AMENDMENT CONCENTRATION

Based on the experimental results from sugarcane pot study that highlights the impact of organic matter on metals in oxidizable fraction (BCR3); the effect of amendment concentration was then further conducted for the detailed information on metal in organic-bound fraction.

3.4.1 Experimental setup: effect of amendment concentration

- The single application of BA, FC and VN was conducted on 1.5 kg of soil (< 3 mg Cd kg^{-1}). The application rates were 5, 10, 20 and 40% (w/w) as the following;
    - 5%, 10%, 20% and 40% FC
    - 5%, 10%, 20% and 40% BA
    - 5%, 10%, 20% and 40% VN
- Thirteen experimental pots included control (Ctrl) were performed and conducted for 4 months (from T0 to T4), corresponding with the previous sugarcane pot experiment.

- Soil samples were collected monthly (from T1 to T4 where T1, T2, T3, T4 represent 1, 2, 3 and 4 month, respectively).



Sugarcane samples

a) Leaves:

b) Shoots:

c) Underground stem:

d) Roots:

Figure 3.3 Different parts of sugarcane (a-d) for metal determination

- Experimental pots were arranged randomly and conducted indoor at the 11th floor Petroleum building, Chulalongkorn University.
- Deionized water (DI) was added to the pots regularly to maintain soil moisture content.
- Total and bioavailable Cd and Zn concentrations, pH, and organic matter content of the amended soils were determined every sampling period (T0 to T4).

3.3.3 Sample collection: effect of amendment concentration

Soil samples from each treatment were collected for the determination of soil pH, organic matter content and metal analysis including total and bioavailable metal determination using *aqua regia* and BCR sequential extraction, respectively. After collection, the deionized water was added in each experimental pot according to the initial soil moisture content and thoroughly mixed at weekly intervals.

3.4 CADMIUM AND ZINC ANALYSIS

For determination of total Cd and Zn concentrations in the treated soils, *aqua regia* digestion according to the Standard US EPA Method 3052 (1996) was performed. For determination of bioavailable Cd and Zn concentrations in treated soils, the three-step BCR sequential extraction proposed by the Standards, Measurements and Testing programme of the European Union (SM&T) (Quevauviller, 2002) with the additional residue extraction (BCR4) were performed that partition metals into specific forms/fractions which are exchangeable (BCR1), reducible (BCR2), oxidizable (BCR3) and residual (BCR4) fraction, respectively. Single extraction using DTPA was determined in the soil pot experiment only for every incubation periods (T0 to T84).

Cd and Zn concentration in different parts of sugarcane were determined by different digestion procedures according to the standard methods; a summary of analytical procedure for Cd and Zn determination in soil and sugarcane is described in Table 3.3. More details for analytical procedures for metal analysis used in this study are described in Appendix A.

Table 3.3 Analytical procedures for determination of Cd and Zn in treated soils and in sugarcane samples

Soil Analysis	Analytical procedure	Associated Forms
1.Total Cd and Zn determination		
1.1 Total digestion	*Aqua regia* (3:1 v/v) of HCl: HNO_3 (ISO 11466 method, 1995)	Total
2. Bioavailable Cd and Zn determination		
2.1 DTPA extraction	(0.005 mol L^{-1} DTPA + 0.01 mol L^{-1} TEA+ 0.01 mol L^{-1} $CaCl_2$, adjust to pH ± 7.3)	Available, soluble
2.2 Three-step BCR sequential extraction (SM&T) & residual extraction (BCR4)		Bioavailable
1). Exchangeable fraction: BCR1	0.11 mol L^{-1} CH_3COOH (acetic acid)	Bound to colloidal/particulate material or soil surface by relatively weak electrostatic interactions, susceptible to change of pH, released by changes in ionic composition and affected by production or consumption of protons
2). Reducible fraction: BCR2	0.5 mol L^{-1} $HONH_2{\cdot}HCl$ hydroxylamine hydrochloride	Bound to Fe/Mn oxides, instability under anoxic conditions and dissolution of metal-oxide phases under controlled ORP and pH.

3). Oxidizable fraction: BCR3	8.8 mol L^{-1} H_2O_2 (hydrogen peroxide) and 1.0 mol L^{-1} NH_4OAc (ammonium acetate)	Bound to the various forms of organic matter and sulfides. The degradation of organic matter under oxidizing conditions is responsible for releasing trace elements.
4). Residual fraction: BCR4	*Aqua regia* (3:1 v/v) of HCl: HNO_3	Bound to the mineral lattices

Sugarcane Analysis	Analytical procedure
Roots	*Aqua regia* extraction (3:1 ratio of 12 ml HCl and 4 ml HNO_3) in inert Teflon vessels with a microwave system
Underground stems	*Aqua regia* extraction (3:1 ratio of 12 ml HCl and 4 ml HNO_3) in inert Teflon vessels with a microwave system
Shoots and leaves	H_2O_2: HNO_3 (1:3) (v/v) (Jamali et al. 2008)

After cooling, the digestion solutions were filtered with filter paper No.41 and diluted to 50 ml and 25 ml for soil and plant samples, respectively. The digested solutions were stored in polyethylene bottles at 4 °C until trace element analysis is performed. Cd concentration was determined using graphite furnace atomic absorption spectrometry (GFAAS) and Zn concentration was determined using flame atomic absorption spectrometry (FAAS) (AAS-ZEEnit 700 Analytik).

3.6 METHOD VALIDATION

For method validation, the accuracy of the analytical procedures was checked using a soil certified reference material (CRM025-050, RTC) with four replicates. CRM025-050 is the moderately contaminated soil available from the Western United States (Peter Matúš et al., 2005). In addition, percentage recovery was also performed for the inter check as it is a comparison between the sum of the extracted Cd and Zn in all BCR fractions (BCR1, BCR2, and BCR3) plus the residual fraction (BCR4) and the total metal concentration from *aqua regia* digestion.

$$\text{Recovery (\%)} = \frac{\text{BCR1 + BCR2 + BCR3 + BCR4}}{\text{Total concentration}} \times 100$$

3.7 DATA ANALYSIS

Translocation index (TI%), the percentage between metal mass in aboveground parts of sugarcane (mg plant^{-1}) and whole sugarcane (mg plant^{-1}), was determined in this study and used to indicate the plant efficiency of heavy metal transfer to the aboveground parts of sugarcane. The aboveground part of sugarcane represents the leaves and shoots; where the underground parts are the summation of underground stem and roots.

Additionally, transfer factor (TF), which is the ratio of metal concentrations in sugarcane (mg kg^{-1}) to total metal concentrations in soils (mg kg^{-1}), was determined with the intention to study the effectiveness of sugarcane waste-products for metal stabilization in relation to the transfer and accumulation of metal from soil to sugarcane.

Data were expressed as means with standard deviation (mean ± SD). The statistical analysis was based on one-way analysis of variance (ANOVA) for the comparison of statistically significances between treatments using the SPSS (Statistical Product and Service Solutions) version 17.0 software, the Duncan test at $*p<0.05$ was used. Pearson’s correlation analysis was also determined to evaluate the relationship between the variables.

CHAPTER IV

RESULTS AND DISCUSSIONS:
SOIL POT EXPERIEMNT

4.1 PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS AND AMENDMENTS

Characterization of the two discrete levels of Cd contaminated soils (unamended soils) and the specified amendments used in this study are given in Table 4.1. These two types of soils were slightly acidic with low plant essential nutrients (total.N, avail.P and exc.K). The soils are generally classified as loam based on the percentage of clay, silt and sand composition and in Order *Ultisols*, Suborder *Ustults*, according to the Soil Survey and Classification Division, the Land Development Department (2010).

For the amendments, vinasse is acidic with the pH value of 4.45, boiler ash is alkaline (pH = 8.39), and filter cake is slightly acidic (pH = 6.42). The filter cake showed higher CEC and OM than boiler ash by the order of five and three folds, respectively. The concentration of Cd in the three sugarcane waste-products is negligible. In terms of plant essential nutrients, filter cake had the highest total nitrogen (total.N) and available phosphorous (avail.P) with 1.39% and 7,097 mg kg^{-1}, respectively. Among the applied amendments, the highest amount of exc.K was added by the boiler ash (23,600 mg kg^{-1}), while vinasse had substantial amounts for all plant essential nutrients (total.N, avail.P and exc.K).

Table 4.1 Physico-chemical characteristics of two studied soils (LCdS and HCdS) and sugarcane waste-products (boiler ash, filter cake, and vinasse).

Soils and amendments	Soil texture^a			pH^b	OM^c	Total.N^d	Avail.P^e	ExcK^f	Cd^g	Zn^g	CEC^f
	Sand	Silt	Clay								
	---------- % ---------				%	%	------------ mg kg^{-1} ------------				cmol$^{(+)}$kg^{-1}
LCdS	24.9	47.6	27.4	6.53	5.64	0.08	16	118	<3	188	12.7
HCdS	38.5	47.9	13.6	6.35	4.47	0.09	22	114	~15	507	15.3
Filter cake		-		6.42	17.4	1.39	7097	4746	<0.01	208	16.0
Boiler ash		-		8.39	6.20	0.12	834	23600	0.005	143	2.9
Vinasse		-		4.45	24.2	1.2	3000	17000	0.003	5.57	-

[a]determined using the pipetting method; [b]soils; determined by 1:2 soil:deionized water, amendments; determined by 1:5 soil:deionized water; [c]OM; organic matter, determined using the *Walkley-Black Method*; [d]Total-N; total nitrogen, determined using the *Kjeldahl Method*; [e]Avail.-P; available phosphorous, determined *by BrayII*; [f]Exc.-K; exchangeable potassium, and CEC; cation exchange capacity, determined from 1M NH_4-acetate extraction and atomic absorption; [g]determined by using aqua regia extraction

4.2 PHYSICO-CHEMICAL CHARACTERISTICS OF TREATED SOILS

4.2.1 Total Cd and Zn concentration (mg kg^{-1}) of treated soils

Total Cd and Zn concentrations in all treatments after the addition of the amendments (T = 0 day) were determined by *aqua regia* digestion (Table 4.2). The addition of sugarcane waste-products did not cause the increase of the total metal load in the soil. The total Cd concentrations ranged from 0.73 to 1.19 mg kg^{-1} and 11.7 to 14.2 mg kg^{-1} for LCdS and HCdS, respectively. The average Cd concentrations were slightly lower than the controls in LCdS and HCdS. Differences of total Cd concentrations in each set of the soil (LCdS and HCdS) were not expected and showed little variation of Cd concentrations as compared to the controls and among different treatments, possibly due to the negligible amount of Cd in the amended materials. However, in LCdS average Zn concentrations in all treatment were slightly higher than the control (LC), these may be because Zn concentration in the amendment materials, especially filter cake and boiler ash that had relatively high Zn content. Nevertheless, since the amendment adding was in the small amount, the statistical analysis considers they are not significantly different with those of the controls. Similar case was found for Zn concentration of HCdS. Total Zn concentrations ranged from 172 to 215 mg kg^{-1} and 459 to 528 mg kg^{-1} for LCdS and HCdS, respectively.

Table 4.2 Total concentration of Cd and Zn (mg kg^{-1}) in the treated soils (LCdS and HCdS)

Treatments	(FC: BA: VN)	Total concentration (mg kg^{-1})	
	(g · pot $^{-1}$)	Cd	Zn
L1	(0:0:60)	0.88±0.20^{a}	215±10.98^{a}
L2	(0:60:0)	0.88±0.22^{a}	200±0.63^{a}
L3	(60:0:0)	0.73±0.08^{a}	201±3.59^{a}
L4	(0:30:30)	1.01±0.16^{a}	197±1.64^{a}
L5	(30:30:0)	1.15±0.76^{a}	204±50.47^{a}
L6	(30:0:30)	0.86±0.40^{a}	172±3.25^{a}
L7	(20:20:20)	1.11±0.46^{a}	180±10.32^{a}
LC	(0:0:0)	1.19±0.34^{a}	188±6.18^{a}
H1	(0:0:60)	12.4±2.54^{bc}	528±29.34^{c}
H2	(0:60:0)	13.4±1.07^{bc}	508±61.85^{bc}
H3	(60:0:0)	11.7±0.82^{b}	486±26.17^{bc}
H4	(0:30:30)	12.1±0.51^{bc}	511±48.15^{bc}
H5	(30:30:0)	11.9±1.00^{bc}	527±54.56^{c}
H6	(30:0:30)	14.2±2.30^{c}	459±2.79^{b}
H7	(20:20:20)	12.0±0.75^{bc}	485±30.74^{bc}
HC	(0:0:0)	12.9±2.99^{bc}	507±35.63^{bc}

Mean values ± SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at *p<0.05.

4.2.2 Cation Exchange Capacity (CEC) and plant essential nutrients (N, P and K)

The CEC and plant essential nutrients (N, P and K) of treated soils after amendment incorporation are shown in Table 4.3. The result showed that the treated soils have a moderate level of CEC, ranged from 8.1-15.3 $cmol_c kg^{-1}$. Treatments in high Cd contaminated soil (H1-HC) showed a higher CEC (12.1-15.3 $cmol_c kg^{-1}$) than the treatments in low Cd contaminated treatment (L1-LC) (8.1-13.9 $cmol_c kg^{-1}$) and this probably due to its higher pH of the HCdS. Increase in CEC of soil after organic amendment application has been previously described due to the organic matter incorporation. The extent of the increase depends on factors such as soil texture, initial soil organic matter content and CEC, nature of organic amendments and period since last application. However, this study showed the contrasting result as the control treatments (LC and HC) exhibited rather high in CEC (12.7 and 15.3 $cmol_c kg^{-1}$, respectively) as compared to the other treatments.

According to the results, sugarcane waste-products as a soil amendment had a beneficial effect on the plant nutrition (Table 4.3). The addition of soil amendments significantly increased the avail.P and exc.K content in all treated soils (LCdS and HCdS). Available phosphorous (avail.P) were found to increase in all treatments, up to five-fold higher as compared to the control treatments in both LCdS and HCdS. For avail.P, moderate to high avail.P concentrations in the two controls was observed (16 and 22 mg kg^{-1} in LC and HC, respectively), but increased significant after amendments had been added, especially in the filter cake treatments (L3, L5, L6, L7 and H3, H5, H6, H7). Filter cake had the highest avail.P concentration (7097 mg kg^{-1}) as compared to vinasse (3000 mg kg^{-1}) and boiler ash (834 mg kg^{-1}). Since N and P are essential elements for the plant growth and being recognized as primary nutrients, significant amount of plant essential nutrients (total.N, avail.P and exc.K concentrations) increase in all treated soils after soil amendment incorporation indicated the effectiveness of using sugarcane waste-products as a soil amendments for amelioration soil quality, especially for soil fertility improvement.

Table 4.3 Cation Exchange Capacity (CEC) and plant essential nutrients (N, P and K) composition of the treated soils

Treatments	(FC: BA: VN)	CEC	Total N	Avail.P	Exc.K
	(g · pot $^{-1}$)	(cmol$_c$kg^{-1})	(%)	(mg kg^{-1})	(mg kg^{-1})
L1	(0:0:60)	12.0		25	354
L2	(0:60:0)	10.7		35	466
L3	(60:0:0)	10.0		85	195
L4	(0:30:30)	13.9		76	316
L5	(30:30:0)	10.0		24	356
L6	(30:0:30)	10.8		68	269
L7	(20:20:20)	8.1		61	330
LC	(0:0:0)	12.7	0.08	16	118
H1	(0:0:60)	12.1		29	382
H2	(0:60:0)	12.3		44	470
H3	(60:0:0)	14.8		98	167
H4	(0:30:30)	14.3		79	400
H5	(30:30:0)	14.3		44	428
H6	(30:0:30)	14.0		73	240
H7	(20:20:20)	13.7		70	368
HC	(0:0:0)	15.3	0.09	22	114

Note: Avail.P = available phosphorous, Exc.K= exchangeable potassium

4.2.3 pH of the treated soils: soil pot experiment

The change of pH values with time of treated soils measured at T0, T28 and T84 after the amendment incorporation is shown in Table 4.4. Incorporating amendments into the soils (at T0) caused insignificant change to the soil pH as compared to the controls (±0.6 and ±0.2 pH unit for LCdS and HCdS, respectively). Results showed that pH values of treated soils over time (from T0 to T84) were changed significantly (*p<0.05). At the beginning of the pot experiment (T0), soil pH of the treated soils are slightly acidic ranged from 5.9 to 6.5 and 6.5 to 6.7, with an average of 6.3 and 6.6 for LCdS and HCdS, respectively. After 28 days of incubation (T28), pH was generally found to increase slightly in all treatments including the controls then dropped to near-neutral, and after 84 days averaged slightly higher than the original pH (see Table 4.4). Comparatively, the pH variations of the LCdS were larger than those of the HCdS.

Table 4.4 Soil pH of all treatments in two studied soils at T = 0, 28 and 84 days of incubation as affected by different amendments.

Treatments	(FC: BA: VN)	pH		
	(g · pot $^{-1}$)	T= 0 day	T= 28 days	T= 84 days
L1	(0:0:60)	5.9±0.14^{b}	6.8±0.02^{a}	6.2±0.11^{b}
L2	(0:60:0)	6.5±0.04^{b}	7.2±0.03^{a}	6.6±0.02^{b}
L3	(60:0:0)	6.4± 0.01^{a}	7.3±0.01^{b}	6.8±0.05^{c}
L4	(0:30:30)	6.5±0.02^{a}	7.1±0.02^{b}	6.7±0.03^{c}
L5	(30:30:0)	6.3±0.07^{b}	6.8±0.01^{a}	6.4±0.08^{b}
L6	(30:0:30)	6.1±0.01^{a}	7.0±0.01^{b}	6.5±0.01^{c}
L7	(20:20:20)	6.2±0.06^{a}	7.0±0.01^{b}	6.5±0.10^{c}
LC	(0:0:0)	6.5±0.01^{a}	6.7±0.03^{b}	5.9±0.02^{c}
H1	(0:0:60)	6.6±0.02^{a}	7.6±0.06^{b}	7.0±0.05^{c}
H2	(0:60:0)	6.7±0.08 ^{a}	7.5±0.06^{b}	7.1±0.06^{c}
H3	(60:0:0)	6.6±0.02^{a}	7.7±0.0^{b}	7.2±0.04^{b}
H4	(0:30:30)	6.6± 0.06^{a}	7.5± 0.03^{b}	7.2± 0.01^{c}
H5	(30:30:0)	6.6± 0.01^{a}	7.5± 0.01^{b}	7.0± 0.00^{c}
H6	(30:0:30)	6.5± 0.04^{a}	7.6± 0.01^{b}	7.1±0.05^{c}
H7	(20:20:20)	6.6± 0.08^{a}	7.5± 0.02^{b}	7.2± 0.01^{c}
HC	(0:0:0)	6.7± 0.04^{a}	7.6± 0.06^{b}	7.2±0.03^{c}

Mean values ± SD denoted by the same letter in rows indicate no significantly difference according to Duncan test at *$p<0.05$

Soil pH is a major factor determining the metal bioavailability and metal speciation in the soil. Aging may cause the change in soil characteristics and thus affecting metal bioavailability. Changes in soil pH will be dependent on soil and amendment properties including the initial soil pH, and buffering capacity of the soil and amendments. Amendment of soils with organic matter and subsequently decomposition of organic matter may change the soil pH and subsequently affect the bioavailability of metals (Cui et al., 2011). Increase, decrease, and no effect of organic amendment application on soil pH have been reported by others (Parkpian et al., 2001; Doelsch et al., 2010). The increase in pH in the first 28 days of incubation (from T0 to T28) followed by decrease (from T28 to T84) can be explained by the initial soils and amendment characteristics. High pH of boiler ash and filter cake may cause the pH increase during the first few weeks of incubation. Then, when mineralization of N content of organic amendments occur, it is possible that this can cause the pH reduction of treated soils. Mineralization is possible an important properties in relation to their effects on soil pH. During the nitrification of NH_4^+ to NO_3^-, the overall process of conversion organic amendment-N to NO_3^- release two H^+ ions, is therefore acidifying.

4.2.4 Organic matter content (% OM) in treated soils

After spreading soil amendments, the chemical and biological conditions of the treated soils can be substantially altered during mineralization of these wastes through changes in the OM and/or physicochemical properties of the treated soils. A change in the organic matter content (% OM) in the treated soils in this study is shown in Table 4.5. The results showed that addition of amendments (at T = 0 day) has led to an increase in organic matter contents in the treated soils as compared to the controls (LC and HC) (see Table 4.5) as expected, almost half of the studied soils have very high organic matter contents (4.14-6.66%) indicating the productivity of the treated soils. The increase in organic matter content following amendment application often results in an improvement in soil physical properties including potentially enhancing fertility, soil structure, porosity, and water retention.

Table 4.5 Changes in organic matter content (% OM) in different treated soils (T0 and T98): soil pot experiment

Treatments	(FC: BA: VN)	% OM at different incubation periods	
	(g · pot $^{-1}$)	T_0 *	T_{98} **
L1	(0:0:60)	4.14	4.27
L2	(0:60:0)	5.16	4.89
L3	(60:0:0)	6.66	5.06
L4	(0:30:30)	5.16	4.89
L5	(30:30:0)	6.29	4.55
L6	(30:0:30)	5.89	4.92
L7	(20:20:20)	5.73	4.17
LC	(0:0:0)	5.64	3.96
H1	(0:0:60)	5.75	5.80
H2	(0:60:0)	6.12	5.62
H3	(60:0:0)	4.93	6.50
H4	(0:30:30)	5.75	6.11
H5	(30:30:0)	5.79	6.21
H6	(30:0:30)	6.17	4.73
H7	(20:20:20)	5.59	5.28
HC	(0:0:0)	4.47	5.26

Note: *$T_{0\,=}$ 0 day of incubation, **$T_{98\,=}$ 98 days incubation

At T = 98 days of incubation, there was a reduction in the organic matter content of the treated soils in low Cd contaminated soil (LCdS), excluding L1; whereas most cases in high Cd contaminated soil an increase in organic matter was observed. The reduction in organic matter is due to the organic matter decomposition. The rate of decomposition and/or mineralization of added amendments depend on the soil physico-chemical properties, environmental conditions and the chemistry of the added organic materials. The variation in organic matter changes of the two studied soils may be due to the differences in Cd and Zn concentrations of the soils studied here because these two soils have similar physico-chemical characteristics, excluding total Cd and Zn concentration. It has been reported that high concentration of heavy metals can have deleterious effects on organic matter decomposition and soil biological processes (Verma et al., 2010). The study of Verma et al. (2010) showed that increase Cd level and incubation times resulted in a reduction the rate of decomposition measured by CO_2 revolution of sugar trash added to the soil. Variation of soil organic matter decomposition can be attributed to the concentration of heavy metals, which would bring down the rate of microbial activity, and thus can have an effect on the metal solubility (Clemente et al., 2006).

4.3 METHOD VALIDATION

The sum of the extracted Cd and Zn concentration (mg kg^{-1}) from each step of the BCR extraction (BCR1, BCR2, and BCR3) plus the residual fraction (BCR4) was compared with the pseudo-total concentration obtained from *aqua regia* digestion of the soils (see Appendix B). According to the results, it can be seen that the sum of the four steps is in good agreement with the total metal concentration. Percentage recovery for Zn varied between 70% and 94% for the soil samples, indicating good laboratory working condition and reliability of the BCR sequential extraction procedure. However, Cd recovery ranged from 72% to 218% (86-218% in LCdS and 72-96% in HCdS), the deviation is low at high Cd concentration and increases at low Cd concentration. The sum of Cd concentrations in all BCR fractions for the low Cd contaminated soil (LCdS) is generally higher than the total Cd concentration detected, which was considered mainly due to the cumulative of metal during fractionation.

4.4 THE FRACTIONATION/DISTRIBUTION OF CADMIUM AND ZINC IN TREATED SOILS

The application of sugarcane waste-products as an amendment in soil can possibly introduce the loads of trace elements in soils. However, the mobile components rather than the total amounts of toxic trace elements are likely to cause adverse effects to the environment. To investigate the chemical speciation of trace elements in soil that can be readily and sequentially absorbed and uptake by plants, sequential extraction was done here by the three-step BCR sequential extraction. The percentage distribution of Cd and Zn in each fraction relative to their sum in all fractions from varying treated soils of LCdS and HCdS were determined according to their aging periods (Figs. 4.1 - 4.4). However, the data at T84 for Zn in LCdS was not available, only the treatments in HCdS are reported because of the accident during the metal analysis.

Based on the overall percentage distribution of Cd and Zn in the treated soils of all aging periods (at T = 0, 14, 28, 56, and 84 days), Cd was predominantly in the exchangeable (BCR1) and reducible (BCR2) fractions (Figs. 4.1 and 4.2). Very small amounts of Cd were in the oxidizable (BCR3) and residual (BCR4) fractions. Sequential extraction of Cd and Zn showed no difference between treated soils and the controls for both set of the studied soils (LCdS and HCdS). Higher Cd extractability in the exchangeable and reducible fraction suggested that Cd can be identified as easily mobilize and potentially bioavailable for plant uptake. Portions of metal in the exchangeable fraction are weakly adsorbed to the colloidal and particulate material of the soil, and susceptible to pH changes (Filgueiras et al., 2002; Gleyzes et al., 2002). Relative high Cd in reducible fraction is probably related to the formation of Fe/Mn oxide and hydroxides which precipitation and co-precipitation act as important metal sink (Hlavay et al., 2004; Purushothaman and Chakrapani, 2007). This fraction represents the metals bound to Fe/Mn oxides that would be released and/or mobilized by increasing reducing and anoxic conditions in the environment (Manskinen et al., 2011). The present in significant amount of Cd in these two fractions (BCR1 and BCR2) of this study indicated that Cd can be readily transferred to the environment, and thus great care must be taken.

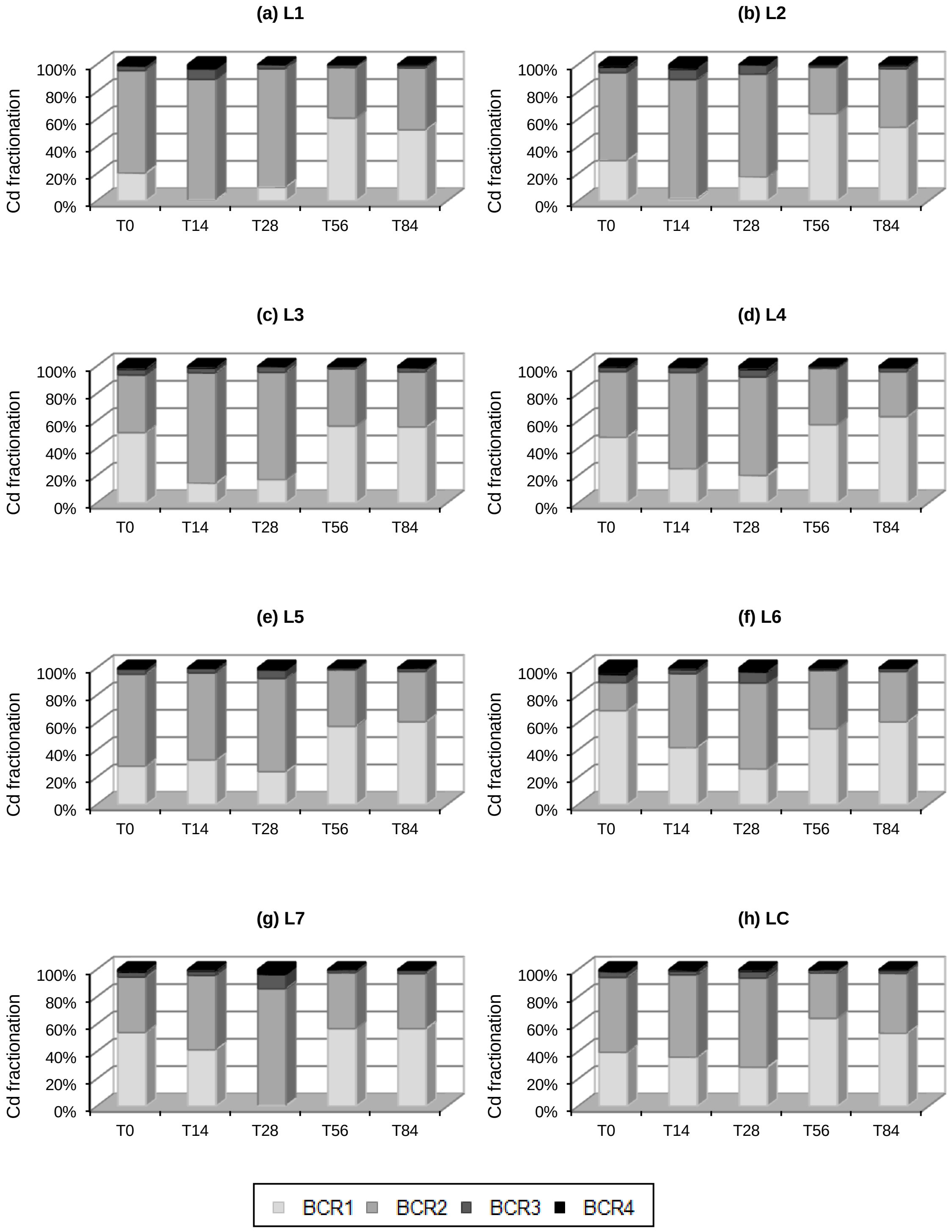


Figure 4.1 Cd fractionation change over time (T0, T14, T28, T56 and T84) by different sugarcane waste-products amended into the low Cd contaminated soil (L1, L2, L3, L4, L5, L6, L7 and LC)

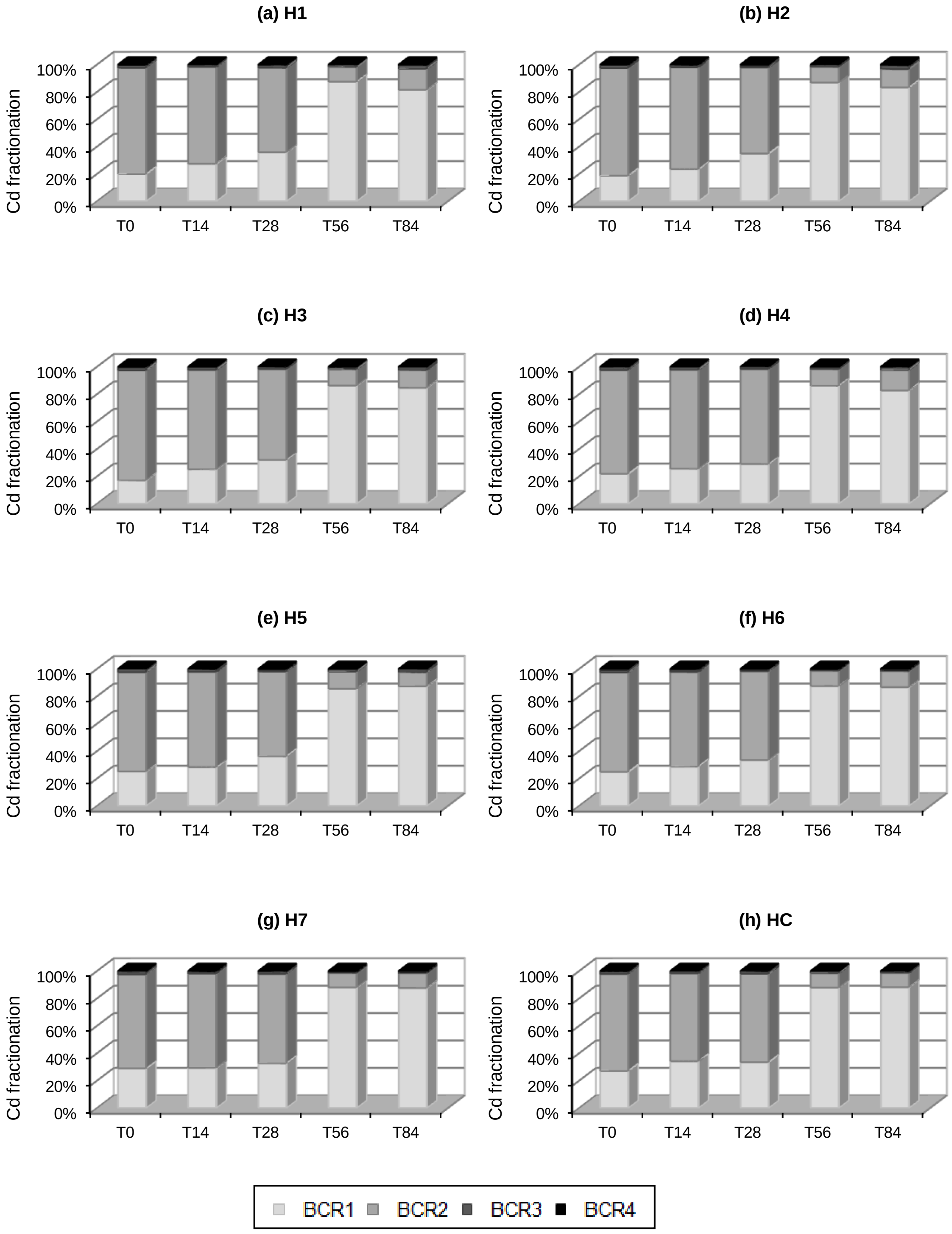


Figure 4.2 Cd fractionation change over time (T0, T14, T28, T56 and T84) by different sugarcane waste-products amended into the high Cd contaminated soil (H1, H2, H3, H4, H5, H6, H7 and HC).

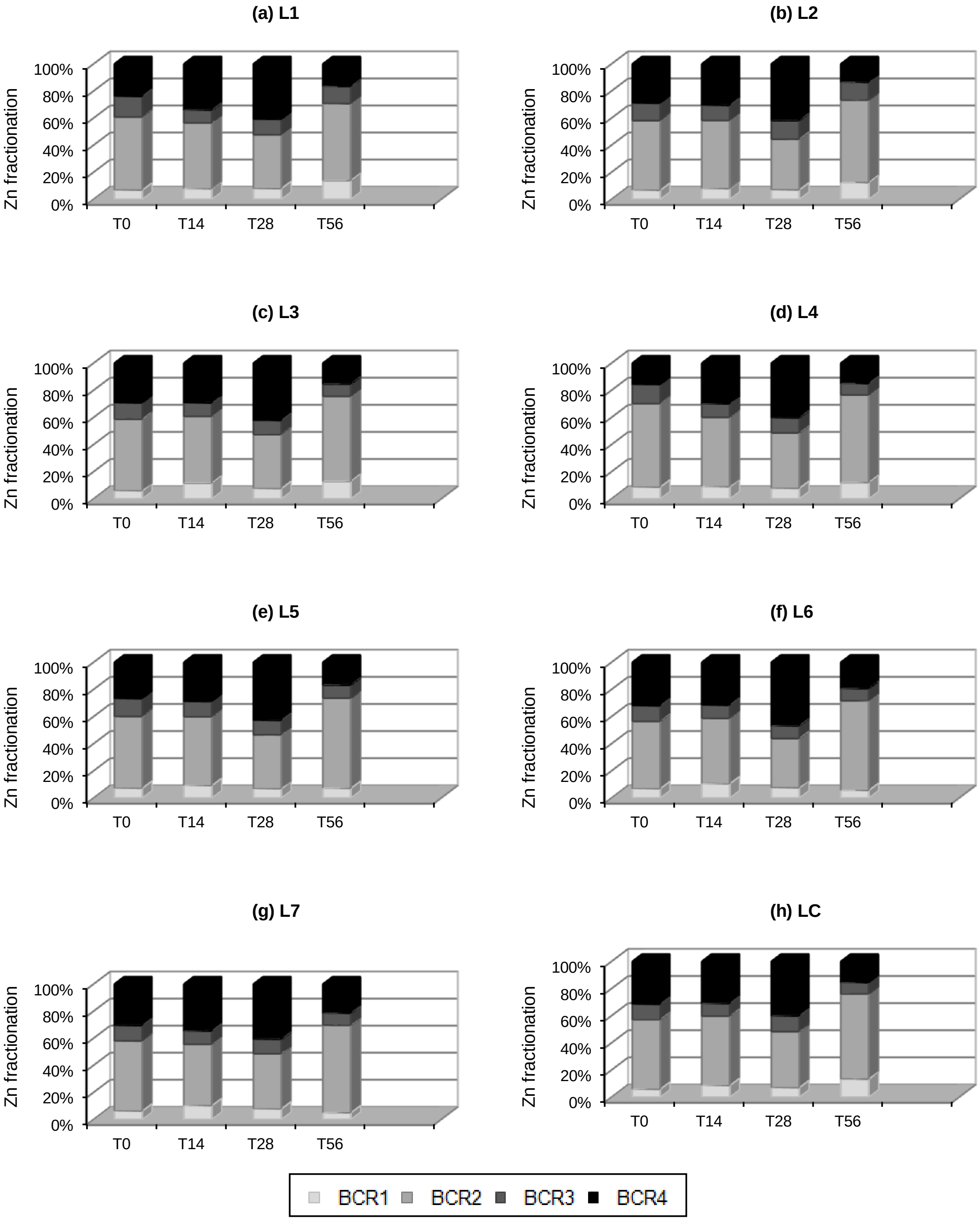


Figure 4.3 Zn fractionation change over time (T0, T14, T28, T56 and T84) by different sugarcane waste-products amended into the low Cd contaminated soil (L1, L2, L3, L4, L5, L6, L7 and LC).

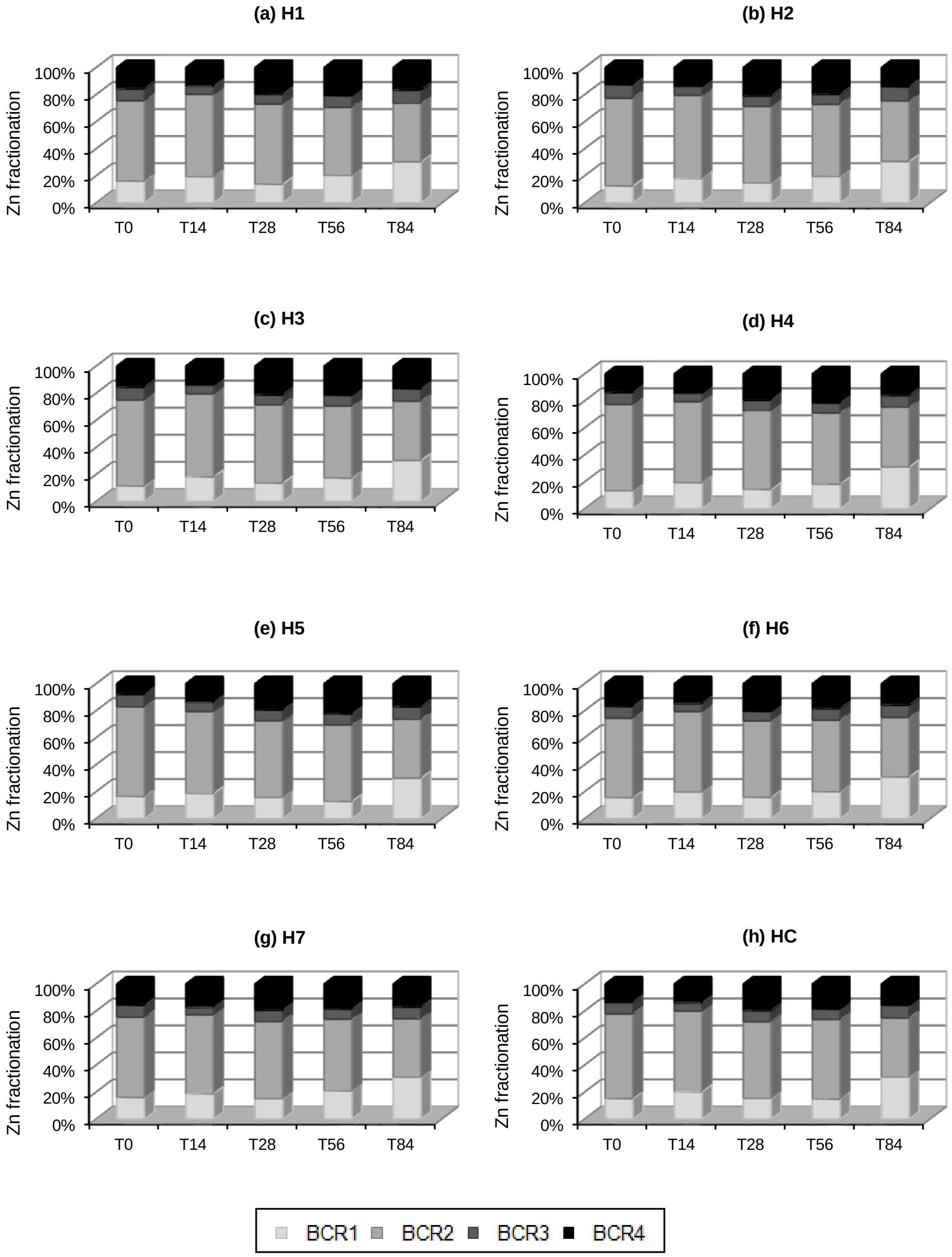


Figure 4.4 Zn fractionation change over time (T0, T14, T28, T56 and T84) by different sugarcane waste-products amended into the high Cd contaminated soil (H1, H2, H3, H4, H5, H6, H7 and HC)

Alternatively, the highest Zn distribution was in the reducible and residual fractions (Figs. 4.3 and 4.4). The percentage distributions of Zn in the exchangeable and oxidizable fractions were relatively similar and contributed to relatively low Zn proportions in all treatments. High Zn in the residual and reducible fractions in this experiment corresponded well with those reported by Pérez-de-Mora et al. (2007) who found that Zn was mainly associated with the second (BCR2) and the fourth (BCR4) fractions. It appears that results obtained from HCdS have the higher percentage of exchangeable Zn as compared to the LCdS, which suggests that binding of Zn may be of minor at high Cd and Zn concentrations. The higher contribution of the exchangeable fraction in HCdS may be possibly attributed to high metal loads in this soil. Considering the relative high amount of clay present in the soil (27.44 and 13.63% in LCdS and HCdS, respectively) (Table 4.1), adsorption to clay can be one of important mechanism that control the fractionation of Zn (Jalali and Khanlari, 2006). Higher clay mineral present in LCdS would have higher potential in terms of metal sorption capacity comparing to HCdS, thus attribute to lower and higher proportion of exchangeable and residual fractions in LCdS, respectively. The present finding is similar with other studies, for example, Achiba et al. (2010) and Janoš et al. (2010) who showed similar fractionation patterns when the BCR sequential extraction was applied. These authors found that Cd is mainly extracted in the first two fractions (BCR1 and BCR2), whereas Zn was principally distributed between the reducible and residual fractions. Sánchez-Martín et al. (2007) reported a similar pattern of Zn distribution in sewage sludge treated soils that the residual fraction exhibited the highest distribution and to lesser extent in Fe/Mn oxide fraction. Anju and Banerjee (2010) also studied the soils collected from different mining areas in India and found that Cd was present in significantly amount in the acid soluble fraction and reducible fraction whereas Zn was predominantly associated with residual fraction.

In this study, Cd displayed relatively high mobility at the end of experiment (T84), contributed in the exchangeable and reducible fractions (BCR1+2) is up to 97.4% (L1) and 98.5% (HC) of the total Cd in LCdS and HCdS, respectively, while Zn exhibited lower in percentage distribution of these highly mobility fractions than Cd (up to 66.8% (L3) and 75.4% (H4) of the total Zn in LCdS and HCdS, respectively). The fractionation patterns of Cd and Zn at T84 can be summarized in the order as following; Cd: BCR1 > BCR2 > BCR3 > BCR4 and Zn: BCR2 > BCR4 ≥ BCR1 > BCR3.

4.5 THE EFFECT OF AGING ON THE TRANSFORMATION OF CADMIUM AND ZINC FRACTIONATION

Significant difference of treatments across times in each fraction (BCR1, BCR2, BCR3 and BCR4) were determined using *Duncan's* test ($*p<0.05$) to investigate the effect of aging. The changes of Cd and Zn across times (T0 to T84) are illustrated in Figs. 4.5 and 4.6, respectively. Generally, the exchangeable Cd (BCR1) in accordance with time (T = 0 to 84 days incubation) was observed to be slightly decreased ($*p <0.05$) during the first month of the pot experiment (from T = 0 to 28 days) in LCdS, and then subsequently increased to T56 then leveled off to T84 (Fig. 4.5 (a)). This is in accord with the initial increase of pH in the first few weeks and subsequently decreases (Table 4.4). The effects of pH on bioavailability and/or fractionation showed the most prevalent in influencing the Cd transformation during the aging in this study. Alternatively, the exchangeable Cd in HCdS gradually increased from T0 to T28, then steeply increased to T56 and then leveled off to the end of incubation period. The reducible fraction of Cd in LCdS (BCR2-Cd), in contrary, exhibits the fluctuation during the first two months of the pot experiment (from T0 to T56; Fig. 4.5 (b)), while the reducible fraction of Cd in HCdS gradually decreased in the first few weeks (T0-T28) followed by sharp decrease through the end of the experiment (Fig. 4.5 (f)).

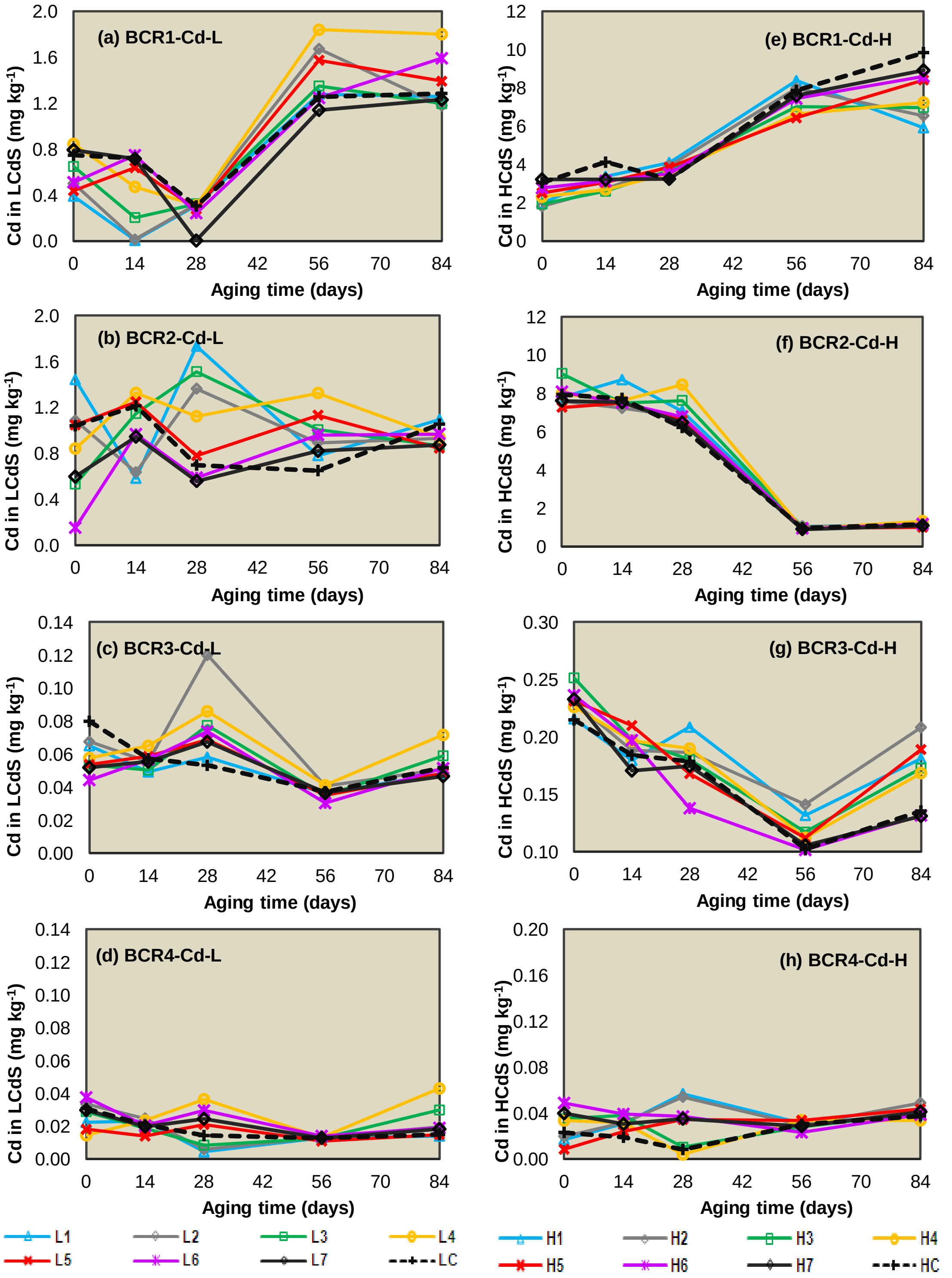


Figure 4.5 Effect of sugarcane waste products amended into the low and high Cd contaminated soils on Cd fractionation (BCR1, BCR2, BCR3 and BCR4) over time (T0, T14, T28, T56 and T84)

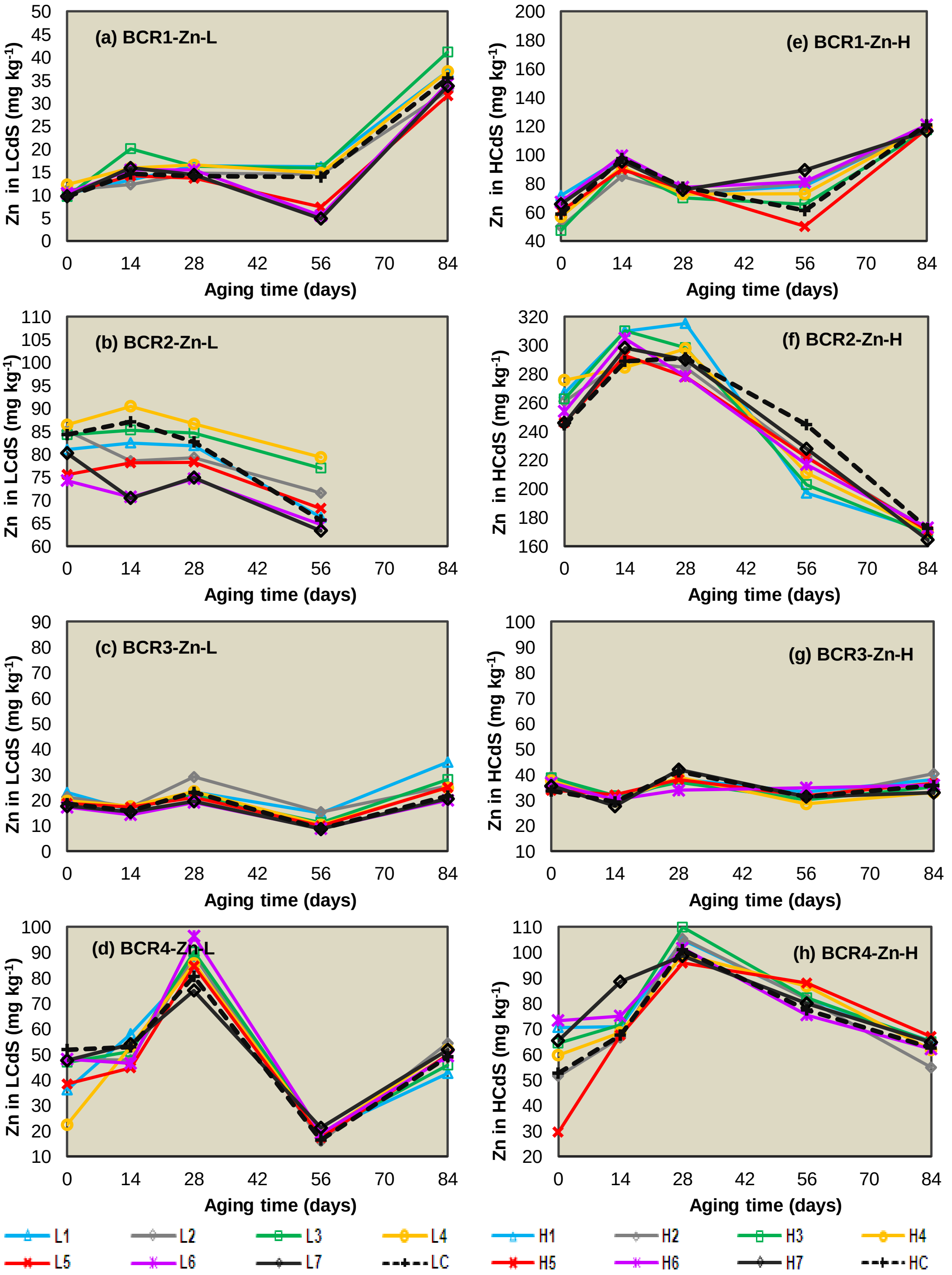


Figure 4.6 Effect of sugarcane waste products amended into the low and high Cd contaminated soils on Zn fractionation (BCR1, BCR2, BCR3 and BCR4) over time (T0, T14, T28, T56 and T84)

This observation may be explained by the reciprocal redistribution of Cd between the exchangeable and the reducible fractions during the entire period of incubation in accordance with the increasing and decreasing of the pH. The difference in Cd distribution between fractions after the addition of sugarcane waste-products was observed and consistent with the study of Wu et al. (2012) those found the changes of Cd distribution of exchangeable and reducible fractions in soils when pig manure was applied with the significant increase in exchangeable fraction. Amounts and changes of Cd concentration in the oxidizable and residual fractions (Figs. 4.5 (c), (d), (g), (h)) due to the aging showed no clear trend on the transformation of Cd across times, except oxidizable Cd in HCdS decreased from T0 to T56 and sharply increased until T84. The gradual decrease of oxidizable fraction (BCR3) with the incubation time (from T0 to T56) could be ascribed to the dissolution and decomposition of metals associated with organic matter in the treated soils, leading to an increase in the concentration of other fraction, especially in the exchangeable fraction (BCR1). pH played an important role for Cd in the transformation and/or redistribution during the aging in this study.

Moreover, the aging showed the similar effect in Zn as was shown in Cd in all fractions. The exchangeable fraction of Zn in all treated soils in both LCdS and HCdS showed variable increase at T14 ($*p<0.05$), decreased at the T28 until T56, and increased to T84. However, Zn in reducible fraction for both studied soils (LCdS and HCdS) (data at T84 for LCdS is not available) tended to decrease significantly until T84. The results here are partially consistent with the results obtained by Lu et al. (2005), who studied the fractionation of Cd and Zn on typical Chinese soils incubated for over 3 weeks and found that Zn concentration in the exchangeable fraction decreased as time prolonged. In contrast to the first two fractions, aging caused the redistribution in the first two fractions, while the more refractory fractions (BCR3 and BCR4) remained unchanged. The increase in the exchangeable with the decrease in the reducible fractions of Zn with the unchanged in non-mobile fraction (BCR3 and BCR4) explained the reciprocal redistribution of Zn between these two fractions. The finding of this study showed that aging has an effect on bioavailability of Cd and Zn in soils, especially in the mobile fractions (BCR1 and BCR2), and significantly affected by the soil pH.

4.6 THE EFFECT OF THE AMENDMENTS ON CADMIUM AND ZINC BIOAVAILABILITY

The effects of the amendments on bioavailability of metals in soil were determined to be soil and dose dependent. A study of the effect of aging under the application of sugarcane waste-products as soil amendments was observed in the first two fractions of the sequential extraction (BCR1 and BCR2) for both metals (Cd and Zn). It was noted that the trend of changing for Cd and Zn fractionation could be explained by the reciprocal redistribution of metals between exchangeable and reducible fractions. In this study, special attention was given to the first fraction (BCR1), the most labile, easily mobilized and highest potential for the plant uptake. Cadmium and Zn concentrations in the exchangeable fraction (BCR1) at all incubation periods are given in Tables 4.6-4.7. The results showed significant differences in Cd concentrations between the control (LC and HC) and the treated soils for both studied soils. Regarding the treatment response on the reduction of Cd in the exchangeable fraction (BCR1) (Table 4.6), treatments L2, L3, L4 and H2, H3, and H4 (in LCdS and HCdS, respectively) are expected to be the optimum ratio of amendments at doses used in this study. Alternatively, no treatment response was observed for Zn in exchangeable fraction (BCR) of HCdS for all incubation periods, except H2 and H3 at T0 (Table 4.7).

The observed reduction in exchangeable fraction of Cd in the treated soils as compared to the control highlights the implications when sugarcane waste-products are applied. Despite a reduction of exchangeable Cd of treatment L1 and H1, which is a single application of vinasse, this waste may negatively affect the physico-chemical properties of soil due to the lower of pH, its high viscosity, and its high salt content. Due to the sugarcane waste-products characteristics, successful addition of single vinasse itself as soil amendments would be very difficult and may causes undesirable effects without the addition of other organic sources. Too low pH of vinasse may affect the soil properties and hence the bioavailability of the metals. Precaution should be taken when applying these waste-products. Despite the plant essential nutrients in vinasse, this waste-product may negatively affect the soil physico-chemical properties of the soils.

Table 4.6 Exchangeable-Cd concentrations (mg kg^{-1}) (BCR1) for all treated soils for low and high Cd contaminated soils (LCdS and HCdS).

Treatments	Cd concentration in exchangeable fraction-BCR1 (mg kg^{-1})				
LCdS	T0	T14	T28	T56	T84
L1	0.39^{a}	0.01^{a}	0.20^{cd}	1.28^{ab}	1.26^{a}
L2	0.50^{ab}	0.01^{a}	0.32^{d}	1.68^{bc}	1.18^{a}
L3	0.65^{abc}	0.20^{a}	0.32^{d}	1.36^{ab}	1.20^{a}
L4	0.85^{c}	0.47^{b}	0.31^{cd}	1.84^{c}	1.80^{a}
L5	0.44^{a}	0.64^{bc}	0.28^{bc}	1.58^{abc}	1.39^{a}
L6	0.51^{ab}	0.75^{c}	0.24^{b}	1.26^{ab}	1.59^{a}
L7	0.79^{bc}	0.72^{c}	0.003^{a}	1.15^{a}	1.23^{a}
LC	0.75^{bc}	0.72^{c}	0.31^{cd}	1.26^{ab}	1.29^{a}
HCdS	T0	T14	T28	T56	T84
H1	1.99^{ab}	3.37^{b}	4.10^{d}	8.37^{a}	5.92^{a}
H2	1.81^{a}	2.74^{ab}	3.92^{cd}	7.98^{a}	6.55^{ab}
H3	1.93^{ab}	2.60^{a}	3.68^{bc}	7.02^{a}	6.98^{b}
H4	2.31^{abc}	2.69^{a}	3.54^{ab}	6.64^{a}	7.23^{b}
H5	2.51^{bcd}	3.06^{ab}	3.86^{bcd}	6.43^{a}	8.41^{c}
H6	2.76^{cde}	3.13^{ab}	3.51^{ab}	7.44^{a}	8.60^{c}
H7	3.22^{e}	3.21^{ab}	3.25^{a}	7.60^{a}	8.92^{c}
HC	3.04^{de}	4.12^{c}	3.22^{a}	7.86^{a}	9.85^{d}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at *$p<0.05$.

*T0, T14, T28, T56, T84 are the time at 0, 14, 28, 56 and 84 days of incubation, respectively.

Table 4.7 Exchangeable-Zn concentrations (mg kg^{-1}) (BCR1) for all treated soils for low and high Cd contaminated soils (LCdS and HCdS).

Treatments	Zn concentration in exchangeable fraction-BCR1 (mg kg^{-1})				
LCdS	T0	T14	T28	T56	T84
L1	10.22^{ab}	13.21^{ab}	16.35^{b}	16.13^{b}	36.90^{ab}
L2	8.96^{a}	12.27^{a}	14.72^{ab}	14.46^{b}	32.65^{ab}
L3	9.65^{a}	20.09^{c}	16.27^{b}	15.83^{b}	41.15^{b}
L4	12.23^{b}	15.85^{b}	16.53^{b}	14.75^{b}	36.91^{ab}
L5	10.34^{ab}	14.14^{ab}	13.63^{a}	7.34^{a}	31.64^{a}
L6	10.36^{ab}	15.45^{ab}	15.48^{bc}	5.48^{a}	34.25^{ab}
L7	9.71^{a}	15.86^{b}	14.36^{ab}	4.85^{a}	33.73^{ab}
LC	9.62^{a}	14.58^{ab}	14.07^{a}	13.89^{b}	35.50^{ab}
HCdS	T0	T14	T28	T56	T84
H1	71.55^{b}	97.58^{a}	73.16^{ab}	78.40^{a}	119.28^{a}
H2	49.95^{a}	85.11^{a}	72.89^{ab}	80.28^{a}	117.71^{a}
H3	47.22^{a}	91.20^{a}	69.92^{a}	65.56^{a}	117.41^{a}
H4	56.75^{ab}	91.34^{a}	72.59^{ab}	72.72^{a}	119.11^{a}
H5	60.72^{ab}	89.82^{a}	76.07^{ab}	50.01^{a}	118.04^{a}
H6	67.01^{b}	99.43^{a}	77.41^{b}	81.12^{a}	120.90^{a}
H7	65.53^{b}	95.31^{a}	75.55^{ab}	89.32^{a}	116.55^{a}
HC	58.63^{ab}	97.34^{a}	77.34^{b}	61.10^{a}	120.82^{a}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at *$p<0.05$.

*T0, T14, T28, T56, T84 are the time at 0, 14, 28, 56 and 84 days of incubation, respectively.

However, the combination of vinasses and boiler ash (L4 and H4) may be a useful treatment for improving soil quality. Boiler ash is alkaline and vinasses are acidic so the mixture of these two materials may have a positive effect on soils. Successful application of sugarcane waste-products to improve soil quality has been previously reported by other (Ossom, 2010). A pot experiment with plant is therefore necessary for further study with these selected ratio of amendments to determine the interaction between soil, amendments and plant where application are expected to continue.

The positive and/or negative effect of using sugarcane waste-products as soil amendments have been reported by others (Parkpian et al., 2001; Meunchang et al., 2005; Resende et al., 2006; Tejada et al., 2007; Bueno et al., 2009). Tejada et al. (2007) reported the negative effect of beet vinasse on structural stability and bulk density of the soil due to high monovalent cations (Na^+) and fulvic acids. Winery vinasse application had been studied by Bueno et al. (2009) and found that vinasses can affect a certain chemical properties (electrical conductivity) in the soil negatively while the aging showed no significant trend for soil pH. The positive effect of vinasse as soil amendment, however, had been reported. Long term effect of vinasse application on the yield of sugarcane plantation in Brazil was studied by Resende et al. (2006). They found that the addition of vinasse at 80 m^3 $ha^{-1}crop^{-1}$ significantly increased cane yield by 12-13% in the two cycles, respectively. They suggested that high K content of the vinasse was the main responsible for the increased yield. Tadsanee D. (2008) and Soongsud P. (2008) also studied the effect of vinasse on the sugarcane growth and also found positive effect on the plant growth. Therefore, treatments L2, L3, L4 and H2, H3, H4 were then selected for further study in the pot experiment growing with the plant.

4.7 DTPA-EXTRACTION

4.7.1 Extractable DTPA-Cd concentration

Diethylenetriaminepentaacetic acid (DTPA) is a chelating agent that transfers metals bound to the solid soil phases into the soil solution by forming water soluble complexes with heavy metals. Single DTPA extraction was selected and used in this study due to the pH of studied soils that are slightly acidic and near neutral (pH 6.35 and 6.53 in LCdS and HCdS, respectively) (Table 4.1). The DTPA-Cd and DTPA-Zn concentration in all treated soils (LCdS and HCdS) incubated at different aging periods (T = 0, 14, 28, 56, and 84 days) was shown in Table 4.8-4.9.

Table 4.8 DTPA-Cd concentrations in low and high Cd treated soil (LCdS and HCdS) at T = 0, 14, 28, 56, and 84 days of incubation (*n* = 3±SD)

Treatments	DTPA-Cd (mg kg^{-1})				
(LCdS)	T_0	T_{14}	T_{28}	T_{56}	T_{84}
L1	0.30 ± 0.03^{a}	0.33 ± 0.07^{ab}	0.25 ± 0.03^{a}	0.20 ± 0.08^{a}	0.45 ± 0.06^{a}
L2	0.29 ± 0.04^{a}	0.28 ± 0.04^{b}	0.24 ± 0.06^{a}	0.16 ± 0.03^{a}	0.51 ± 0.14^{a}
L3	0.25 ± 0.05^{a}	0.25 ± 0.03^{b}	0.20 ± 0.04^{a}	0.06 ± 0.06^{a}	0.66 ± 0.15^{a}
L4	0.31 ± 0.05^{a}	0.31 ± 0.06^{ab}	0.19 ± 0.01^{a}	0.31 ± 0.2^{a}	0.74 ± 0.18^{a}
L5	0.29 ± 0.04^{a}	0.31 ± 0.06^{ab}	0.22 ± 0.03^{a}	0.27 ± 0.14^{a}	0.52 ± 0.19^{a}
L6	0.33 ± 0.04^{a}	0.26 ± 0.02^{b}	0.19 ± 0.05^{a}	0.25 ± 0.16^{a}	0.45 ± 0.05^{a}
L7	0.29 ± 0.02^{a}	0.56 ± 0.22^{a}	0.17 ± 0.02^{a}	0.13 ± 0.02^{a}	0.58 ± 0.01^{a}
LC	0.30 ± 0.00^{a}	0.36 ± 0.04^{ab}	0.18 ± 0.03^{a}	0.28 ± 0.68^{a}	0.48 ± 0.05^{a}
(HCdS)	T_0	T_{14}	T_{28}	T_{56}	T_{84}
H1	0.98 ± 0.16^{a}	0.77 ± 0.15^{a}	0.74 ± 0.05^{a}	1.31 ± 0.55^{a}	1.81 ± 0.37^{a}
H2	0.84 ± 0.07^{a}	0.63 ± 0.11^{a}	0.80 ± 0.15^{a}	1.35 ± 0.28^{a}	1.83 ± 0.37^{a}
H3	0.72 ± 0.08^{a}	0.67 ± 0.13^{a}	0.66 ± 0.04^{a}	0.92 ± 0.13^{a}	1.63 ± 0.34^{a}
H4	0.84 ± 0.19^{a}	0.82 ± 0.07^{a}	0.71 ± 0.14^{a}	1.02 ± 0.23^{a}	1.53 ± 0.16^{a}
H5	0.94 ± 0.19^{a}	0.73 ± 0.36^{a}	0.69 ± 0.27^{a}	1.22 ± 0.12^{a}	1.42 ± 0.17^{a}
H6	0.90 ± 0.15^{a}	0.58 ± 0.08^{a}	0.66 ± 0.12^{a}	1.42 ± 0.13^{a}	1.51 ± 0.05^{a}
H7	0.83 ± 0.20^{a}	0.58 ± 0.28^{a}	0.67 ± 0.03^{a}	1.38 ± 0.31^{a}	1.30 ± 0.17^{a}
HC	0.90 ± 0.03^{a}	0.88 ± 0.06^{a}	0.70 ± 0.09^{a}	1.33 ± 0.21^{a}	1.58 ± 0.70^{a}

Mean values ± SD not connected by same letter in columns are significantly different according to Tukey-Kramer HSD at *$p<0.05$.

According to the results, DTPA-Cd concentration of the treatments in LCdS was very low in all treatments in relation to their respective total Cd concentration, indicated low mobility and bioavailability of Cd. Addition of sugarcane waste-products (BA, FC, and VN) resulted in the similar DTPA-Cd concentrations as the control treatment (LC) at the time of application (T = 0 day incubation). The changes in concentration of DTPA-Cd over time from T = 0 to 84 days exhibited the same pattern among all LCdS treatments. One-way ANOVA analysis was carried out for DTPA-Cd across treatments by times. A statistical means comparison for all pairs of treatments was performed using the Tukey-Kramer multiple comparison test at $*p<0.05$ to determine significant difference among treatments within days (T = 0, 14, 28, 56, 84 days incubation), and within soils (LCdS and HCdS). Results showed that there was no significant difference among treatments for all of incubation periods, indicating different rate of amendments may not have an effect on DTPA-Cd concentration for low Cd contaminated soil. According to time and treatment interaction, there is no significance among treatments for all incubation times (T = 0, 14, 28, 56, 84 days incubation), suggesting that length of incubation is not important.

For high Cd contaminated soil (HCdS), approx. three-fold DTPA-Cd concentration of the treated soils in HCdS was observed as compared to LCdS at the time of amendments application (T = 0 day incubation). DTPA-Cd concentrations in all HCdS treatments tend to increase throughout the incubation period (Table 4.9). According to the Turkey-Kramer multiple comparison, the results showed that there is no significant difference among treatments for every incubation time (T0-T84). This suggests that different ratio of amendments may not have an influence on DTPA-Cd concentrations for high Cd contaminated soil. In other words, the addition of sugarcane waste-products may not have an influence on DTPA-Cd concentration for both studied soils.

4.7.2 Extractable DTPA-Zn concentration

According to the results of extractable DTPA-Zn concentration, aging significantly increased the bioavailability of Zn in both LCdS and HCdS (Table 4.9). The mechanisms involved in the increase of DTPA-Zn concentration due to the aging may be various and depend on both amendments and soil characteristics, which play an essential role in metal bioavailability. DTPA-Zn concentration in all treatments of LCdS exhibited the same trend as in HCdS which tends to increase as time increased. DTPA-Zn concentration in LCdS ranged from 4.35 to 5.57 mg kg^{-1}, while DTPA-Zn concentration in HCdS was from 9.48 to 13.98 mg kg^{-1} when amendments have been applied. Almost double of DTPA-Zn concentration was observed in HCdS as compared to LCdS. DTPA-Zn concentration in HCdS was relatively low as compared to the corresponding total Zn concentration in the soils. Low DTPA-Zn concentration in both LCdS and HCdS indicated the low mobility of metal.

Tukey-Kramer test ($*p<0.05$) was used to determine significant difference among treatments in two studied soils (LCdS and HCdS) for all incubation periods (T = 0, 14, 28, 56, 84 days incubation) for DTPA-Zn and results showed that there is no significant difference among treatments, excluding T = 28 days in LCdS and T = 0 and T = 28 days in HCdS. By comparing with the control (LC) in LCdS, significant difference was observed between the control (LC) and L1, L2, and L3 (at T = 28 days incubation), and L3 (at T = 56 days incubation). For HCdS, control treatment (HC) was significant difference from H1 (at T = 0 day incubation) and H1 and H3 (at T = 28 days incubation). Regarding to the treatments, L1/H1, L2/H2, and L3/H3 are the single application of sugarcane waste-products, namely vinasse (L1 and H1), boiler ash (L2 and H2) and filter cake (L3 and H3), respectively. This suggested that aging time may have an effect on the DTPA-Zn concentration along with the application of single amendment in the two studied soils. In contrast to our results, Zapusek and Lestan (2009) studied the effect of accelerated aging with 5 sequential cycles of 5-day exposure to high temperature (T = 105˚C) followed by 5-day exposure to low temperature (T = -20˚C) on artificial soil mixed with sewage sludge and found a significant decreased in DTPA-Cd and Zn concentrations in artificial soil mixtures. Keller et al (2005) reported the reduced DTPA-Cd and Zn concentration when inorganic amendments (lime, vermiculite and hydroxyapatite) had been added after 8 repeated wet and dry cycles of accelerated aging.

Table 4.9 DTPA-Zn concentrations in low and high Cd treated soil (LCdS and HCdS) at T = 0, 14, 28, 56, 84 days of incubation

Treatments	DTPA-Zn (mg kg^{-1})				
(LCdS)	T$_0$	T$_{14}$	T$_{28}$	T$_{56}$	T$_{84}$
L1	4.37±0.57^{a}	4.01±0.42^{b}	7.19±0.96^{a}	6.29±0.66^{ab}	6.33±0.38^{a}
L2	4.88±0.20^{a}	3.69±0.57^{b}	6.90±0.73^{ab}	6.04±0.05^{ab}	6.50±0.69^{a}
L3	4.35±0.54^{a}	3.93±0.63^{b}	6.07±1.09^{abc}	5.40±0.26^{b}	7.10±0.58^{a}
L4	5.57±0.33^{a}	4.02±0.11^{b}	5.02±0.25^{bcd}	6.54±0.93^{ab}	7.09±0.46^{a}
L5	5.08±0.98^{a}	2.32±0.28^{b}	4.73±1.04^{cd}	6.65±0.64^{ab}	6.67±0.41^{a}
L6	5.50±0.41^{a}	2.57±0.83^{b}	3.33±0.35^{d}	6.65±0.68^{ab}	6.92±0.35^{a}
L7	4.69±0.03^{a}	8.25±3.42^{a}	3.22±0.39^{d}	5.97±0.17^{ab}	7.40±0.06^{a}
LC	4.73±0.26^{a}	5.75±0.44^{ab}	3.25±0.32^{d}	7.03±1.10^{a}	7.34±0.13^{a}
(HCdS)	T$_0$	T$_{14}$	T$_{28}$	T$_{56}$	T$_{84}$
H1	13.98±0.94^{a}	9.76±1.36^{a}	8.56±0.64^{c}	14.25±4.42^{a}	17.05±1.53^{a}
H2	10.77±0.78^{ab}	7.60±0.63^{a}	8.82±0.34^{bc}	11.12±0.51^{a}	18.54±1.96^{a}
H3	9.48±0.82^{b}	7.91±0.74^{a}	7.48±0.23^{c}	12.60±4.09^{a}	18.59±1.31^{a}
H4	10.64±1.79^{ab}	9.05±0.20^{a}	16.39±2.73^{a}	14.73±2.11^{a}	19.91±0.23^{a}
H5	10.98±1.33^{ab}	8.22±3.54^{a}	16.07±2.57^{a}	15.77±1.19^{a}	18.62±0.93^{a}
H6	11.21±0.50^{ab}	7.41±0.70^{a}	14.76±1.74^{a}	18.77±0.55^{a}	18.71±1.16^{a}
H7	10.36±2.62^{ab}	6.83±4.19^{a}	13.79±1.57^{a}	18.29±1.67^{a}	16.02±1.13^{a}
HC	10.05±0.41^{b}	8.59±0.64^{a}	13.40±1.11^{ab}	15.98±2.45^{a}	17.49±5.17^{a}

Mean values ± SD not connected by same letter in columns are significantly different according to Tukey-Kramer HSD at *$p<0.05$.

4.8 CORRELATION OF BCR-DTPA EXTRACTION

BCR and DTPA extraction were used in this study to identify and evaluate the mobility and bioavailability of the metals in soil samples that are believed to be available for the plant uptake. Correlation analysis was conducted for all incubation periods of time (at T0, T14, T28, T56 and T84 days incubation) to determine the relationship between these two procedures. Pearson correlation coefficient (*r*) was used in this study by means of the BIVARIATE procedure in the SPSS software package version 17.0 in order to determine the relationship between DTPA extractable metal concentration, exchangeable metal concentration (BCR1) and the sum of the first two fraction of BCR sequential extraction (BCR1+2) for all incubation periods (Table 4.10).

Table 4.10 Correlation analysis of DTPA-BCR of Cd and Zn concentrations in LCdS and HCdS

Incubation period-Cd	Pearson correlation coefficient (*r*)	
	Cd (BCR1)	Cd (BCR1+2)
T0-DTPA-Cd	0.881^{**}	0.933^{**}
T14-DTPA-Cd	0.797^{**}	0.797^{**}
T28-DTPA-Cd	0.934^{**}	0.908^{**}
T56-DTPA-Cd	0.838^{**}	0.836^{**}
T84-DTPA-Cd	0.835^{**}	0.835^{**}
Incubation period-Zn	Pearson correlation coefficient (*r*)	
	Zn (BCR1)	Zn (BCR1+2)
T0-DTPA-Zn	0.935^{**}	0.930^{**}
T14-DTPA-Zn	0.710^{**}	0.721^{**}
T28-DTPA-Zn	0.821^{**}	0.782^{**}
T56-DTPA-Zn	0.767^{**}	0.872^{**}
T84-DTPA-Zn	0.959^{**}	0.957^{**}

**Correlation is significant at the 0.01 level (2-tailed)

According to the results, DTPA-Cd and Cd extracted from BCR1 showed a good correlation at $*p<0.01$ for all aging periods. This is also in the case for Zn in which DTPA-Zn and Zn in BCR1 was also significantly positive. DTPA is the chelator which is able to complex with most metal in soils, the exchangeable fraction of BCR (acetic acid) is the metal that associated with the carbonates and weakly bound to the solid phase particle. The quantity of metal extracted either by the BCR sequential extraction or by the DTPA can be considered as a potential prediction of phytoavailability of the metals. The good correlation from the correlation analysis (Table 4.10) implied the usefulness of using these procedures (DTPA and BCR sequential extraction) in metal bioavailable determination in this study. Moreover, good correlation between DTPA and (BCR1+2) was also observed for both metals. Therefore, the use of single (DTPA) and sequential extraction (BCR) would be very useful in this study to provide useful information for prediction of trace element mobility and providing further evaluation on the metal uptake by plants.

4.9 CORRELATION OF BCR1-Avail.P AND Exc.K

Correlation of BCR1-concentration (mg kg^{-1}) of two metals (Cd and Zn) and essential plant nutrients (Avail.P and Exc.K) were determined to identify and evaluate the relationship of these nutrients via the waste application through the metal bioavailability. Correlation analysis was conducted for all incubation periods of time (at T0, T14, T28, T56 and T84 days incubation) and shown in Table 4.11.

Table 4.11 Correlation analysis of BCR1-Avail.P and BCR1-Exc.K of Cd and Zn concentrations in LCdS and HCdS

Incubation period	Pearson correlation coefficient (*r*)			
	Cd		Zn	
	AvailP	ExcK	AvailP	ExcK
BCR1 T0	0.171	-0.036	0.118	0.111
BCR1 T14	0.053	-0.038	0.184	0.043
BCR1 T28	0.149	0.139	0.161	0.084
BCR1 T56	0.117	0.097	0.196	0.129
BCR1 T84	0.154	-0.011	0.182	0.067

It has been shown that there is the weak positive relationship between exchangeable Cd and Zn with Avail.P and Exc.K but not significant at *$p<0.01$. Therefore, relationship cannot be clearly defined based on the correlation analysis of these variables.

4.10 CONCLUSION: SOIL POT EXPERIMENT

The observed reduction in exchangeable fraction of Cd (BCR1) at the 3% (w/w) amendment application, the low total metal concentrations, and the significant amount of plant essential nutrients (N, P, K) in the sugarcane waste-products treated soils highlights the implications of sugarcane waste-products as soil amendment. Among different treatments of the application rates used in this study, the most effective in terms of reducing the potential bioavailability of Cd and Zn (BCR1) were chosen. Treatments L2 and H2 (single application of boiler ash at 3%), L3 and H3 (single application of filter cake at 3%), and L4 and H4 (combination between boiler ash and vinasse, 1.5%+1.5%) were selected as the optimum ratio of amendments and used further in the next experiment to determine the interaction between soil, amendments and plant where application are expected to continue. Correlation analysis between the two extraction procedures (BCR and DTPA) showed the good correlation exhibited the feasibility and validity for metal bioavailable determination. However, due to the very low DTPA-metal concentrations and no treatment response was observed, only the BCR sequential extraction was selected and used for the next experiment.

CHAPTER V

RESULTS AND DISCUSSIONS: SUGARCANE POT EXPERIEMNT

This chapter covers the experimental results and discussions from the sugarcane pot experiment which are separated into three different sub-sections as 1) the physico-chemical characteristics of the soils and amendments used in this sugarcane pot experiment; 2) the results from the sugarcanes grown in low Cd contaminated soil (LCdS), and 3) the results from the sugarcanes grown in high Cd contaminated soil (HCdS). The capitals L- and H- of the treatment names indicate treatments in low Cd and high Cd contaminated soils, respectively.

5.1 PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS AND AMENDMENTS

Soils and sugarcane waste-products used in this experiment were collected at different periods from the previous study (soil pot experiment). Therefore, soils and amendments analysis were then performed again to determine their characteristics prior to be used in the experiment. Characterization of the initial soils (LCdS and HCdS) and the amendments (BA, FC, VN) used in this study are given in Table 5.1. The studied soils are classified as sandy clay loam and slightly acidic (pH 6.4 and 6.6, respectively). The soils have moderate level of organic matter content (2.79% and 2.58%), low level of total N (0.08% and 0.09%), available P (6 and 11 mg kg^{-1}), and exchangeable K (87 and 74 mg kg^{-1}). The soils contain moderately high levels of Cd (3-5 mg kg^{-1} and 19 mg kg^{-1} in LCdS and HCdS, respectively) and Zn (211-329 mg kg^{-1} and 914 mg kg^{-1}) with the CEC of 15.5 and 12.2 cmol^{+} kg^{-1}. In comparison, BA is alkaline (pH 8.85), FC and AV are slightly acidic (pH 6.02 and 6.58, respectively). These sugarcane wastes-products of BA, FC, and AV contained very high organic matter content (6.20%, 17.4%, 15.2%), available P (6964 mg kg^{-1}, 1041 mg kg^{-1}, 3000 mg kg^{-1}) and exchangeable K (3325 mg kg^{-1}, 5350 mg kg^{-1}, 20000 mg kg^{-1}), respectively as compared to the soils. Considering the beneficial effect on plant essential nutrients (avail.P and exc.K) of these sugarcane waste-products and the properties related to soil quality (OM), these amendments offer an alternatively suitable option for the use as soil conditioner to improve the nutritional status of the soils. The incorporation of these amendments into the soils at the ratio used in this study (3% w/w) did not significantly change the total Cd and Zn concentrations in the treated soils as compared to the non-amended soil ($^*p<0.05$), due to the relatively low Cd and Zn concentrations of the wastes-products (0.01-0.54 mg Cd kg^{-1} and 59.8-162.7 mg Zn kg^{-1}). The total Cd and Zn concentration after incorporation of sugarcane waste-products is given in Appendix C. Even though some studies reported a certain degree of total metal increase in amended soils, however, in our study metal loads remained unchanged after amendment incorporation.

Table 5.1 Physico-chemical characteristics of initial soils and amendments.

	pH	OM	Total.N	Avail.P	Exc.K	Cd	Zn
		(%)	(%)	--------------------(mg kg^{-1})---------------------			
LCdS*	6.40	2.79	0.08	6	87	3-5	211-329
HCdS**	6.60	2.58	0.09	11	74	16-18.8	756-821
BA	8.85	6.20	0.12	6964	3325	0.44	116.5
FC	6.02	17.4	1.39	1041	5350	0.54	162.7
VN	3.95	15.1	0.31	3000	20000	0.01	59.8

*LCdS= low Cd contaminated soil; **HCdS= high Cd contaminated soil

5.2 SUGARCANES GROWN IN LOW CADMIUM CONTAMINATED SOIL (LCdS)

5.2.1 Effect of amendments on sugarcane growth: LCdS

The biomass production of sugarcane, expressed in dry weight basis (g plant^{-1}), grown in different treated soils of LCdS included aboveground part and root dry weight (g plant^{-1}), shoot height (cm) and shoot diameters (cm) at the end of the pot experiment (4 months) is shown in Table 5.2. The aboveground part dry weight (g plant^{-1}) was the summation between the leaves and shoots dry weight (g plant^{-1}) due to the very low biomass production and unspecified parts of sugarcane of the control treatment (Ctrl). It was found that sugarcane grows well in the treated soils and no visual symptoms of metal toxicity were observed during at harvest, and most of stalks (shoot height) were generally more than 1.5 m long when sugarcanes were harvested. There was significant difference in plant biomass production ($*p<0.05$) of sugarcane grown in the treated soils as compared the non-amended soil (Ctrl); 6 and 3-fold higher for the aboveground parts (from 8.5 to 57.6 g plant^{-1}), and root (from 2.1 to 6.59 g plant^{-1}), respectively. The highest biomass production was found in plants grown in the LF and LAV treatments, which is the single application of filter cake (3% w/w) and the combination between boiler ash and vinasse (1.5% BA and 1.5%VN w/w), respectively. Increased biomass production may be attributed to the large amount of available-P, exchangeable-K and organic matter which contained in the amendments that could cause higher leaf growth and thus resulted in an increase of sugarcane weight.

Table 5.2 The effect of soil amendments incorporation on the biomass of sugarcane grown in LCdS at 4 month period.

Treatments	Mean biomass dry weight (g plant^{-1}) ± SD		Height (cm)	Diameter (cm)
	Aboveground	Root		
Ctrl	8.5 ± 5.7^{a}	2.10 ± 1.9^{a}	117 ± 26^{a}	1.30^{*a}
LA	40.4 ± 17^{b}	4.76 ± 3.3^{ab}	181 ± 6.1^{b}	2.13 ± 0.35^{b}
LF	57.6 ± 6.3^{b}	6.11 ± 0.8^{b}	214 ± 14^{c}	2.00 ± 0.0^{b}
LAV	55.6 ± 3.7^{b}	6.59 ± 0.9^{b}	172 ± 14^{b}	2.10 ± 0.30^{b}

* = no SD available; mean values ± SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at $*p<0.05$.

Improved biomass production of sugarcanes grown in LCdS in this study revealed the effectiveness of sugarcane wastes-products in promoting sugarcane growth due to significant amount of plant essential nutrients in the amendments.

Besides the great contribution of plant essential nutrients of the soil amendments, especially P and K, the treated soils also contained significant amounts of Zn (274-308 mg kg^{-1}) which is an essential nutrient and required for the plant growth. The high Zn concentration in soils and its preferential uptake of Zn in the plant may cause the higher in biomass production. An increase in biomass production due to the application of Zn was observed by other (Gurmani et al., 2012). Table 5.3 shows Zn/Cd ratio in the whole part of sugarcane (using mass uptake, mg) at the 4 month growth stage.

Table 5.3 Zn/Cd ratio in the whole part of sugarcane in LCdS

Treatments	Zn/Cd in the whole part of sugarcane
Ctrl	85±18^{a}
LA	129±50^{a}
LF	103±11^{a}
LAV	89±9^{a}

Mean values ± SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at *p<0.05.

It has been shown that Zn/Cd ratios in sugarcanes of the treated soils (LA, LF, and LAV) were higher than that of the non-amended soil (Ctrl) (but not significant at *p<0.05) which may resulted in the greater preferential uptake of Zn in comparison with Cd. Higher Zn uptake may result in the higher sugarcane growth and caused significant difference in biomass production (*p<0.05) of sugarcane in the treated soils. In addition, the increase in biomass production by the addition of soil amendments has been also reported by others (Wei et al., 2010; Chaiyarat et al., 2011; Xu et al., 2012)

5.2.2 Accumulation of Cd and Zn in sugarcane: LCdS

Based on the total dry mass (kg) of the whole sugarcane parts (roots, underground setts, shoots and leaves) and the relative metal concentration (mg kg^{-1}), total Cd and Zn accumulation (µg) in sugarcane is shown in Figures 5.1 and 5.2. The results of the present study clearly showed the plateau effect in metal accumulation of sugarcane during the time, as hypothesized.

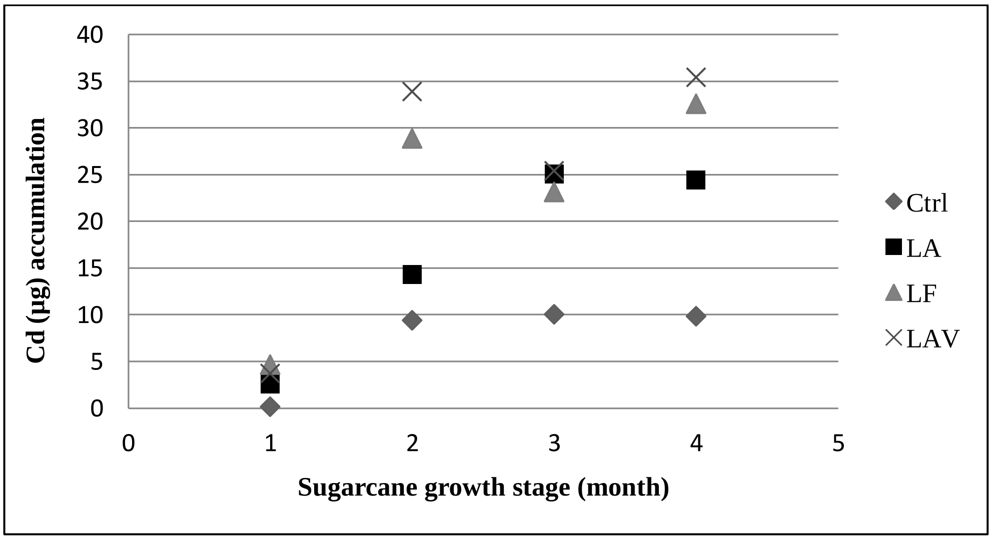


Figure 5.1 Total Cd accumulation (µg) in sugarcanes grown in LCdS.

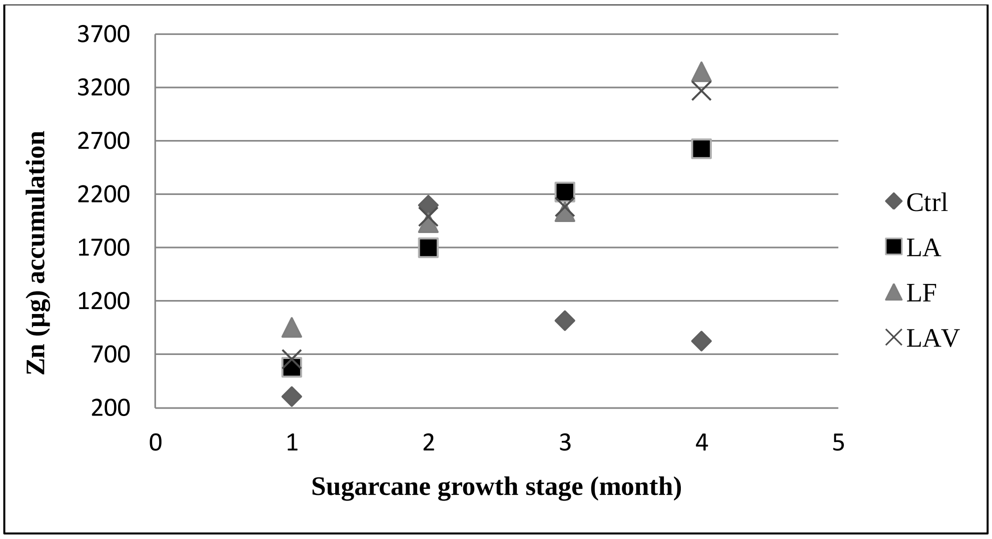


Figure 5.2 Total Zn accumulation (µg) in sugarcanes grown in LCdS.

According to the results, the uptake and/or accumulation of Cd and Zn (µg) were initially increased linearly during the first three months and reached a plateau at the end of the pot experiment (4 month growth stage). The greater in metal uptake at the early sugarcane growth can be confirmed by the transfer index (TI %), the percentage between metal mass (mg plant^{-1}) in aboveground parts of sugarcane and whole sugarcane, was determined and shown in Table 5.4.

Table 5.4 Transfer index (TI %) of Cd and Zn (LCdS and HCdS) in sugarcane pot experiment

Treatments	Cd-TI %				Zn-TI %			
	T1	T2	T3	T4	T1	T2	T3	T4
LC	45	10	48	24	11	3	40	25
LA	10	23	74	57	5	16	74	60
LF	13	6	67	60	6	24	67	65
LAV	22	16	77	76	9	49	76	58
HC	16	47	61	52	9.2	26	51	43
HA	5.2	26	64	35	3.5	40	56	32
HF	0.4	62	64	63	5.7	73	59	48
HAV	4.5	56	71	62	3.2	61	62	45

It was found that the TI values increased as time increased for both metals, especially during 1-3 months. This indicates that an increase in metal translocation in the aboveground parts of sugarcane at the early growth stage will be followed by a reduction in the mature growth stage. Moreover, the Cd-TI% and Zn-TI% at the end of the pot experiment (T4) (24-76% and 25-65%, respectively) were below the TI values reported from other studies for other plant species (Santos et al., 2010). Cleide et al. (2012) showed that the TI values obtained for boron (B) and Zn of the castor oil plants *(Ricinus communis* L.) are 98% and 68%, respectively when peat and filter cake added to the soil contaminated with heavy metals. Therefore, with the purpose of using sugarcane waste-products for *in-situ* stabilization of heavy metals to reduce the risks associated with Cd transfer to the food chain, the application of amendments at early plant growth stage is essential due to greater metal uptake. However, this highlights a need for a further experiment with respect to metal uptake by sugarcane to further quantify impacts of sugarcane waste-products application in order to confirm which mechanisms might be responsible for any observed plateau effect. In addition, Cd and Zn concentrations (mg kg^{-1}) in different parts of sugarcane (leaves, shoots, underground setts, and roots) at the plant growth stage of 4 months (T4) were determined and shown in Table 5.5.

Table 5.5 Cd and Zn concentration (mg kg^{-1}) in different parts of sugarcane grown in LCdS and the transfer factor (TF) of Cd and Zn at 4 month period

	Cd concentration (mg kg^{-1})			
	Ctrl	LA	LF	LAV
Aboveground*	0.1839^{a}	0.3975^{bc}	0.3396^{b}	0.4837^{c}
Underground**	0.7319^{a}	0.6992^{a}	0.7755^{a}	0.6805^{a}
Total-sugarcane***	0.4796^{a}	0.4801^{a}	0.4402^{a}	0.5205^{a}
Total-soil	1.452^{a}	1.817^{a}	1.323^{a}	1.557^{a}
TF	0.33	0.26	0.33	0.33
	Zn concentration (mg kg^{-1})			
	Ctrl	LA	LF	LAV
Aboveground*	37.4^{a}	39.0^{a}	37.7^{a}	33.5^{a}
Underground**	44.4^{a}	71.5^{ab}	69.4^{ab}	104.4^{c}
Total-sugarcane***	39.9^{a}	48.1^{a}	44.8^{a}	46.6^{a}
Total-soil	207^{a}	216^{a}	214^{a}	215^{a}
TF	0.19	0.22	0.21	0.22

Mean values denoted by the same letter in row indicate no significant difference according to Duncan test at *$p<0.05$. *denotes leave + shoot; **denotes underground sett + root; ***denotes all parts of sugarcane; underground + aboveground.

According to Table 5.5, metal concentrations (mg kg^{-1}) in the aboveground part of sugarcane represents the metal concentrations in leaves and shoots; where the underground parts are the summation of metal concentrations in the underground sett and roots. It was found that the translocation of Cd and Zn are mainly in underground parts of sugarcane in all treatments. Both Cd and Zn showed high accumulation in roots and underground setts, resulting in lower concentration uptake in shoots and leaves (data not shown).

The results here showed that the addition of amendments caused no significant difference in the total Cd and Zn concentration (mg kg^{-1}) in sugarcane as compared to the control (Ctrl), except that Cd in the aboveground and Zn in the underground parts of sugarcane, especially in the LAV treatment ($*p<0.05$) (see Table 5.5). The lowest increase in the treated soil pH throughout the experiment of the LAV treatment (+ 0.7 pH unit) as compared to other treatments (+ 1.1 pH units) may have caused the formation of soluble Cd organic complex that increase the metal solubility and thus its uptake to sugarcane (Putwattana et al., 2010). The pH of the treated soils after incorporation of sugarcane is given in Appendix C. Although the LAV treatment resulted in higher growth and biomass production of sugarcane, it also caused an increase in the aboveground Cd accumulation. Therefore, the potential harmful effect on metal accumulation due to high biomass production should be carefully considered. Despite no statistically significant difference ($*p<0.05$) in the total Cd and Zn concentration (mg kg^{-1}) in sugarcane, it seems that the addition of filter cake (LF at 3% w/w) turned out to be the best treatment to reduce total Cd uptake in sugarcane.

Regarding the total metal concentrations (mg kg^{-1}) in the whole sugarcane sample (Table 5.5), Cd and Zn accumulations in sugarcane (0.4402-0.5205 mg Cd kg^{-1} and 39.9-48.1 mg Zn kg^{-1}) are below the maximum concentrations found in some plants (2.0 and 400 mg kg$^{-1,}$ Cd and Zn, respectively) (Abreu et al., 2012). TF value, which is the ratio of metal concentrations (mg kg^{-1}) in sugarcane to the total metal concentrations (mg kg^{-1}) in soils, indicates the capability and the transfer of metals from soil to the plants. Transfer factor (TF) was determined in this study with the intention to study the effectiveness of sugarcane waste-products in metal stabilization in relation to the metal transfer and accumulation in sugarcane (Table 5.5). In this study, even though TF of all treatment are higher than the control, all of the TF values of Cd and Zn were below 1 (0.22-0.33 and 0.19-0.22 for Cd and Zn, respectively), indicating low metal uptake and metal transfer from soil to sugarcane. In accordance to the results obtained, TF values of Cd and Zn in this study have lower values compared to other studies (Putwattana et al., 2010; Singh et al., 2010; Baraud and Lydia, 2012; Xu et al., 2012)

5.2.3 The effect of amendments on Cd and Zn bioavailability in soil: LCdS

5.2.3.1 Fractionation of Cd and Zn in BCR1+2 fraction: LCdS

Previous work of soil pot experiment with the same Cd level showed that using sugarcane waste-products as soil amendments significantly reduced the exchangeable fraction of Cd (BCR1) in soils. In this study, the feasibility and the effectiveness of using sugarcane waste-products as soil amendments in stabilization of Cd and Zn in soil was studied with the cultivation of sugarcane. Special attention was given to the first two fractions, exchangeable (BCR1) and reducible (BCR2), of the BCR sequential extraction which are considered to be the most mobile, easily mobilized and high potential for the plant uptake as identified as the direct effect fractions (Filgueiras et al., 2002; Pensiri and Chantra, 2010; Paradelo et al., 2011). Among the chemical forms, the exchangeable (BCR1) and reducible (BCR2) fractions of the BCR sequential extraction can be used to determine the environmental risk associated with the plant uptake due to it represent the most mobile and bioavailable fraction (Pensiri and Chantra, 2010) and could be used to evaluate the effect of the incorporated amendments on metal transformation and immobilization. The summation of Cd and Zn concentrations (mg kg^{-1}) in soil of exchangeable and reducible fractions (BCR1+2) at all incubation periods are given in Table 5.6 with the significant difference of treatments test using Duncan test ($*p<0.05$) was studied.

The results showed that the addition of sugarcane waste-products influences the reduction of potential bioavailable fractions (BCR1+2) of Cd in the low Cd contaminated soils throughout the experiment (from T0 to T4), while no effect on Zn is seen ($*p<0.05$). The highest reduction of the most bioavailable fraction of Cd at T4 was observed in the LAV treatment followed by the LF and LA treatments (54.5%, 39.9%, 35.4%, respectively). The non-amended soil (Ctrl) showed the higher concentration of the most bioavailable Cd concentration (BCR1+2) in most of the harvesting periods (not statistical difference), illustrating the effectiveness of sugarcane waste-products for *in-situ* metal immobilization. Moreover, the results from previous studies on soil pot experiments showed the consistent finding of non-significant difference of exchangeable Zn (BCR1-Zn) in the treated soils as compared to the controls.

Table 5.6 The exchangeable and reducible Cd and Zn concentrations (BCR1+2) (mg kg^{-1}) in soils of LCdS at different harvesting times (from T0 to T4).

Treatments	Cd concentration in BCR1+2 (mg kg^{-1})				
	T0	T1	T2	T3	T4
Ctrl	1.26± 0.10^{a}	2.19± 0.15^{b}	1.38±0.14^{a}	1.44±0.01^{a}	1.98±0.41^{b}
LA	1.16± 0.07^{a}	1.75± 0.00^{a}	1.40± 0.04^{a}	1.53±0.03^{b}	1.28±0.53^{a}
LF	1.17± 0.03^{a}	1.74± 0.14^{a}	1.34±0.03^{a}	1.56±0.03^{b}	1.19±0.21^{a}
LAV	1.16± 0.04^{a}	1.74±0.06^{a}	1.28±0.10^{a}	1.65±0.06^{c}	0.90±0.18^{a}
Treatments	**Zn concentration in BCR1+2 (mg kg^{-1})**				
	T0	T1	T2	T3	T4
Ctrl	194±26^{a}	118±19^{a}	35±5^{a}	28±1.6^{a}	31±1^{a}
LA	216±52^{a}	141±9^{a}	36±1^{a}	30±0.7^{a}	35±2^{b}
LF	167±28^{a}	119±17^{a}	36±1^{a}	29±0.8^{a}	35±2^{b}
LAV	188±1^{a}	130±19^{a}	34±1^{a}	28±1.5^{a}	34±3^{b}

Mean values ± SD denoted by the same letter in column indicate no significantly difference according to Duncan test at $*p<0.05$ (T1, T2, T3, T4 are 1, 2, 3 and 4 month growth stage of sugarcane, respectively).

Even without significant difference by the addition of amendments in the total Cd and Zn concentration in sugarcanes ($*p<0.05$), the reduction of the most bioavailable Cd concentration (BCR1+2) in soil was observed, especially during T3 and T4. This contrasting finding can be explained by the metal restriction from soil to plant, which the sugarcanes in this study exhibited higher metal accumulation in underground parts compared to aboveground (Table 5.5). The incorporation of sugarcane waste-products with significant amounts of organic matter and the higher pH of the treated soils might play an important role in the observed reduction in bioavailability of Cd that caused the metal sorption and complexation in the soil. In addition, an increase in soil pH was observed throughout the experiment, ranged from

6.40 to 6.58 at the beginning (T0) and 7.34 to 7.51 at the end of the pot experiment (T4), respectively. In this case, the reduction of the most bioavailable Cd concentration in the treated soils (BCR1+2), the lower transfer of heavy metal to plants (TF) and the high metal accumulation in underground parts of sugarcane confirm that the mechanism of metal bioavailability reduction was probably due to the association of Cd with organic matter and high soil pH.

5.2.3.2 Fractionation of Cd and Zn in BCR3 fraction: LCdS

Based on the above consideration, the transformation and/or fractionation of metals in terms of quantitative organic matter bound fraction (BCR3) was also considered. The percentage distribution of Cd and Zn in oxidizable fraction (% BCR3), relative concentration of BCR3 with the sum of metal concentration in all fractions (BCR1+BCR2+BCR3+BCR4), is shown in Table 5.7.

It was observed that the % organic bound fraction (% BCR3) of both metals increase significantly as time increased. The significant difference in organic bound fraction of Cd (% BCR3) between the control (Ctrl) and the treated soils was observed (*$p<0.05$), indicates the treatment effect on the binding of metals. The difference in metal distribution over time could be related to the properties of the soils and the added soil amendments. This may be due to the increase of soil organic matter, pH, and available P (Mohamed et al., 2010). The increment of soil organic matter content encouraged the metal redistribution from soluble/exchangeable to the fraction associated with organic matter and the formation of stable metal-organic complexation which could diminish the solubility of the metal ions in the soils. The effect of organic matter on metal mobility has been reported by others (Janoš et al., 2010; Pérez-Estebana et al., 2011; Hernandez-Soriano and Jimenez-Lopez, 2012). These findings highlighted the conversion and transformation of soluble/exchangeable Cd (BCR1+2) to other insoluble fraction, especially organic matter-bound fraction (BCR3) after the application of amendments.

Table 5.7 The percentage of oxidizable fraction (% BCR3) of Cd and Zn in soils of LCdS at different harvesting times (from T0 to T4).

Treatments	% BCR3-Cd				
	T0	T1	T2	T3	T4
LC	4.88^{a}	4.82^{a}	7.82^{b}	4.01^{a}	5.02^{a}
LA	4.87^{a}	3.10^{a}	8.55^{b}	4.49^{a}	8.79^{b}
LF	3.84^{b}	2.93^{a}	7.51^{c}	3.94^{a}	7.27^{c}
LAV	4.32^{a}	3.38^{a}	7.53^{b}	3.72^{a}	7.72^{b}
	% BCR3-Zn				
Treatments	T0	T1	T2	T3	T4
LC	6.0^{a}	6.6^{a}	15.0^{a}	16.9^{a}	36.4^{b}
LA	7.5^{a}	4.4^{a}	13.4^{b}	15.9^{b}	26.6^{c}
LF	5.4^{a}	5.2^{a}	14.7^{b}	14.5^{b}	24.8^{c}
LAV	4.0^{a}	7.2^{a}	13.7^{ab}	14.3^{ab}	21.8^{b}

The same letter in row indicate no significantly difference according to Duncan test at $*p<0.05$ (T1, T2, T3, T4 are 1, 2, 3 and 4 month growth stage of sugarcane, respectively).

5.3 SUGARCANES GROWN IN HIGH CADMIUM CONTAMINATED SOIL (HCdS)

5.3.1 *Effect of amendments on soil pH: HCdS*

As soil pH is one of the most important factors affected metal speciation and transformation, soil pH of pot experiment was determined every sampling period of sugarcane growth stage (from T0 to T4) (Figure 5.3). It was shown that the pH of treated soils significantly increased ($*p<0.05$) with the residence time from the first sampling (T0) with the highest pH occurred at the end of incubation period (T4) (+1.0 to +1.5 pH unit increase). The trend of initial increase in pH (from T0 to T2) with the following slightly pH reduction (at T3) (not statistical significance at $*p<0.05$) is consistent with the previous work of the soil pot experiment conducted on the agricultural Cd contaminated soils with the same Cd levels. Cui et al. (2011) showed similar pattern of pH change with initial increase followed by a decrease and then remained constant after incubating soils for a period of 6 months with Indian mustard. The initial increase in pH may be contributed by the alkaline amendments and organic matters in amended materials used in this study that may release and modify soil pH and that depending on the composition of organic matter and soil physic-chemical properties.

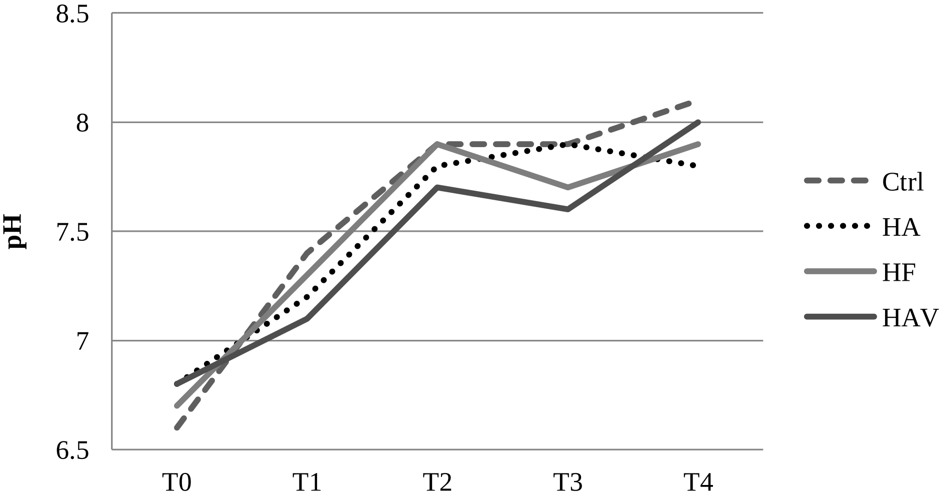


Figure 5.3 Values of pH for different soil treatments at different incubation periods (T0-T4)

5.3.2 Effect of amendments on sugarcane growth: HCdS

The biomass production of the sugarcane, expressed in dry weight basis (g plant^{-1}), grown in different treated soils of HCdS were measured at the end of the pot experiment (4 months) (Table 5.8). Results showed that without any soil amendment (HC) sugarcane could grow well in this contaminated soil as shoot, leave, and root biomass (g plant^{-1}) remained very high. However, high metal loading in this high metal contaminated soil may have an influence on sugarcane growth as illustrated by no improvement in biomass production caused by the addition of amendments. The addition of 3% (w/w) of BA and FC (HA and HF treatment) resulted in a negative growth response (not significant, $*p<0.05$) when comparing to the control (HC). However, the highest biomass production was found in plants grown in HAV with the higher and larger in number of leaves (Appendix C) and reached 23% increase in the leave dry matter yield (Table 5.8). The elevated biomass production in HAV reflected an improvement in soil fertility and the supply of nutrients, especially K in the vinasse, that would promote and facilitate the plant growth. No toxic symptom was observed during the harvesting time, when sugarcane was harvested 120 days after planting, most of stalks (height) were generally more than 1.3 m long. Zn/Cd ratio in the whole sugarcanes grown in HCdS can be found in Appendix C.

Table 5.8 The effect of soil amendments incorporation on the biomass of sugarcane grown in HCdS at 4 month period.

Treatments	Mean biomass dry weight (g plant^{-1}) ±SD			Height	Diameter
	Leave	Shoot	Root	(cm)	(cm)
HC	26.9±3.6^{a}	16.9±4.5^{a}	11.8±4.4^{a}	172±9.6^{a}	1.93±0.21^{a}
HA	11.9±10.1^{a}	13.4±3.8^{a}	5.80±2.3^{a}	134±51^{a}	1.80±0.14^{a}
HF	13.1±16.5^{a}	11.2±10.5^{a}	4.28±5.2^{a}	122±39^{a}	1.23±0.81^{a}
HAV	33.1±8.3^{a}	16.2±2.8^{a}	11.9±6.2^{a}	166±3.8^{a}	2.20±0.10^{a}

Mean values ± SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at *$p<0.05$.

5.3.3 Accumulation of Cd and Zn in sugarcane: HCdS

Cd and Zn concentration (mg kg^{-1}) in different parts of sugarcane at the plant growth stage of 4 months has been determined (Table 5.9) as the following order; root > shoot ≥ underground sett > leave; and root > underground sett > shoot > leave, respectively. In this study, Cd and Zn were accumulated mainly in the roots of sugarcane. Many of plant samples growing in polluted soils showed the higher metal concentrations mainly in roots due to soil-plant transfer processes (Putwattana et al., 2010; García-Salgado et al., 2012). Regarding the total metal concentrations in the whole sugarcane samples (Table 5.9), Cd and Zn accumulation in sugarcane (0.5684-0.8737 and 75.1-117 mg kg^{-1} for Cd and Zn, respectively) is below the maximum concentration normally found in plant (2.0 and 400 mg kg^{-1}, Cd and Zn, respectively) (García-Salgado et al., 2012). Additionally, transfer factor (TF) showed that TF values of Cd and Zn were below 1 even in the control treatments (0.04-0.07 and 0.14-

0.23, respectively), indicating low metal uptake and effectiveness of sugarcane waste-products for *in-situ* immobilization.

Table 5.9 Cd and Zn concentration (mg kg^{-1}) in different parts of sugarcane grown in HCdS and the transfer factor (TF) of Cd and Zn at 4 month period.

Treatments	Cd concentration (mg kg^{-1})						TF
	Leave	Shoot	Sett	Root	Total^{sugar*}	Total^{soil**}	
HC	0.3976^{b}	0.9349^{a}	0.8121^{a}	1.7464^{ab}	0.8362^{a}	12.9^{a}	0.06
HA	0.0273^{a}	0.7009^{a}	0.8087^{a}	2.2681^{b}	0.8737^{a}	12.7^{a}	0.07
HF	0.1678^{ab}	0.8761^{a}	0.7278^{a}	0.8121^{a}	0.5684^{a}	12.9^{a}	0.04
HAV	0.3762^{b}	0.7813^{a}	0.7488^{a}	0.8323^{a}	0.5975^{a}	13.4^{a}	0.04
	Zn concentration (mg kg^{-1})						TF
	Leave	Shoot	Sett	Root	Total^{sugar*}	Total^{soil**}	
HC	30.4^{a}	104^{ab}	140^{a}	222^{a}	95.1^{a}	520^{b}	0.18
HA	27.0^{a}	57.2^{a}	134^{a}	251^{a}	117^{a}	499^{a}	0.23
HF	26.5^{a}	120^{b}	135^{a}	257^{a}	89.3^{a}	523^{b}	0.17
HAV	26.2^{a}	91^{ab}	77^{a}	168^{a}	75.1^{a}	527^{b}	0.14

Note: *= total concentration in sugarcane; **= total concentration in soil;
Mean values denoted by the same letter in column indicate no significantly difference according to Duncan test at *$p<0.05$.

Base on plant dry weight (kg) and the relative metal concentration (mg kg^{-1}), mass of Cd and Zn accumulation (µg) in the aboveground and underground part was determined (Table 5.10). It was found that metal uptake of these two metals changed over time (from T1 to T4); accumulated more in underground parts in the first two month (T1-T2) and then moved up to aboveground parts at the end of harvesting periods (T3-T4). As expected, total Cd and Zn accumulations (µg) were highest at the end of pot experiment (T4) due to plant growth and metal accumulation. The metal increase in sugarcane was supposed to result from uptake by plants during the growth period. Moreover, the results showed that the addition of 3% (w/w) of BA and FC (HA and HF treatments) were effective in reducing Cd uptake (µg) in the aboveground part of sugarcane at the end of the pot experiment, showing the 62.68% and 53.73% reduction, respectively as compared to the HC. However, non-significant difference in Zn uptake (*$p<0.05$) was observed in this study, except HA treatment.

Table 5.10 Cd and Zn uptake (µg) in aboveground and underground parts of sugarcane grown in HCdS at different harvesting times (from T1 to T4).

	Metal uptake (µg)							
	T1		T2		T3		T4	
Cd	Above ground	Under ground	Above ground	Under ground	Above ground	Under ground	Above ground	Under ground
HC	0.752^{ab}	4.07^{a}	3.64^{a}	4.04^{a}	13.5^{a}	8.73^{a}	26.8^{b}	24.9^{b}
HA	0.299^{b}	5.49^{ab}	2.56^{a}	7.36^{b}	13.2^{a}	7.54^{a}	10.2^{a}	18.9^{b}
HF	0.018^{a}	4.33^{ab}	8.38^{a}	5.23^{ab}	17.1^{a}	9.65^{a}	13.7^{a}	7.29^{a}
HAV	0.553^{ab}	11.6^{b}	7.84^{a}	6.14^{ab}	23.6^{a}	9.76^{a}	25.2^{b}	15.4^{a}
Zn	T1		T2		T3		T4	
HC	66^{ab}	650^{a}	388^{a}	1171^{ab}	1767^{a}	1723^{a}	2489^{b}	3324^{a}
HA	27^{a}	841^{a}	366^{a}	824^{a}	1939^{a}	1503^{a}	1156^{a}	2661^{a}
HF	42^{ab}	702^{a}	1016^{a}	1189^{ab}	2142^{a}	1468^{a}	1645^{ab}	1766^{a}
HAV	58^{ab}	1757^{a}	1142^{a}	1719^{b}	2216^{a}	1380^{a}	2298^{ab}	2797^{a}

Mean values denoted by the same letter in column indicate no significantly difference according to Duncan test at *$p<0.05$.

5.3.4 The effect of amendments on Cd and Zn bioavailability in soil: HCdS

5.3.4.1 Fractionation of Cd and Zn in BCR1+2 fraction: HCdS

Cd and Zn concentration (mg kg^{-1}) of the treated soils in BCR1+2 fraction is shown in Table 5.11. According to the results, the reduction of the most bioavailable Cd and Zn concentrations (BCR1+2) (mg kg^{-1}) at the end of experiment (T4) was observed in HA, HF and HAV (not significant difference), ranged from 4.0% to 9.6% and 5.5% to 6.3%, respectively. Two hypotheses can explain this phenomenon. Firstly, the direct effect of amendment addition that raising the pH of the treated soils throughout the experiment (Figure 5.3). The precipitation of metal hydroxides and carbonates may cause metals to be less mobile in this study. Secondly, the binding of metals to the added amendments which are known to be rich in metal binding sites could occur. The results from this study clearly revealed that the pH increase, caused by the addition of amendments, had a major role in reducing the metal bioavailability in soils and thus its uptake by sugarcanes.

Table 5.11 The exchangeable and reducible Cd and Zn concentrations (BCR1+2) (mg kg^{-1}) in soils of HCdS at different harvesting times (from T0 to T4).

Treatments	BCR1+2 Cd Concentration (mg kg^{-1})				
	T0	T1	T2	T3	T4
HC	26.8±2.2^{b}	14.3±1.3^{a}	4.96±0.8^{a}	5.32±0.3^{b}	3.54±0.1^{a}
HA	16.5±5.5^{ab}	11.7±1.4^{a}	4.80±0.4^{a}	4.64±0.2^{a}	3.30±0.2^{a}
HF	24.3±2.2^{ab}	13.9±1.2^{a}	4.61±0.2^{a}	4.69±0.3^{a}	3.40±0.3^{a}
HAV	19.2±5.3^{a}	14.3±1.3^{a}	5.27±0.5^{a}	4.78±0.3^{ab}	3.20±0.4^{a}
Treatments	**BCR1+2-Zn Concentration (mg kg^{-1})**				
	T0	T1	T2	T3	T4
HC	647±67^{b}	404±38^{b}	64±2.1^{ab}	182±6.0^{a}	128±6.6^{a}
HA	223±169^{a}	333±10^{a}	63±1.1^{a}	180±6.1^{a}	120±2.4^{a}
HF	526±20^{b}	402±43^{b}	63±0.8^{a}	174±6.4^{a}	121±8.6^{a}
HAV	468±145^{b}	415±27^{b}	66±1.4^{b}	182±4.4^{a}	120±6.9^{a}

Mean values ± SD denoted by the same letter in column indicate no significantly difference according to Duncan test at *$p<0.05$.

5.3.4.2 Fractionation of Cd and Zn in BCR3 fraction: HCdS

In accordance with the reduction of the most mobile fraction of Cd and Zn (BCR1+2), that showed the evolution with time toward form of lower bioavailability. The effect of organic matter on metal mobility and speciation in terms of quantitative organic matter bound fraction (BCR3) was considered in this study to explain the influence of organic matter in metal complex formation. Transformation and the change of % BCR3 of Cd and Zn throughout the experiment (from T0 to T4) were then determined and illustrated in Figure 5.4

According to the results, the percentage distribution of Cd and Zn in oxidizable fraction (% BCR3), relative concentration of BCR3 with the sum of all fractions, was increased significantly as time increases (Figure 5.4). The increment of soil organic matter content of the treated soils encouraged the formation of stable metal-organic complexation which could diminish the solubility of the metal ions in the soils. The decrease of the bioavailable pool of Cd in the treated soils (BCR1+2) in this study revealed the potential use of sugarcane waste-products for metal stabilization and this could be attributed to metal complexation by organic matter. The finding of this study is in agreement with Mohamed et al. (2010) those evaluated the influence of rice straw, green manure and pig manure on the retention of Cd and found that the addition of organic amendments significant reduced the soluble Cd with the increases in organic-bound fraction.

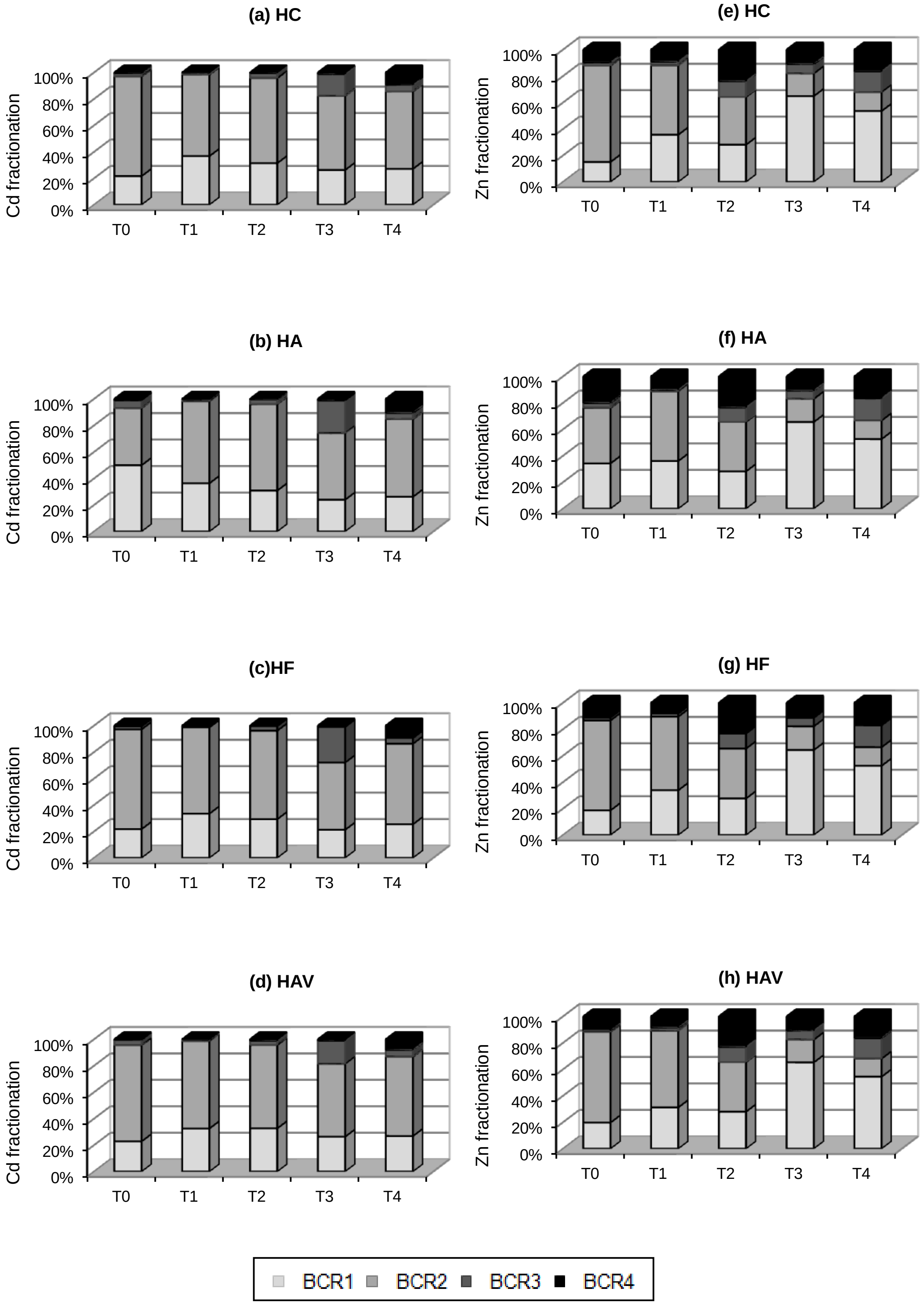


Figure 5.4 Cd and Zn fractionation in high Cd contaminated soils (HCdS) at different harvesting periods (T0-T4)

5.4 CONCLUSIONS: SUGARCANE POT EXPERIMENT

Two studied soils with two different Cd concentration level (LCdS and HCdS) showed different effects of the amendment application. In LCdS, the incorporation of 3% (w/w) sugarcane waste-product as a soil amendment plays an important role in promoting the growth of sugarcane, especially in the LF and LAV treatments. The reduction in bioavailable Cd (BCR1+2) concentration in soil was observed in every treatment (from 35.4% to 54.5%) even no reduction of Cd uptake in sugarcanes was observed. Alternatively, the addition of single boiler ash (HA) and filter cake (HF) to highly Cd and Zn contaminated soil (HCdS) contributed to the reduction of Cd uptake in aboveground part of sugarcane and bioavailability of Cd (BCR1+2) in soil (not significantly at $*p<0.05$). However, no positive effect on plant growth was seen in this study probably due to high metal loading of this contaminated soil (HCdS). However, even in low or high Cd contaminated soil used in this study, no visual toxicity was observed in the sugarcanes grown in the treated soils which may have been due to the improved soil fertility resulted from high plant essential nutrients (N, P, K) of the waste-products. This suggests the alternative use and reutilization of sugarcane waste-products in agricultural land, especially in high Cd and Zn contaminated sites, nevertheless, the long term effect of repeated application of these materials still required in depth investigation.

CHAPTER VI

RESULTS AND DISCUSSIONS: EFFECT OF AMENDMENT CONCENTRATION ON Cd AND Zn FRACTIONATION

Based on the experimental results from sugarcane pot study (Chapter V) that highlights the impact of pH and organic matter on metals transformation; the extra soil pot experiment was then further conducted for four-month incubation in accordance with the time of the sugarcane pot experiment. This chapter is focused on the effect of sugarcane waste-products at different rates (5%, 10%, 20% and 40%) on metal bioavailability and metals associated with organic matter and how different rates of amendments influence the change in soil parameters (pH and OM). Therefore, the bioavaialble/exchangeable fraction (BCR1) and the organic bound fraction (BCR3) are of the interest in this section. The amendments used in this study came from the same batch as used in the sugarcane pot study; therefore the amendment characteristics can be found in Chapter V.

6.1 pH OF THE TREATED SOILS: EFFECT OF AMENDMENT CONCENTRATION ON Cd AND Zn FRACTIONATION

pH of the treated soils was determined for every incubation periods (from T0 to T4) and the results is given in Table 6.1. According to the results, it can be seen that sugarcane waste-products incorporation caused the variation of the treated soil pH. The application of BA caused the increase in the soil pH, the highest pH (7.41) was observed in the highest percentage (%) of BA application (40BA). Alternatively, the application of FC and VN showed contrasting effect exhibited the significant pH decrease as the percentage (%) of the application increases. The pH of FC treatments (from 5% to 40%) ranged from moderately acid to slightly acid (pH 5.65-6.24); whereas the application of VN caused the soils to be very strong acid when 40% VN is applied (pH ranged from 6.11 to 4.57).

Table 6.1 pH of the treated soils at all incubation periods (T0 to T4): effect of amendment concentration on Cd and Zn fractionation

Treatments	T0	T1	T2	T3	T4
Ctrl	6.35 ± 0.05^{g}	7.26 ± 0.04^{a}	6.48 ± 0.58^{a}	7.67 ± 0.14^{a}	6.94 ± 0.06^{a}
5FC	6.24 ± 0.04^{fg}	7.60 ± 0.02^{b}	7.52 ± 0.11^{b}	7.93 ± 0.03^{b}	7.28 ± 0.03^{b}
10FC	6.08 ± 0.06^{e}	7.68 ± 0.07^{c}	7.59 ± 0.07^{bc}	8.18 ± 0.07^{cd}	7.38 ± 0.02^{bc}
20FC	5.89 ± 0.05^{d}	7.85 ± 0.02^{d}	7.70 ± 0.04^{bcd}	8.24 ± 0.05^{d}	7.35 ± 0.06^{bc}
40FC	5.65 ± 0.04^{c}	7.70 ± 0.02^{c}	7.83 ± 0.07^{bcde}	8.09 ± 0.00^{c}	7.45 ± 0.04^{c}
5BA	6.80 ± 0.20^{h}	7.90 ± 0.05^{d}	7.87 ± 0.12^{cde}	8.23 ± 0.08^{d}	7.88 ± 0.03^{def}
10BA	6.91 ± 0.09^{h}	8.04 ± 0.08^{e}	7.93 ± 0.12^{de}	8.27 ± 0.14^{d}	7.87 ± 0.14^{de}
20BA	7.22 ± 0.14^{i}	8.25 ± 0.01^{f}	8.11 ± 0.11^{ef}	8.55 ± 0.06^{e}	8.00 ± 0.03^{f}
40BA	7.41 ± 0.10^{j}	8.51 ± 0.02^{h}	8.27 ± 0.08^{f}	8.72 ± 0.08^{f}	8.20 ± 0.05^{g}
5VN	6.11 ± 0.08^{ef}	8.00 ± 0.01^{e}	7.75 ± 0.05^{bcd}	8.31 ± 0.08^{d}	7.79 ± 0.03^{de}
10VN	5.64 ± 0.08^{c}	8.25 ± 0.01^{f}	8.01 ± 0.08^{def}	8.57 ± 0.03^{e}	7.90 ± 0.03^{ef}
20VN	5.07 ± 0.03^{b}	8.33 ± 0.02^{g}	8.19 ± 0.05^{ef}	8.66 ± 0.07^{ef}	7.77 ± 0.08^{d}
40VN	4.57 ± 0.09^{a}	8.70 ± 0.02^{i}	8.30 ± 0.06^{g}	9.25 ± 0.02^{g}	8.36 ± 0.15^{h}

Mean values ±SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at $*p<0.05$.

Moreover, it can be seen that aging has an effect on the pH of the treated soils, increased in all treatments including control (Ctrl). The aging showed significant trend of pH increase from the beginning (T0) until the end of pot experiment (T4); 1.0 to 1.8, 0.8 to 1.1 and 1.7 to 3.8 pH unit increases in the FC, BA, and VN treatments, respectively, where the highest pH increase was observed in the 40VN treatment (+3.8 pH unit). Soil pH of the Ctrl, alternatively, remained relatively constant during the incubation period (+0.59 pH unit) as compared to the treated soils. The magnitude of pH variation depends on the soil properties, the rates applied and the characteristics of waste used. Difference in pH values of the three amendments used, BA (pH 8.5); FC (pH 5.33); and VN (pH 4.48), contributed to the difference in soil pH. The VN had the highest K^{+} content (exc.K = 11663 mg kg^{-1}) more than the BA and FC (3325 and 5350 mg kg^{-1}, respectively). The significant amount of the basic cations in VN may lead to significant pH increase of the VN treatments during the experiment as compared to the BA and FC treatments.

In the present study, the increase of soil pH occurred immediately after the addition of BA with the reduction of pH when VN and FC were applied, before the waste decomposition had occurred. Such initial pH change was due to the alkali nature of BA and acidic condition of VN and FC. However, the increase in pH was observed in all treated soils as compared to the initial value and remaining higher at the end of the experiment than the Ctrl. This result demonstrated that the little variation in pH of the Ctrl throughout the experiment and the significant increase in the pH of the treated soils, especially in BA and VN treatments could be explained by the significant amount of excess cations (exc.K) that has a great impact on soil pH equilibrium. In addition, depending on pH buffering capacity, the pH increase is generally greater in lower pH soil than in higher pH ones, as demonstrated by Xu et al. 2006, that the VN treatments showed the lowest pH at the initial (4.57-6.11) as compared to other treated soils (Table 6.1). However, Conde et al. (2009) showed the contrasting results as the application of wine vinasse at the increased rates of 1 ml, 5 ml, 10 ml, 20 ml, 40 ml, 70 ml in 200 g soil (equiv. 7.5, 37.5, 75, 150, 300, 525 ml per 1500 g soil) incubated for 4 months caused no significant change in soil pH, falling less than one pH unit.

6.2 BCR SEQUERNTIAL EXTRACTION

6.2.1 Cd and Zn fractionation

The BCR sequential extraction procedure was carried out in soil samples amended with different rates of sugarcane waste-products to study the effect of increasing application rate on Cd and Zn fractionation. Figure 6.1-6.2 illustrated Cd and Zn fractionation pattern among different BCR fractions of different treated soils at all incubation periods (T0 to T4). The results showed that Cd concentrates mostly in the first two fractions (BCR1 and BCR2) of BCR sequential extraction, revealing the high mobility and bioavailability of the metal. Alternatively, Zn was predominantly in reducible fraction (BCR2), but significant amount of Zn was also associated with the residual fraction (BCR4), which agrees with the results of other studies (Achiba et al., 2010; Pérez-Esteban et al., 2011). The fractionation pattern of

Zn, however, differed from that of Cd in which exhibited more association with residual fraction (BCR2 > BCR4 > BCR1 > BCR3). No significant difference was observed in the Zn distribution of the treated soils included the control. In addition, the fractionation pattern of Cd and Zn in the treated soils of the extra soil pot experiment is similar of what we have in the previous study of soil pot experiment that the majority of Cd concentration was among the first two fraction (BCR1 and BCR2) where Zn was concentrated mainly in the reducible and residual fraction (BCR2 and BCR4).

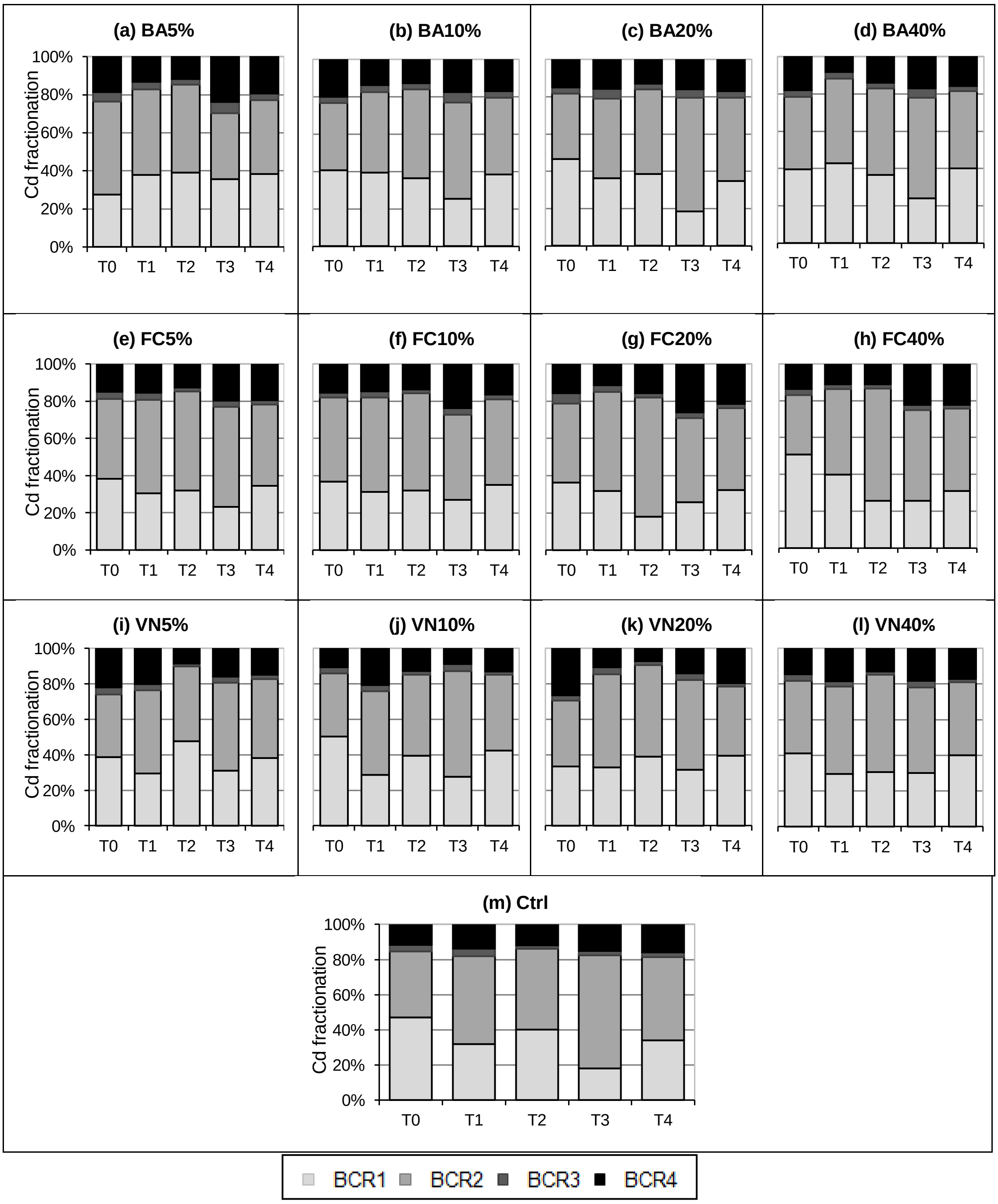


Figure 6.1 (a-m) Cd fractionation in different treatments of extra soil pot experiment at different incubation periods (T0 to T4)

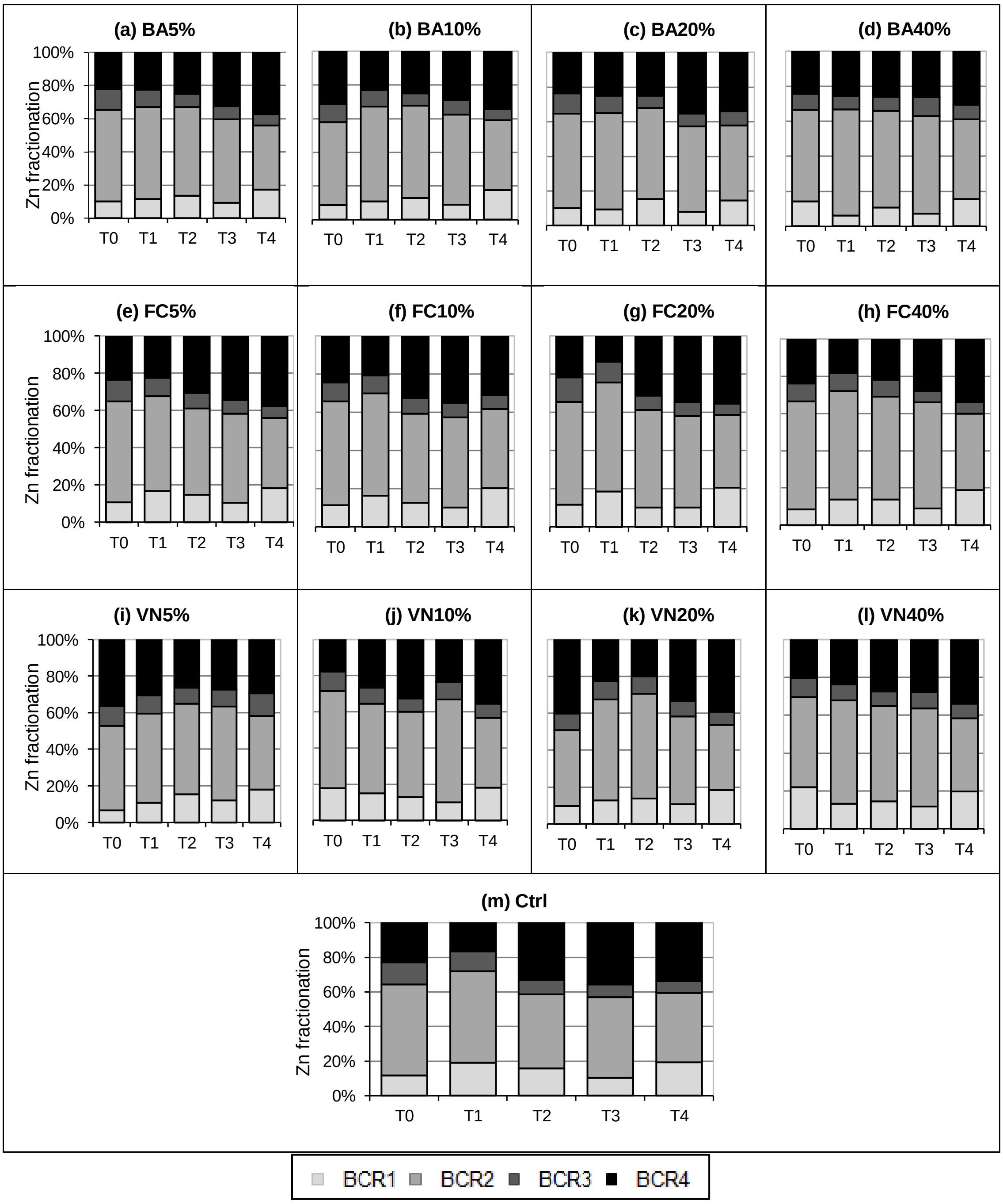


Figure 6.2 (a-m) Zn fractionation in different treatments of extra soil pot experiment at different incubation periods (T0 to T4)

6.2.2 Cd in BCR1 and BCR3 of BCR sequential extraction

Cd concentrations (mg kg^{-1}) in the first and the third fraction of BCR sequential extraction (BCR1 and BCR3) of the treated soils at different rates was determined and showed in Table 6.2 and 6.3, respectively.

Table 6.2 The exchangeable Cd concentration (BCR1) (mg kg^{-1}) in different treated soils at all incubation periods (T0 to T4): effect of amendment concentration on Cd and Zn fractionation

Treatments	BCR1-Cd (mg kg^{-1})				
	T0	T1	T2	T3	T4
Ctrl	1.4482^{e}	0.7343^{abc}	2.3562^{ef}	0.8392^{g}	1.4643^{c}
Filter cake-5FC	0.9824^{bc}	0.7680^{abc}	1.5542^{bcd}	0.7606^{ef}	1.3218^{bc}
10FC	0.8760^{bc}	0.7478^{abc}	1.5180^{bcd}	0.6946^{cde}	1.2061^{b}
20FC	0.7235^{ab}	0.6418^{a}	0.7056^{a}	0.6456^{bc}	1.0014^{a}
40FC	1.2689^{de}	0.9560^{cde}	1.0042^{cde}	0.5686^{a}	0.8927^{a}
Boiler ash-5BA	0.5091^{a}	1.0679^{e}	1.6970^{e}	0.8543^{g}	1.3902^{bc}
10BA	0.9960^{bc}	1.0112^{de}	1.4579^{de}	0.6820^{cde}	1.2706^{bc}
20BA	1.1205^{cd}	0.8408^{abcd}	1.4401^{abcd}	0.6638^{bcd}	1.2290^{b}
40BA	0.8082^{b}	0.9120^{bcde}	1.1911^{bcde}	0.5893^{ab}	1.2119^{b}
Vinasse-5VN	0.9827^{bc}	0.7666^{abc}	2.8462^{abc}	0.9025^{h}	1.3254^{bcc}
10VN	1.3076^{de}	0.7522^{abc}	2.0300^{abc}	0.7381^{de}	1.6866^{d}
20VN	0.8935^{bc}	0.7248^{abc}	2.0595^{abc}	0.8222^{fg}	1.4605^{c}
40VN	0.9008^{bc}	0.6884^{ab}	1.3496^{ab}	0.7083^{cde}	1.3688^{bc}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at *$p<0.05$.

The results showed that there is a significant reduction (*$p<0.05$) in exchangeable fraction (BCR1) of Cd as compared to the control (Ctrl) throughout the experiment. It was found that BCR1-Cd decreased significantly as the increased rate of amendments, especially during T2 to T3. Aging caused the exchangeable Cd concentration (BCR1) increases in all treatments, except FC40 treatment. At the end of the pot experiment (T4), the reduction in exchangeable Cd was found, especially in FC and BA treatments, as compared to the Ctrl. The reduction could be explained as results of increasing pH of the treated soils as the application rates of wastes increases (from pH 7.28 to 7.45 and 7.88 to 8.20 in FC and BA treatments, respectively) (Table 6.1). However, this effect was not pronounced for VN treatments.

Table 6.3 The oxidizable Cd concentration (BCR3) (mg kg^{-1}) in different treated soils at all incubation periods (T0 to T4): effect of amendment concentration on Cd and Zn fractionation

Treatments	BCR3-Cd (mg kg^{-1})				
	T0	T1	T2	T3	T4
Ctrl	0.1151^{c}	0.1023^{fg}	0.1072^{cd}	0.1148^{fg}	0.1162^{e}
Filter cake-5FC	0.0996^{bc}	0.0927^{ef}	0.1007^{bc}	0.1031^{de}	0.0888^{d}
10FC	0.0590^{a}	0.0804^{cd}	0.0960^{bc}	0.0873^{c}	0.0794^{cd}
20FC	0.1105^{bc}	0.0694^{ab}	0.0894^{ab}	0.0755^{b}	0.0656^{ab}
40FC	0.0840^{abc}	0.0605^{a}	0.0894^{ab}	0.0628^{a}	0.0546^{a}
Boiler ash-5BA	0.0917^{abc}	0.1116^{gh}	0.1295^{e}	0.1431^{h}	0.1293^{f}
10BA	0.0801^{abc}	0.0939^{ef}	0.1291^{e}	0.1538^{i}	0.1217^{ef}
20BA	0.0824^{abc}	0.1175^{h}	0.12^{01de}	0.1620^{i}	0.1298^{f}
40BA	0.0745^{ab}	0.0753^{bc}	0.0955^{bc}	0.1238^{g}	0.0831^{cd}
Vinasse-5VN	0.0972^{bc}	0.0909^{de}	0.1037^{e}	0.1064^{ef}	0.0831^{cd}
10VN	0.0888^{abc}	0.0863^{cde}	0.0972^{bc}	0.1069^{ef}	0.0779^{cd}
20VN	0.0818^{abc}	0.0858^{cde}	0.1071^{cd}	0.1952^{cd}	0.0715^{bc}
40VN	0.0806^{abc}	0.0673^{ab}	0.0795^{a}	0.0848^{bc}	0.0639^{ab}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at $*p<0.05$.

For the oxidizable fraction (BCR3), even there was a very low concentration of Cd found in this fraction (BCR3); significant difference was observed between the control and the treated soils for most of incubation periods, especially during T1 to T4. Aging had no effect on this fraction but Cd concentration (mg kg^{-1}) in this fraction seems to increase in BA treatment. Alternatively, the FC and VN treatments showed no significant difference throughout the experiment (from T0 to T4).

6.2.3 Zn in BCR1 and BCR3 of BCR sequential extraction

Table 6.4-6.5 showed Zn concentration (mg kg^{-1}) in BCR1 and BCR3 of the treated soils at different incubation periods, respectively.

Table 6.4 The exchangeable Zn concentration (BCR1) (mg kg^{-1}) in different treated soils at all incubation periods (T0 to T4): effect of amendment concentration on Cd and Zn fractionation

Treatments	BCR1-Zn (mg kg^{-1})				
	T0	T1	T2	T3	T4
Ctrl	23.45^{e}	35.35^{g}	31.55^{d}	21.91^{ef}	36.84^{d}
Filter cake-5FC	$20{\cdot}30^{d}$	31.79^{f}	29.33^{cd}	21.93^{ef}	36.09^{d}
10FC	21.69^{de}	30.75^{f}	25.70^{bcd}	21.00^{de}	35.08^{cd}
20FC	20.77^{d}	31.23^{f}	19.70^{ab}	19.84^{cde}	35.02^{cd}
40FC	15.24^{ab}	23.97^{de}	24.49^{bc}	18.10^{bc}	35.57^{cd}
Boiler ash-5BA	19.67^{cd}	21.25^{cd}	25.16^{bcd}	19.87^{cde}	33.06^{c}
10BA	17.43^{bc}	19.14^{c}	24.10^{bc}	18.62^{bcd}	30.54^{b}
20BA	17.43^{bc}	15.67^{b}	28.69^{cd}	16.49^{b}	25.65^{a}
40BA	29.28^{f}	10.28^{a}	17.02^{a}	12.72^{a}	24.00^{a}
Vinasse-5VN	14.17^{a}	22.14^{cd}	31.38^{d}	26.75^{h}	34.75^{cd}
10VN	34.72^{g}	31.10^{f}	27.67^{cd}	21.49^{ef}	34.82^{cd}
20VN	21.66^{de}	25.70^{e}	29.72^{cd}	23.67^{fg}	35.89^{d}
40VN	41.03^{h}	26.53^{e}	28.23^{cd}	25.21^{gh}	36.45^{d}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at *$p<0.05$.

It was found that aging caused the increase in exchangeable Zn (BCR1) concentration in all treatments similarly to Cd. Significant reduction in exchangeable Zn concentration was observed in this study, the addition of BA at different rates caused the significant reduction of Zn in this fraction as compared to other treatments throughout the experiment. In comparison, the FC and VN treatments showed no difference in this fraction in most of incubation periods and less pronounced at the end of the pot experiment.

Alternatively, Zn in oxidizable fraction (BCR3) (Table 6.5) showed the reduction of Zn concentration throughout the experiment (from T0 to T4). The aging caused the reduction of Zn oxidizable fraction and this was in accordance with the reduction in organic matter content of the treated soils as time increased (Appendix D). Moreover, all the treated soils exhibited the reduction in BCR3-Zn concentration as the rates of application increases (but not significant difference).

Table 6.5 The oxidizable Zn concentration (BCR3) (mg kg^{-1}) in different treated soils at all incubation periods (T0 to T4): effect of amendment concentration on Cd and Zn fractionation

Treatments	BCR3-Zn (mg kg^{-1})				
	T0	T1	T2	T3	T4
Ctrl	26.14^{d}	21.22^{c}	16.35^{cde}	16.16^{bcd}	13.51^{a}
Filter cake-5FC	21.72^{bcd}	18.97^{bc}	16.38^{cde}	15.46^{bc}	12.11^{a}
10FC	18.34^{a}	17.36^{abc}	16.66^{de}	15.39^{bc}	12.41^{a}
20FC	22.79^{bcd}	18.58^{bc}	14.30^{abcd}	13.84^{ab}	10.42^{a}
40FC	17.48^{ab}	16.56^{abc}	15.85^{bcde}	12.117^{a}	11.07^{a}
Boiler ash-5BA	24.71^{cd}	19.68^{bc}	14.74^{abcd}	17.48^{cde}	12.66^{a}
10BA	21.97^{bcd}	17.90^{abc}	13.80^{abc}	18.27^{def}	11.34^{a}
20BA	20.1^{4bcd}	16.91^{abc}	13.44^{ab}	15.14^{bc}	14.35^{a}
40BA	19.44^{bcd}	13.08^{ab}	13.11^{a}	19.41^{ef}	12.91^{a}
Vinasse-5VN	22.84^{bcd}	20.56^{c}	18.13^{ef}	20.49^{f}	23.84^{b}
10VN	20.95^{bcd}	18.39^{bc}	15.91^{bcde}	20.65^{f}	14.49^{a}
20VN	19.78^{bcd}	19.85^{bc}	20.12^{f}	18.47^{def}	13.73^{a}
40VN	18.98^{bc}	16.95^{a}	14.93^{abcd}	17.99^{de}	14.09^{a}

Mean values denoted by the same letter in columns indicate no significantly difference according to Duncan test at $*p<0.05$.

6.3 CORRELATION ANALYSIS BETWEEN pH-BCR1, OM-BCR1, and OM-BCR3

The Pearson correlation analyses were determined between the pH&BCR1, OM&BCR1, and OM&BCR3 for Cd and Zn in all incubation periods with the purpose to investigate the relationship between these two important factors (pH and OM) that affecting the metal bioavailability and metal adsorption via organic matter, respectively (Table 6.6). Organic matter content (%) in the treated soils was determined for every incubation periods and given in Appendix D. Soil pH is an important factor that affects the metal bioavailability. There are many studies reporting the effect of pH on metal bioavailability and showed that the high pH caused the reduction of metal bioavailability through the increase of metal sorption onto the negative sites (Guo et al., 2011; Houben et al., 2012; Sun et al., 2013). The precipitation and sorption of metals are the major mechanisms controlling the retention of metals in the treated soils with the alkaline materials. Therefore, we expect the negative correlation between pH and BCR1 (that represent the most bioavailable metal concentration), and that the higher the pH would lessen the metal bioavailability.

According to the results, it was found that pH of the treated soils was negatively correlated to the bioavailable metal concentration (BCR1) for both metals, especially Zn with significantly correlated ($^{**}p<0.01$). This result confirmed that pH has a strong influence on metal bioavailability in this study, exhibited the negative correlation with the metal bioavailability. The high significant negative correlation coefficient between pH and the exchangeable Cd and Zn (BCR1) suggested that the pH induced by the amendment incorporation was one of the key parameters controlling metal bioavailability of Cd and Zn in this study. Houben et al., 2012 found the consistent results of the significant negative correlation between pH and metals (Cd and Zn) when soils was treated with six cost-effective amendments ($CaCO_3$, iron grit, fly ash, manure, bentonite and bone meal) at 5% (w/w).

Table 6.6 Correlations between pH-BCR1 and OM-BCR3 at different incubation times (T0-T4): effect of amendment concentration on Cd and Zn fractionation

	Pearson's coefficient (*r*)					
	pH		OM		OM	
	BCR1-Cd	BCR1-Zn	BCR1-Cd	BCR1-Zn	BCR3-Cd	BCR3-Zn
T0	-0.107	-0.419**	-0.202	0.561**	-0.218	-0.521**
T1	-0.029	-0.550**	-0.175	0.379*	-0.637**	-0.107
T2	-0.351*	-0.250	-0.569**	-0.274	-0.568**	-0.026
T3	-0.188	0.037	-0.484**	0.000	-0.754**	-0.682**
T4	0.170	-0.494**	-0.652**	0.281	-0.647**	-0.278

* = significant at $p<0.05$ level; ** = significant at $p<0.01$ level

Surprisingly, the results from correlation analysis of BCR1 and OM showed the contrasting results between the two studied metals (Cd and Zn). The negative correlation between these two parameters for Cd was found in every incubation periods, with positive correlated in Zn. On other hands, the correlation analysis was also done between BCR3 and OM because this fraction (BCR3) represents the metals bound and/or associated with organic matter and we believe that the higher the organic matter in the treated soils via the higher % of waste-products addition might cause the lower metal bioavailability by metal sorption into the particles. According to the results, it was surprised to find the negative correlation between the OM and the metals associated with the organic matter (BCR3). We hypothesized the positive correlation between these two factors (OM and BCR3) that the higher the OM in the treated soils, the higher the metals associated with, and the higher the metal released in BCR3 fraction.

Based on the above information, it can be concluded that pH is the important factors affecting the available pool of metals in this study. However, the results from the OM correlation analysis cannot be used in refining any relationship between the bioavailable fraction (BCR1) and with the organic-matter bound fraction (BCR3) because of the contrasting results observed in this study that cannot clearly interpreted.

6.4 CONCLUSION: EFFECT OF AMENDMENT CONCENTRATION ON Cd AND Zn FRACTIONATION

In conclusion, by increasing the rate of amendment application from 5% to 40%, the bioavailable or exchangeable concentration of Cd and Zn (BCR1) decreased accordingly to the rate of application. The FC and BA treatments were effective in reducing bioavailable Cd concentration; while only BA was more pronounced for Zn. The results from this experiment revealed the important role of soil pH in influencing the bioavailability of Cd and Zn (BCR1) in this study. Even the addition of the FC and VN at the beginning caused the reduction of soil pH, however, aging caused the pH of the treated soils increased throughout the experiment and thus resulting in the reduction of the exchangeable fraction of Cd and Zn in the treated soils as compared to the control. No clear relation between the organic matter contents (OM) and BCR1 and BCR3 existed; this could lead to the undefined relationship between OM and the metal pool in this study.

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS: SOIL POT EXPERIEMENT

The study of the soil pot experiment used sugarcane waste-products from ethanol production plant as soil amendments at 3% (w/w) in investigating the effects of Cd and Zn bioavailability in soils showed that the application of sugarcane waste-products caused the redistribution of metals, especially in the first-two fractions (BCR1 and BCR2) of BCR sequential extraction, resulting in the variation of bioavailability of Cd and Zn in soils. No change in metal load of the treated soils was observed after amendment incorporation. Aging had an influence on fractionation of Cd and Zn and caused the reduction of exchangeable Cd (BCR1) during the first few weeks of incubation period. The reduction of bioavailable Cd, represented by the exchangeable fraction of BCR, was ranged from 2.3% to 4.7% in LCdS and 9.4% to 39.9% in HCdS.

Based on the reduction of the exchangeable Cd concentrations of the BCR sequential extraction, treatment L2, L3, L4 and H2, H3, H4, which are the single application of BA (3% w/w), FC (3% w/w) and a combination between BA and VN (1.5%+1.5% w/w), respectively, were selected as the optimum ratio at the doses used in the this study. Even though treatments L1 and H1 (3% w/w), which is the single application of vinasse, showed the reduction in the exchangeable Cd concentration, they were excluded from the ratio selection as they may negatively affect the physico-chemical properties of soil in the long term application due to the vinasse properties of the low pH, high viscosity, and high salt content. Despite the good correlation between the BCR and DTPA extraction, using Pearson correlation analysis *(r)*, was observed in determining metal bioavailability, the procedure of BCR sequential extraction was only selected for further study in sugarcane pot experiment due to the low extractable metal concentrations from the DTPA and no treatment effect in DPTA extraction was observed.

Considering the beneficial effect on plant essential nutrients (total.N, avail.P and exc.K) and the properties related to soil quality (OM and CEC) of sugarcane waste-products, the use of these amendments might be an alternatively suitable option for reclamation of contaminated soils in terms of soil fertility improvement. The information from this study is essential in developing a guideline for using sugarcane waste-products as soil amendments. However, in order to determine the effectiveness of using these sugarcane waste-products in a real scenario for *in-situ* metal stabilization; a further study of an experiment with plant is of importance to determine the metal accumulation and translocation and its response in plant. A pot experiment with plant is therefore necessary with these selected ratios of amendments to determine the interaction between soil, amendments and plant.

7.2 CONCLUSIONS: SOIL SUGARANE EXPERIEMENT

The selected ratios of sugarcane waste-products as soil amendments from the previous study of soil pot experiment were then used in the sugarcane pot experiment with the two Cd concentration levels of contaminated soils (LCdS and HCdS).

7.2.1 Conclusion: low Cd contaminated soil (LCdS)

The incorporation of sugarcane waste-products [BA at 3% (w/w); FC at 3% (w/w) and BA&VN (1.5%+1.5% w/w)], as a soil amendment at the ratio used in this study plays an important role in promoting the growth of sugarcane in LCdS; all treated soils showed significantly higher biomass production (g plant^{-1}) as compared to non-amended soils, especially in the LF and LAV treatments. In addition, no visual toxicity was observed in the sugarcanes grown in the treated soils of LCdS which may have been due to the improved soil fertility resulting in higher plant biomass production (6 and 3-fold higher for the aboveground parts and roots, respectively). With regards to metal bioavailability, a decrease in the most bioavailable Cd concentration (BCR1+2) in the treated soils (LAV, LF, LA) at the end of the pot experiment was observed (54.5%, 39.9%, 35.4%, respectively). This finding could not be explained as a result of the depletion of total metal accumulation in sugarcane; instead, we hypothesize that Cd reduction in soils could be resulted from the transformation of exchangeable/soluble metals into more resistant form (organic matter bound fraction, BCR3). The decrease of the most bioavailable pools of Cd in the treated soils, the lower transfer of Cd and Zn to plants (TF<1), the high metal accumulation in underground parts of sugarcane, and the conversion/transformation of metal to insoluble fraction (organic matter bound fraction, BCR3) suggests the alternative use and reutilization of these sugarcane waste-products when applied to agricultural lands, especially in contaminated sites at normal Cd and Zn level (< 3 mg Cd kg^{-1}).

7.2.2 Conclusion: high Cd contaminated soil (HCdS)

The overall, the addition of single boiler ash (BA) and filter cake (FC) at 3% (w/w) to highly Cd and Zn contaminated soil (HCdS) (15-20 mg Cd kg^{-1}) contributed to the reduction of Cd uptake (µg) in aboveground part of sugarcane (62.68% and 53.73% reduction, respectively) and bioavailability of Cd (BCR1+2) in soil (not significant, 6.8% and 4.0%, respectively) as compared to the control, while little effect on speciation of Zn was seen. However, no positive effect on plant growth and metal toxicity visually seen in this high Cd contaminated soil experiment. Both Cd and Zn were accumulated mainly in underground parts of sugarcane (root > shoot ≥ underground sett > leave; and root > underground sett > shoot > leave, respectively) and the translocation factor (TF) were below 1, indicating low metal uptake. The results suggested that even sugarcane waste-products insignificantly promote the sugarcane growth in this high Cd and Zn contaminated soil (15-20 mg Cd kg^{-1}); however, they can be used in agricultural due to the low metals accumulation in sugarcane and the reduction in metal bioavailability in soil.

7.3 CONCLUSIONS: EFFECT OF AMENDMENT CONCENTRATION ON Cd AND Zn FRACTIONATION

The characteristics of the treated soils in this experiment included pH and OM content and their behavior due to the aging was similar as in the previous study of soil pot experiment. The results of the experiment in this part revealed that soil pH is a key factor influencing the bioavailability of Cd and Zn (BCR1) in this study, while the undefined relation between OM and the metal pool (BCR1 and BCR3) was observed. This results are then used to confirm the effect of pH on metal bioavailability from the previous experiments as the increased in soil pH resulted in the reduction of bioavailable pool of metals. In addition, increasing the rate of amendment application from 5% to 40%, the bioavailable or exchangeable concentration of Cd and Zn (BCR1) decreased accordingly to the rate of application. The FC and BA treatments were effective in reducing bioavailable Cd concentration; while only BA was more pronounced for Zn.

7.4 CONCLUSIONS: OVERALL

The results from two different Cd concentration levels of contaminated soils (LCdS and HCdS) from all the experiment showed that the application of sugarcane waste-products can reduce bioavailable Cd concentration in the studied soils. The application of BA at 3% (w/w) can effectively reduce bioavailable Cd concentration in soils in all experiments; both in the LCdS and HCdS; while other treatment tend to show less pronounced results. Using BA as soil amendments in metal stabilization showed good results as compared to other sugarcane waste-products due to its composition (high silica content, aluminum, and alkaline earth oxides) and characteristics (high pH) of the waste itself. The majority of the composition of BA consisted mainly of SiO_2, Al_2O_3, FeO_3, and CaO, while the composition of bagasse and vinasse is of the cellulose fiber with high moisture content and biodegradable organic content, respectively. Boiler ash contains high concentrations of aluminum oxides and hydroxides which might be used as adsorbing materials for heavy metals; (1) once incorporated into the soils, it can form or complex and adsorb with Cd^{2+} (the form of Cd in soil solution) and thus stabilize metal in the soil systems (2) (Figure 7.1). This suggests the alternative use and reutilization of sugarcane waste-products especially BA, in agricultural land.

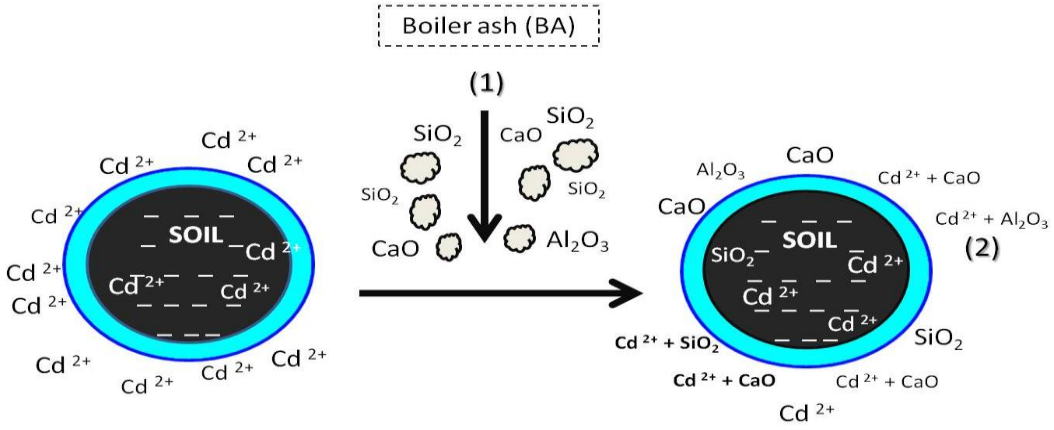


Figure 7.1 Proposed mechanisms of boiler ash in metal stabilization in soil solution

7.5 RECOMMENDATIONS

The use of sugarcane waste-products as soil amendment in this study showed the potential use in the agricultural, however, there are some gaps involving including;

- The application was only done for sugarcane cultivation; therefore, in order to utilize and reuse the waste-products for the effective management, other plant species should be studied.
- This study is mostly limited to the pot scale investigation, the long term effect of repeated application of these waste-materials still required in depth investigation and the long term studies are necessary to determine the validity and feasibility of these waste-products before applying to the real field sites to assure the experimental result.
- The potential harmful effect on metal accumulation due to high biomass production should be carefully considered.
- The works from this study showed an impact of soil amendments on contaminant extractability by BCR sequential extraction; however, it does not provide insight via complementary approaches for example molecular spectroscopy into the mechanisms of contaminant stabilization due to the very low Cd concentration used in this study (<

15 mg kg^{-1}). Therefore, spectroscopic analysis by the X-ray absorption spectroscopy (XAS) cannot be used in this study to obtain information on the properties of materials, the quantitative information is used for analyzing the data instead.

- Ethanol production from sugarcane is a seasonal operation, the waste-products availability is often a constraint and limited resources sometimes, and therefore, alternative materials from other industrial waste-products (from fruit and vegetable proceesing) should be studied and utilized for this purpose.

Despite this research is relatively short conducted in a period of 4 month of growing sugarcanes, however, the results could provide useful information for predicting metal mobility in the future and providing further evaluation of recycling sugarcane waste-products generated from ethanol production plant on agricultural lands. An understanding on sugarcane waste-products as soil amendments and soil constitutes interaction might be useful for developing management practice within the particular area for recycling the nutrients, waste management along with the protection the environment from heavy metals translocation.

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APPENDICES

APPENDIX A

Appendix A: Supplementary information for research methodology

A-1: Sugarcane sampling and analysis

Sugarcane analysis at different plant growth stages:

Sugarcane at 1-2 month (T1 and T2):

- Fresh weight (g), dry weight (g), shoot height (cm), and number of leaves of individual sugarcane grown in each pot was determined.
- The aboveground part (any part of sugarcane grown above the underground sett, underground sett and root were used for metal determination.

Sugarcane at 3 month (T3):

- Fresh weight (g), dry weight (g), shoot height (cm), and number of leaves of individual sugarcane grown in each pot was determined.
- Four different part of sugarcane (leaves, shoots, underground sett, and roots) were used for metal determination.

Sugarcane at 4 month (T4):

- Fresh weight (g), dry weight (g), shoot height (cm), and number of leaves of individual sugarcane grown in each pot was determined.
- Shoot diameter (cm) was also determined at this harvesting time.
- Four different part of sugarcane (leaves, shoots, underground sett, and roots) were used for metal determination.

Shoot height (cm) is measured from the top of underground sett to the tip of the longest leaf.

Shoot diameter (cm) is measured at sugarcane stalk 15 cm above the underground sett.

Aboveground part = the summation of leaves and shoots of sugarcane

Underground parts = the summation of underground sett and roots of sugarcane

A-2: Determination of total Cd and Zn concentration in soil samples

Soil samples are digested using the microwave-assisted acid digestion procedure. Three replicates of 0.5 g of representative samples are accurately weighed and digested with a mixture of acid (3:1 ratio of 12 ml HCl and 4 ml HNO_3) (Milestone, Shelton, CT, USA) (ISO 11466 method). Temperature of the vessel in the Microwave Digestion System is raised to 180±5 °C remaining for 9.5 min to ensure the completion of specific reaction.

A-3: Determination of the bioavailable Cd and Zn concentration

A-3-1: BCR sequential extraction

BCR1: Exchangeable and weak acid soluble fraction: 0.5 g soil sample is extracted with 20 mL of 0.11 mol L^{-1} acetic acid solution by shaking in a mechanical, end-over-end shaker at 30±10 rpm at 22±5 ºC for 16 h. The extract is separated by centrifugation at 3000 g for 20 min, collected in polyethylene bottles and stored at 4 ºC until analysis. The residue is washed by shaking for 15 min with 20 mL of doubly deionized water and then centrifuged, discarding the supernatant.

BCR2: Reducible fraction: 20 mL of 0.5 mol L^{-1} hydroxylamine hydrochloride solution is added to the residue from the first step, and the mixture is shaken 30±10 rpm at 22±5 ºC for 16 h. The acidification of this reagent is by the addition of a 2.5% (v/v) 2 mol L^{-1} HNO_3 solutions (prepared by weighing from a suitable concentrated solution). The extract is separated and the residue will be washed as in the first step.

BCR3: Oxidizable fraction: 5 mL of 8.8 mol L^{-1} hydrogen peroxide solution is carefully added to the residue from the second step. The mixture is digested for 1 h at 22±5 ºC and for 1 h at 85±2 ºC, and the volume is reduced to less than 1 mL. A second aliquot of 5 mL of H_2O_2 is then added, the mixture is continual digested for 1 h at 85±2 ºC, and the volume is reduced to about 1 mL. Twenty milliliter of 1 mol L^{-1} ammonium acetate solution, adjusted to pH 2.0, is then added to the residue, shaken at 30±10 rpm and 22±5 ºC for 16 h. The extract is separated and the residue is washed as in previous steps.

BCR4: Residual fraction: the residue obtained from BCR3 is used to continue digested with *aqua regia*, 3:1 (v/v) of 12 mL HCl and 4 mL of HNO_3 on the hot plate until no fume and clear solution. The sample is then cooled down and filtered by using a Whatman No. 41 filter paper. The volume is then adjusted to 25 mL with deionized water.

A-3-2: DTPA extraction

Ten-gram soil samples are mixed with 20 mL of DTPA solution (0.005 mol L^{-1} DTPA with 0.01 mol L^{-1} TEA and 0.01 mol L^{-1} $CaCl_2$, adjust to pH ± 7.3). The mixture is mixed by shaken at 30±10 rpm at 22±5 ºC for 2 h. The extractant is then be separated from the residue by 10 min., centrifugation at 3000 g, and decanted into polyethylene container.

APPENDIX B

Appendix B: Supplementary information for soil pot experiment

Table B-1 The % recovery of Cd and Zn from the pseudo-total concentration and sum of all BCR fractions (BCR1+2+3) included residual fraction (BCR4) (*n*=3).

LCdS	Treatments							
	L1	L2	L3	L4	L5	L6	L7	LC
Cd (mg kg^{-1})								
-*Aqua regia*	0.880	0.885	0.729	1.01	1.15	0.861	1.11	1.19
-BCR1+2+3+4	1.92	1.81	1.26	1.76	1.76	0.744	1.47	1.90
-% recovery	218	204	173	175	153	86	132	159
Zn (mg kg^{-1})								
-*Aqua regia*	215	200	201	197	204	172	180	188
-BCR1+2+3+4	150	167	160	141	143	150	155	164
-% recovery	69.8	83.1	79.6	71.3	67.0	87.0	86.0	87.4

HCdS	Treatments							
	H1	H2	H3	H4	H5	H6	H7	HC
Cd (mg kg^{-1})								
-*Aqua regia*	12.4	13.4	11.7	12.1	11.9	14.2	12.0	12.9
-BCR1+2+3+4	10.0	9.65	11.3	10.6	10.0	11.1	11.1	11.2
-% recovery	80.8	71.9	96.1	87.1	84.5	78.3	92.8	86.9
Zn (mg kg^{-1})								
-*Aqua regia*	528	508	486	511	527	459	485	507
-BCR1+2+3+4	448	401	413	430	370	431	412	389
-% recovery	84.7	79.1	84.9	84.1	70.2	93.8	85.0	76.7

APPENDIX C

Appendix C: Supplementary information for sugarcane pot experiment

Table C-1 Total Cd concentration (mg kg^{-1}) in the treated soils (LCdS and HCdS): sugarcane pot experiment

Treatments	Total-Cd (mg kg^{-1}) in soil				
	T0	T1	T2	T3	T4
LC	4.30	4.36	2.35	1.45	1.45
LA	4.50	3.56	1.72	1.82	1.82
LF	2.99	2.66	1.34	1.32	1.32
LAV	3.02	2.75	1.55	1.56	1.56
HC	20.7	16.0	13.5	12.9	12.9
HA	16.0	14.5	12.7	12.7	12.7
HF	18.8	16.5	12.8	12.9	12.9
HAV	18.2	15.1	13.1	13.4	13.4

Table C-2 Total Zn concentration (mg kg^{-1}) in the treated soils (LCdS and HCdS): sugarcane pot experiment

Treatments	Total-Zn (mg kg^{-1}) in soil				
	T0	T1	T2	T3	T4
LC	299	195	199	268	268
LA	308	187	200	220	220
LF	274	176	183	215	214
LAV	294	187	187	219	219
HC	914	544	510	520	520
HA	821	492	500	499	499
HF	756	524	495	523	523
HAV	771	522	526	527	527

Table C-3 Values of pH for different soil treatments (LCdS and HCdS) at different incubation periods: sugarcane pot experiment

Treatments	pH				
	T0	T1	T2	T3	T4
LC	6.4±0.09^{a}	7.0±0.14^{bc}	7.2±0.11^{c}	6.9±0.17^{b}	7.5±0.05^{d}
LA	6.4±0.15^{a}	6.9±0.02^{b}	7.5±0.06^{d}	7.2±0.22^{c}	7.5±0.07^{d}
LF	6.4±0.08^{a}	7.1±0.14^{b}	7.5±0.13^{c}	7.3±0.03^{b}	7.5±0.08^{c}
LAV	6.6±0.02^{a}	6.9±0.10^{a}	7.3±0.05^{b}	7.4±0.46^{b}	7.3±0.10^{b}
HC	6.6±0.01^{a}	7.4±0.06^{b}	7.9±0.16^{c}	7.9±0.13^{c}	8.1±0.10^{c}
HA	6.8±0.12^{a}	7.2±0.02^{b}	7.8±0.08^{c}	7.9±0.15^{c}	7.8±0.04^{c}
HF	6.7±0.06^{a}	7.3±0.09^{b}	7.9±0.03^{d}	7.7±0.10^{c}	7.9±0.04^{d}
HAV	6.8±0.05^{a}	7.1±0.05^{b}	7.7±0.19^{c}	7.6±0.07^{c}	8.0±0.09^{d}

Mean values ± SD denoted by the same letter in rows indicate no significantly difference according to Duncan test at $p<0.05$.

Table C-4 Height of sugarcane plants (cm) grown in different treatment (LCdS and HCdS) at different incubation periods (T1-T4)

Treatments	Height of plant (cm)			
	T1	T2	T3	T4
LC1	71	90	120	141
LC2	67	no sample	148	90
LC3	no sample	10	144	121
LA1	17	140	no sample	188
LA2	no sample	no sample	161	176
LA3	31	53	170	180
LF1	31	110	185	228
LF2	43	155	187	215
LF3	32	60	173	200
LAV1	60	156	no sample	156
LAV2	40	130	158	181
LAV3	49	no sample	179	180
HC1	53	113	172	182
HC2	65	103	186	163
HC3	75	90	150	170
HA1	32	123	164	78
HA2	no sample	130	no sample	177
HA3	no sample	110	144	146
HF1	43	132	162	110
HF2	32	115	172	90
HF3	53	114	172	166
HAV1	39	135	no sample	163
HAV2	32	110	170	170
HAV3	48	160	167	164

Table C-5 Number of sugarcane leaves in different treatment (LCdS and HCdS) at different incubation periods (T1-T4)

Treatments	Number of leaves			
	T1	T2	T3	T4
LC1	6	7	12	11
LC2	5	no sample	7	5
LC3	no sample	1	8	7
LA1	4	10	no sample	10
LA2	no sample	no sample	12	8
LA3	3	4	11	18
LF1	10	8	11	16
LF2	4	16	11	19
LF3	4	7	12	22
LAV1	5	15	no sample	34
LAV2	4	8	20	25
LAV3	5	no sample	17	20
HC1	5	6	10	16
HC2	5	9	14	16
HC3	6	11	8	18
HA1	4	13	10	4
HA2	no sample	8	no sample	8
HA3	no sample	7	10	16
HF1	5	16	10	6
HF2	5	10	13	6
HF3	5	9	15	23
HAV1	4	13	no sample	20
HAV2	3	7	21	30
HAV3	4	20	12	25

Table C-6 Zn/Cd ratio in the whole part of sugarcane in HCdS at T4

Treatments	Zn/Cd in the whole part of sugarcane
Ctrl	116.2±29^{a}
HA	123.5±38^{a}
HF	178.8±1.2^{a}
HAV	124.8±43^{a}

Mean values ± SD denoted by the same letter in columns indicate no significantly difference according to Duncan test at *p<0.05.

Table C-7 Correlation analysis between BCR1-Cd and -Zn in soils and total-Cd and -Zn concentration in sugarcane at T4

	Pearson' correlation analysis			
	BCR1-Cd	BCR1-Cd	BCR1- Zn	BCR1- Zn
BCR1-Cd in soil	1	0.933**	0.674**	0.686**
BCR1- Zn in soil	0.933**	1	0.637**	0.705**
Cd Conc.-sugarcane	0.674**	0.637**	1	0.823**
Zn Conc.-sugarcane	0.686**	0.705**	0.823**	1

**correlation is significant at the 0.01 level (2-tailed).

APPENDIX D

Appendix D: Supplementary information for the effect of amendment concentration on Cd and Zn fractionation experiment

Table D-1 Changes in organic matter content (% OM) in different treated soils at all incubation periods (T0 to T4): the effect of amendment concentration on Cd and Zn fractionation

Treatments	% OM				
	T0	T1	T2	T3	T4
Ctrl	2.91	4.19	3.69	3.19	1.51
Filter cake5FC	4.82	5.30	5.37	4.93	2.91
10FC	7.45	7.57	7.44	6.41	3.46
20FC	11.1	9.29	9.45	8.41	4.92
40FC	17.0	11.6	11.6	11.9	7.28
Boiler ash-5BA	3.51	3.31	3.88	2.85	2.12
10BA	3.13	3.23	3.68	3.40	2.02
20BA	3.26	2.52	3.62	3.55	1.92
40BA	3.33	3.86	3.72	2.72	2.33
Vinasse-5VN	3.68	2.73	3.41	3.74	2.05
10VN	3.84	3.84	3.79	3.51	2.51
20VN	4.87	4.87	4.65	4.46	2.75
40VN	4.69	4.69	7.29	5.69	2.79

Table D-2 Correlation analysis between pH and OM at different incubation times (T0-T4): the effect of amendment concentration on Cd and Zn fractionation

Incubation periods	Pearson's correlation coefficient (*r*)
T0	-0.840*
T1	-0.321*
T2	0.057
T3	-0.130
T4	-0.263

* = significant at $p<0.05$ level

BIOGRAPHY

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2000-2001:	High School Certificates (Year 10-12) Burwood Girls' High School, Sydney, Australia
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