ประสิทธิภาพของถ่านชีวภาพในการตรึงแคดเมียมในดินปนเปื้อน

นายทรงกฤษณ์ ประภักดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาวิทยาศาสตร์สิ่งแวดล้อม (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

EFFICIENCY OF BIOCHAR FOR CADMIUM IMMOBILIZATION IN CONTAMINATED SOIL

Mr. Songkrit Prapagdee

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Environmental Science
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2014

Thesis Title	EFFICIENCY OF BIOCHAR FOR CADMIUM IMMOBILIZATION IN CONTAMINATED SOIL
By	Mr. Songkrit Prapagdee
Field of Study	Environmental Science
Thesis Advisor	Associate Professor Somkiat Piyatiratitivorakul, Ph.D.
Thesis Co-Advisor	Associate Professor Amorn Petsom, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Graduate School
(Associate Professor Sunait Chutintaranond, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Somchai Pengprecha, Ph.D.)
.....Thesis Advisor
(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)
.....Thesis Co-Advisor
(Associate Professor Amorn Petsom, Ph.D.)
.....Examiner
(Assistant Professor Narong Praphairaksit, Ph.D.)
.....Examiner
(Associate Professor Thares Srisatit, Ph.D.)
.....External Examiner
(Assistant Professor Nukoon Tawinteung, Ph.D.)

ทรงกฤษณ์ ประภักดี : ประสิทธิภาพของถ่านชีวภาพในการตรึงแคดเมียมในดินปนเปื้อน (EFFICIENCY OF BIOCHAR FOR CADMIUM IMMOBILIZATION IN CONTAMINATED SOIL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. สมเกียรติ ปิยะธีรธิติวรกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. อมร เพชรสม, 291 หน้า.

ถ่านชีวภาพเป็นวัตถุดูดซับทางเลือกที่มีต้นทุนต่ำและมีประสิทธิภาพในการกำจัดปริมาณแคดเมียมออกจากสารละลายด้วยคุณสมบัติที่มีความพรุนสูงประกอบกับคุณลักษณะของหมู่ฟังก์ชั่นทางเคมีที่สำคัญบนพื้นผิวถ่านชีวภาพ ถ่านชีวภาพที่ใช้ในการศึกษาผลิตจากเหง้ามันสำปะหลังและแกลบซึ่งเป็นของที่เหลือใช้จากภาคเกษตรกรรม อุณหภูมิที่ใช้เผาถ่านชีวภาพประกอบด้วย 300 400 และ 500 องศาเซลเซียส การกระตุ้นถ่านชีวภาพด้วยกระบวนการทางเคมี ฟิสิกส์ โดยการใช้สารละลายโพแทสเซียมไฮดรอกไซด์เข้มข้น 1.63 โมลาร์ และตามด้วยการเผาซ้ำในครั้งที่สอง ผลจากการทดสอบการดูดซับแคดเมียมในสารละลายพบว่าประสิทธิภาพของการกำจัดแคดเมียมของถ่านเหง้ามันสำปะหลังเพิ่มขึ้นจากร้อยละ 52.43-59.17 เป็น 79.28-84.45 และถ่านแกลบเพิ่มขึ้นจากร้อยละ 13–20 เป็น 95–97 โดยถ่านชีวภาพทั้งสองแบบที่เตรียมจากอุณหภูมิ 300 องศาเซลเซียส ให้ค่าการดูดซับแคดเมียมสูงสุดกว่าการเตรียมถ่านจากช่วงอุณหภูมิอื่น

การศึกษาผลของการใช้ถ่านชีวภาพผสมในดินที่มีการปนเปื้อนของแคดเมียมซึ่งมีระดับของสังกะสีในปริมาณสูง การทดลองในสภาวะโรงเรือนปลูกพืชโดยใช้ถั่วเขียว (*Vigna radiata* L.) ปลูกในดินผสมถ่านชีวภาพในอัตราส่วนร้อยละ 5 10 และ 15 ผลจากการศึกษาแสดงให้เห็นถึงความสามารถของถ่านชีวภาพในการเร่งการเจริญเติบโตของถั่วเขียว โดยที่ดินผสมถ่านชีวภาพในสัดส่วนร้อยละ 10 ให้ผลในการกระตุ้นให้เกิดการเจริญเติบโตของถั่วเขียวและผลผลิตมากที่สุด แต่ในทางกลับกันดินที่ผสมถ่านชีวภาพในอัตราร้อยละ 15 ให้ผลต่อการลดการเจริญเติบโตของถั่วเขียว ถ่านชีวภาพมีคุณสมบัติในการลดค่าความพร้อมใช้ทางชีวภาพของแคดเมียมและสังกะสีโดยการเพิ่มอัตราส่วนของถ่านชีวภาพเป็นผลให้ค่าความพร้อมใช้ทางชีวภาพลดลง แต่ในทางกลับกันการเพิ่มอัตราส่วนของถ่านชีวภาพกระตุ้นให้ถั่วเขียวสามารถดูดดึงแคดเมียมเข้าสะสมในถั่วเขียวมากขึ้น ทั้งนี้การเพิ่มปริมาณของถ่านชีวภาพไม่มีผลต่อปริมาณการดูดดึงของสังกะสี ผลของการวิจัยแสดงให้เห็นว่าการใส่ถ่านชีวภาพในดินเป็นการได้ผลร่วมกันของการทำให้ถั่วเขียวเจริญเติบโตมากขึ้นและทำให้ถั่วเขียวสามารถดูดดึงแคดเมียมเข้าสะสมในพืชมากขึ้นด้วย ซึ่งการกระตุ้นการดูดดึงด้วยการใช้ถ่านชีวภาพเป็นวิธีการที่ใช้งบประมาณต่ำกว่าการใช้สารเคมีอื่น สำหรับค่าการสะสมของปริมาณแคดเมียมในส่วนของพืชระหว่างรากและดินที่มีค่าเกินกว่า 1 และค่าการเคลื่อนย้ายแคดเมียมจากรากไปลำต้นมีค่าต่ำกว่า 1 แสดงถึงความเหมาะสมของการใช้ถั่วเขียวร่วมกับการใช้ถ่านชีวภาพเพื่อเพิ่มประสิทธิภาพในการตรึงแคดเมียมในระบบรากของถั่วเขียวในดินที่ปนเปื้อนแคดเมียม

สาขาวิชา วิทยาศาสตร์สิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5387773520 : MAJOR ENVIRONMENTAL SCIENCE
KEYWORDS: CASSAVA STEM BIOCHAR / RICE HUSK BIOCHAR / ACTIVATION / CADMIUM / ZINC / BIOAVAILABILITY / VIGNA RADIATA L / PHYTOSTABILIZATION

SONGKRIT PRAPAGDEE: EFFICIENCY OF BIOCHAR FOR CADMIUM IMMOBILIZATION IN CONTAMINATED SOIL. ADVISOR: ASSOC. PROF. SOMKIAT PIYATIRATITIVORAKUL, Ph.D., CO-ADVISOR: ASSOC. PROF. AMORN PETSOM, Ph.D., 291 pp.

Biochar is an alternative cost effective adsorbent for removing of cadmium (Cd) from aqueous solution. The process of carbonization of cassava stem and rice husk were made by pyrolysis at the temperature of 300, 400, and 500ºC. The obtained biochars were activated by an alkaline solution of 1.63 M of KOH plus second pyrolysis. Adsorption efficiencies were studied using the batch static method under laboratory conditions. The Cd removal efficiency of activated cassava stem and rice husk biochar were increased from 52.43-59.17% to 79.28-84.45% and 13-20% to 95-97%, respectively. The activated biochar at a pyrolysis temperature of 300ºC showed the highest Cd removal efficiency both cassava stem and rice husk biochar.

The study on the use of biochar to immobilize Cd in soil was conducted in greenhouse condition. The cassava stem biochar was produced through low temperature pyrolysis was applied to natural Cd contaminated soil that also had a high zinc concentration. The green bean plant, *Vigna radiata* L., was planted in three different application rates of biochar-amended soil, including 5, 10 and 15%, respectively. The results showed the positive effect of biochar on promoting plant growth with 10% biochar-amended soil the optimum concentration for stimulating plant growth and seed yield and 15% biochar-amended soil causing an adverse effect to plant growth. The biochar significantly reduced Cd and Zn bioavailability in soil by increasing biochar concentration. Contradictory, the increase of Cd uptake in plants was observed with increasing biochar concentrations, but cause no significant change for Zn uptake. The results also showed that the combined approach of biochar-amended soil can promote plant growth and increase Cd uptake could be an alternative cost-effective method for decreasing Cd mobility in soil. Cd bioaccumulation in plant root to soil was higher than one, while the translocation factor from root to shoot was less than one. This indicated that cultivation of *V. radiata* L. coupled with biochar addition is an appropriate method for enhancing phytostabilization efficiency of *V. radiata* L. in Cd polluted sites.

Field of Study: Environmental Science
Academic Year: 2014

Student's Signature .....
Advisor's Signature .....
Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deep appreciation and sincere to my major advisor, Assoc.Prof.Dr.Somkiat Piyatiratitivorakul and my co-advisor, Assoc.Prof.Dr.Amorn Petsom for their excellent advice, motivation and valuable guidance throughout my work. I would like to convey my sincere gratitude to my thesis committee members, Assoc.Prof.Dr.Somchai Pengprecha, Assoc.Prof.Dr.Thares Srisatit and Asst.Prof.Dr.Narong Praphairaksit from Chulalongkorn University and the external examiner, Asst.Prof.Dr.Nukoon Tawinteung from King Mongkut's Institute of Technology Ladkrabang for their critical comments and suggestions in this thesis. My appreciation is also given to Asst.Prof.Dr.Charnwit Kositanont for his kindness and encouragement throughout the period of my study.

This research is supported by the grant from the 90th Year Chulalongkorn Scholarship, Graduate School, Chulalongkorn University.

I am indebted to Environmental Research Institute, Chulalongkorn University for facility support in this research. Special thank is expressed to Ms.Waleporn Sripenprapa and Ms.Chantana Kiengmol for their help and participation in some experiments. My sincere thank is extended to all friends from Inter-Department of Environmental Science, Graduate School, Chulalongkorn University for their kindness, friendships and hospitalities.

Finally, I would like to express my sincere gratitude to my parents for their infinite supporting and all the teachers who have taught me since my childhood. Special gratefulness should be expressed to Mrs. Benjaphorn Prapagdee, my wife, for her understanding and encouragement throughout this study.

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CHAPTER 1
INTRODUCTION

1.1 Background

Biochar or bio-charcoal is carbon rich material produced by pyrolysis process of bio-material under limited supplies of oxygen or air with heating temperature below 700ºC (Lehmann and Joseph, 2009). The carbonization process converts biomass to highly porous carbon product by physical and chemical change. In the decade year, the use of biochar for soil improvement as soil amendment is very attractive since porous structure of biochar can act like nutrient reservoir and habitat of beneficial soil microbial. The biochar can adsorb nutrients and retain in soil for plant availability from its adsorption ability. The adsorptions of organic and inorganic substance on biochar surface mainly occur by chemical function groups on biochar surface. Therefore, there are several applications of biochar for environmental management (Uchimiya, Lima, Klasson, et al., 2010). In particular, biochar is regarded as a promising heavy metal adsorbent (Li et al., 2013). The biochar quality depends on the forms of originated biomass and pyrolysis conditions; therefore, the applications of biochar in environmental aspects must be made for specific study (Ngah and Hanafiah, 2008).

Phatad Pha-Daeng sub-district, Mae Sot district, Tak province has high potential area of Zinc ore in Thailand. The case of Cd contamination in soil at Ban Pha Tae of Phatad Pha-Daeng sub-district which had high concentration of Cd in soil exceed the Cd and compounds standard adopted by Pollution Control Department of Thailand (PCD), 37 mg/kg soil (Pollution Control Department of Thailand, 2004). This contaminated area is located in Mae Ku and Mae Tao sub-catchment which located downstream from Zn mining. Mae Tao and Mae Ku canal flow through the Zn mine resulting in accumulation of Cd in the water, particular in suspended particulate solid. The uses of water from these canals are mainly for agricultural purpose and lead to Cd contamination in agricultural soil and finally contaminate in agricultural products. Simmons et al. (2005) reported that the levels of Cd and Zn in the agricultural paddy soil were in a range of 0.5 to 284 mg/kg and 100 to 8,036 mg/kg,

respectively. The results indicate that the contamination is associated with suspended sediment transported to paddy fields via the water supply from Mae Tao and Mae Ku canals. Furthermore, the results found that the Cd contamination in polished rice grain ranged from 0.05 to 7.7 mg/kg. Over 90% of rice grain samples exceeded the maximum permissible level of Cd (0.2 mg/kg; revision in 2013 adopted at 0.4 mg.kg) of the Codex Committee on Food Additives and Contaminants (CCFAC) (Simmons et al., 2005). In this contaminated area, the government had zoning on land uses and adopted hazard red zone in some area which not allow for agricultural activities. The decontamination of Cd from soil was introduced mostly by bioremediation technique. Phytoremediation is one of bioremediation technique which is the direct use of green plants and their associated microorganisms to reduce contamination of heavy metals in soils. The uptake of heavy metals by plant transfer from root and accumulation mostly in shoot can reduce heavy metals in soil. After planting, the biomass will be excavated and eliminated by secured method. On the other hand, phytostabilization is one of phytoremediation techniques which used plant to uptake heavy metal and mostly store in the root system. This technique is used for mitigation of heavy metals transportation into the surrounding areas by run-off or other weathering effects. Therefore, the phytostabilization technique is the method to make heavy metals immobile (Yoon et al., 2006). Soil organic amendments used to immobilize metals are subject to biodegradation (N. Bolan et al., 2014). Biochar has been more attractive for use as a soil amendment due to its persistent in soil (Lehmann and Joseph, 2009). In the past, the environment aspects of biochar addition in soil are for soil improvement and enhancement plant growth. From its adsorption capability, biochar can adsorb heavy metals and stabilize in soil. The biochar amendment soil is actually induced on low level of heavy metals bioavailable form in soil (Prokop et al., 2003).

This research was conducted on biochar prepared from the available biomass of agricultural by-products, namely cassava stem and rice husk. The efficiency of cassava stem and rice husk biochars on Cd removal from aqueous solution was evaluated. The improvement of biochar quality for enhancing Cd removal from aqueous solution by physico-chemical activation was also performed. In addition, the effects of biochar as soil amendment for promoting plant growth, Cd uptake and

phytostabilization by *Vigna radiata* L. (green bean) cultivated in Cd contaminated soil were investigated in the green-house condition.

1.2 Objectives of this study

1) To evaluate the efficiency of biochar on Cd removal from aqueous solution

2) To study the physico-chemical activation of biochar for enhancing Cd removal efficiency from aqueous solution

3) To investigate the effects of biochar amended in soil on Cd phytostabilization by *V. radiata* L.

1.3 Scopes of work

1.3.1 Laboratory study:

1) Biochars were prepared from cassava stem and rice husk by pyrolysis in laboratory furnace.

2) The physico-chemical activation was done by KOH activation followed by the second pyrolysis in laboratory furnace.

3) Cadmium removal efficiency by biochars was tested in the aqueous solution.

1.3.2 Green-house study:

1) Optimum condition on biochar preparation was obtained from Laboratory study part.

2) Cadmium contaminated soil was collected from an agricultural area at Pha Tae village, Phatad Pha-Daeng sub-district, Mae Sot district, Tak province, Thailand.

3) The local plant strain of *V. radiata* L. strain Kamphaeng Saen 1 (KS1) was obtained from Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

1.4 Benefit from this work

The results from this research facilitated the use of biochar as an alternate cost effective adsorbent for Cd removal from aqueous solution. The study of activation technique led to understanding how to enhance the Cd removal efficiency and its adsorption mechanisms. The study of the effects of biochar on plant growth and Cd uptake by *V. radiata L*. provided the understanding of potential of biochar on Cd phytostabilization in contaminated soil and further applied of biochar for Cd phytostabilization in the Cd polluted areas.

CHAPTER 2
LITERATURE REVIEW

2.1 Biochar

Biochar is a porous substance, similar in its appearance to charcoal produced by the combustion of biomass under oxygen-limited conditions. The carbonization temperature is actually below 700^{o}C with low heating rates which are relative slow (Lehmann and Joseph, 2009). In general, the term charcoal is for the uses of char as fuel propose, but in term of biochar was originally associated with a specific type of production for environmental uses. There is a wide variety of char products produced for environmental propose such as activated carbon, which was produced at high temperature, under long heating time and with controlled supply of inert gas or stream. The benefit of biochar for environmental aspect mainly consist of carbon storage in biochar by converted biomass into fix carbon, remove impurity substance from water, Soil improvement for planting, waste management by converse biomass to biochar, produce energy during pyrolysis process, and mitigation of climate change by decrease CO_2 emission (N. Bolan et al., 2014; Lehmann and Joseph, 2009; Park et al., 2011; Regmi et al., 2012).

Pyrolysis is a thermochemical decomposition of organic material in limiting oxygen condition. Pyrolysis involves a cracking process on polymeric structure to convert the biomass into biochar and volatile organic compound (Manya et al., 2013). Carbonization consists of two stages; the first stage is cellulose decomposition and the second stage is biochar formation (Gani and Naruse, 2007). The thermolysis of organic material starts when the temperature was rising. The products characteristics from pyrolysis were determined from pyrolysis conditions such as heating rate until pyrolysis temperatures. **Figure 2.1** shows various products from pyrolysis process. The complex biomass decomposition starts from gas generation, liquid condensed and main product of char forming. Bio-gas such as hydrogen, nitrogen, carbondioxide, carbonmonoxide and oxygen are generated from early stage of pyrolysis. The liquid product such as tars and heavily hydrocarbons and water was condensed to bio-oil.

Pyrolysis make higher carbon ratio content when increasing temperature and burning time and transform to black char after finished carbonization process.

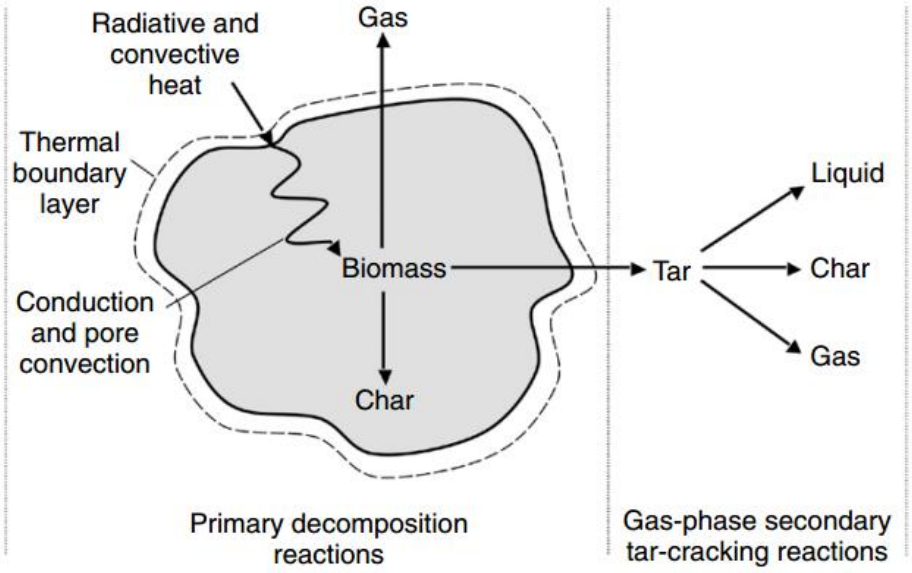


Figure 2.1 Pyrolysis of biomass (Basu, 2010)

Types of pyrolysis mainly divide into three categories, fast pyrolysis, mild pyrolysis and slow pyrolysis (**Table 2.1** and **Table 2.2**). The considerations of pyrolysis type are depending on heating rate of temperature, final heating temperature and holding time for carbonization (Mayhead et al., 2014). Mild pyrolysis is the process for making bio-coal which holding for short heating time. The short period of heating make change on partial physico-chemically properties of biomass. Bio-coal has less mass than origin biomass which economic for transportation and has stable heat capacity for substitute coal for power plant. Furthermore, mild pyrolysis can make wood to hydrophobicity which preserve wood for weathering resistance. Slow pyrolysis is carbonization process of biomass in slow heating rate and long resident time as traditional coal making method. This process generated less smoke char products. The carbonization temperature is actually less than 400°C. The slow pyrolysis generated high char, but less bio-oil. In contrast, fast pyrolysis actually carbonizes biomass in higher temperature, 400-600°C. Fast pyrolysis gives less on char yield, but high bio-oil production (Bruun et al., 2012).

Table 2.1 Types of pyrolysis, pyrolysis condition and products (Mayhead et al., 2014)

Type of pyrolysis	Term used	Temperature (°C)	Resident time	Products
Mild pyrolysis	Torrefaction, torification, airless drying, destructive distillation	200-315	Short (5-30 minutes)	Torrified wood (bio-coal)
Slow pyrolysis	Charcoal making, carbonization	300-400	Long (hours-days)	Charcoal (biochar)
Fast pyrolysis	Bio-oil production	400-600	Short (rapid heating rate <1 second)	Liquid (bio-oil), Char (bio-char), Gases (H_2, CH_4, CO and CO_2), Liquid smoke

Table 2.2 Product comparison from pyrolysis (Mayhead et al., 2014)

Type of pyrolysis	Products yield		
	Liquid	Solid	Gas
Mild pyrolysis	~11	70-90	~2
Slow pyrolysis	30	35	35
Fast pyrolysis	75	12	13

The biomass feed in fast pyrolysis to produce bio-oil should have low moisture content and grind to size between 1-2 mm to save burning energy and fast burning. Bio-oil has 15-35% water content and 35-40% oxygen which make bio-oil has low heat capacity and low pH (pH 2-3 from acetic and formic acid) (Bruun et al., 2012)**.** Biochar produced from low pyrolysis temperature is expected to be high metal adsorption efficiency. The low pyrolysis temperature preserves higher organic carbon contents and aromatic carbon that are important for metal binding on the biochar

surface, while the higher pyrolysis temperature can leach aromatic carbon compound from its surface (Regmi et al., 2012; Song and Guo, 2012). To achieve biochar in different physico-chemical properties for specific uses, it needs consider not only the types of biomass, but also pyrolysis conditions (Uchimiya et al., 2011).

2.2 Cadmium

Cadmium (Cd) is a soft, silver-white or bluish-white surface, less smell and soft enough to be cut with a knife. Cadmium is soluble in acids but not in alkalis and it is similar in many respect to zinc (Zn) and it is located just below Zn in the periodic table; therefore, it's physical and chemical properties are rather similar to Zn, but it forms more complex compounds. It is always found Cd associated with Zn in environment; therefore, Cd is by-product from Zn ore mining and led to disperse Cd from mining activity to the surrounding areas. Cd burns and appears brown amorphous in air in the form of cadmium oxide (CdO). Zn oxide (ZnO) appears in the dark red crystalline form which change color after heating. Other soluble forms when it solutes in acid are cadmium chloride ($CdCl_2$), cadmium sulfate ($CdSO_4$), or cadmium nitrate ($Cd(NO_3)_2$).

2.2.1 Mobility of Cd in the environment

The distributions Cd in the environment occur from natural degradation of the earth crusts and human activities. Naturally, Cd is released into the environment by degradation process. Weathering and erosion of parent rocks releases Cd to soil and water. A major natural atmospheric source of Cd was released through forest fires and volcanoes (UNEP, 2000). Both of the results were transported Cd into the ecosystem. The rest of the cadmium is released through human activities, such as manufacturing. The anthropogenic emission of Cd is major concern due to its related to human health. Cadmium can be distributed in various environments, air, water and soil which Cd can be transported between these three main environments. The distribution of Cd in air leads to washout into soil and water. The anthropogenic activities can disturb Cd in soil and lead to transport Cd into air. The distribution form of Cd in air is usually

found as CaO which combined to suspended aerosol. The main factors influent Cd transported into air such as Cd complex molecule size, aerosol size and metrological condition which enhance transportation Cd far away from source. The use of natural materials contaminated with Cd is one factor that distributes Cd far away from Cd contamination sources. Phosphate fertilizer which is natural product from soil may be the transportation factor of Cd since some fertilizer sources contaminate in high Cd concentration. The household open burning of waste can release Cd and other toxic metals into air. The mining is also the possible source of Cd release into surrounding environment because Cd always combined with other elements in mineral compounds such as Zn and Pb. Cadmium might washout from mine area to outer mining zone, Therefore, the good practice of mining must be concern for protect surrounding environment.

2.2.2 Cadmium level in the environment

Cadmium is found in atmosphere, water and soil. It enters into the environment since Cd cannot be degraded. The risk of environmental exposure to Cd is stable increasing because of its accumulation via the food chain (Registry, 2012). Cadmium level in environment is different which depend on location of Cd sources. In ambient air, the major anthropogenic sources of Cd are metal use for combustion, industry revolution. The concentration of Cd in the air depends on population, urbanization and high densities of traffic (Komarnicki, 2005). Cd levels in ambient air in rural, urban and industrialized areas were ranged from 0.1 to 5 ng/m³, 2 to 15 ng/m³ and 15 to 150 ng/m³, respectively (International Cadmium Association, 2013). World Health Organization (2010) adopted Cd ambient air guidelines at 5 ng/m^3 for annual average. It is obviously seen that Cd levels in ambient air depend on anthropogenic activities related to Cd uses and cause harmful to human life. The Cd in vapor form of CdO is toxic, non-soluble in the water and it can attach to suspended particles in water.

In general, Cd forms in water are in Cd ion form or complex with inorganic substances in water such as carbonates, hydroxides, chlorides and sulphates or as organic complexes with humic acids. Cadmium may enter aquatic environment by several ways such as through weathering and erosion of soil, direct discharge from industrial wastewater, leak from landfill or contaminated site, and the washout from agriculture area that use Cd contaminated fertilizer. Phosphate fertilizer which has Cd contamination is major source that input Cd to soil and compose through the groundwater (Kalicanin, 2009). The growth of industrial lead to discharge cadmium entering fresh waters and it may be adsorbed onto suspended particulate matter and sink into the aquatic environment. Cadmium concentration in water is in usually in range of 10 to 4,000 ng/L depending on the distance from Cd sources. Cadmium in the water contains low levels of dissolved forms and accumulates in sediment (Burger, 2008). In aquatic organisms, mussels and crayfish, Cd can accumulate from feeding sediment which contaminated cadmium via food chain (Bennet-Chambers et al., 1999). Marine environment is an important source of Cd due to Cd from atmospheric deposition, wastewater discharges, sludge and industrial leach to the marine. Thus, marine environment acts as sink of Cd (Torres et al., 1998).

Cadmium contamination in soil divided in to two cases, land for agriculture and land for living. Anthropogenic input of cadmium to soils occurs via the use of Cd contaminated water. The uses of sewage sludge, manure and phosphate fertilizer application directly to soil without analyzed for Cd contamination lead to accumulation Cd in agricultural soil for long-term. Normal level of cadmium in common soil that is far from sites of contamination e.g. industries, mining has 0.1-0.5 mg/kg of soil (Cook and Morrow, 1995;). Anthropogenic sources of Cd contaminated in soil are mining electroplating, smelting and other industrial, and activities artificial phosphate fertilizers (Burger, 2008). Cadmium is much less mobile in soils than in air and water. The major factors governing cadmium speciation, adsorption and distribution in soils are pH, soluble organic matter, clay content and type, presence of organic and inorganic compounds and competition from other metal ions.

Cadmium contamination in soil at Mae Tao sub-catchment, Pha Tae village, Phatad Pha Daeng sub-district, Mae Sot district, Tak Province, Thailand has been discovered since year 2000. This area is Zn ore deposit and Cd occurs in association with Zn. Natural soil erosion and agricultural activities caused Cd distribution and contamination in the water, sediment and soil. In addition, the use of water from irrigation canals which has high Cd content for plantation also causes the Cd contamination into soil. Simmons et al. (2005) studied Cd contamination levels in soil during 2001–2002. The results indicated that Cd levels in soil were in range of 0.5 to 284 mg/kg. Cd concentrations in soil from several places exceeded the standard of Cd in soil for agricultural and living purposes announced by Pollution Control Department of Thailand (37 mg/kg). Furthermore, Cd contamination in suspended solid from canal water which led to Cd accumulation in agricultural soil beside the canal (Simmons et al. (2005). The analysis of Cd levels in rice grain grown in contaminated area found that over 90% of rice grain samples exceed the standard of the CCFAC draft maximum permissible level of Cd in rice grain of 0.2 mg/kg (Now, revision 2013 adopted 0.4 mg/kg). The study of Simmons et al. (2003) found that during 2000-2002, rice and soybean planted in this area contained Cd concentrations which were a ranged from 0.02–5.00 and 1.08–1.71 mg/kg. In 2006, the Land Development Department of Thailand developed master plan for solving Cd contamination in agricultural soil at Mae Tao sub-catchment which consists of land zoning area and Cd remediation plan. The phytoremediation by several plants was introduced to study in these contaminated areas.

2.2.3 Level of Cd in foodstuffs

Several edible crops that grow in Cd polluted soil can easily take up and accumulate Cd, thus transporting contamination to consumers via the food chain. Excessive intake of cadmium-contaminated food causes Itai-itai disease (Makino et al., 2007). In natural condition, the Cd contaminations in food are not very high. The level of Cd in vegetable and rice grain exhibit relatively high values from 30 to 150 ppb. There are some Cd levels in human's food animals such as meat and fish in a range of 5 to 40 ppb. The Cd levels are vary widely with the agricultural practices such as selection of low contamination area and consider the uses of low Cd level in

phosphate fertilizer. At present less Cd contamination in foodstuffs is found than in the past because of better agricultural practice which can introduce quality control since starting the crop until the end of crop monitoring.

2.2.4 Effects of Cd to human

Cd has high toxic to animal and human even though low concentration uptake. The toxicity of Cd to human is depending on the Cd form and the way that Cd enters into the human body. In acute toxicity of Cd, inhalation exposure to high levels of cadmium effects on the lung, such as bronchial and pulmonary irritation. A single acute exposure to high levels of cadmium can result in long-lasting impairment of lung function. The acute toxicity of Cd inhalation through upper respiratory tract occurs within 2-3 h after inhalation and may lead to infection at lower respiratory tract. The maximum permissible value for worker inhalation according to German law is 15 μg/L of air. For comparison, non-smokers show an average cadmium blood concentration of 0.5 μg/L. The uptake through the human gastrointestinal tract is approximately 5% of an ingested amount of Cd, depending on the uptake dose and nutritional composition (Godt et al., 2006). The uptake Cd by food may lead chronic effect to liver and kidney because body mechanisms try to eliminate Cd. The Itai-Itai disease was first notice at Jhinzu River, Toyama Prefecture, Japan. However, it was not defined as Cd poison disease until 1960. On the upper land of contaminated area has mine activity which dispersed Cd through downstream. The people consumed the water in river and rice which contaminated with Cd for a long time. The cadmium poisoning caused softening of the bones and kidney failure.

The Environmental Protection Agency, the United State of America (USEPA) mentioned an association between cadmium exposure and an increased risk of lung cancer, but these studies are inconclusive due to confounding factors. The EPA has classified cadmium as a group B1, probable human carcinogen.

2.3 Adsorption

Adsorption process is fundamentally the attachment of one type of material onto surface of another material type. Adsorption occurs by either a physical process or chemical process that involves the transfer between 2 matrixes such as liquid: liquid, gas: liquid, gas: solid and liquid: solid. The adsorbate or solute is the material being adsorbed while adsorbent is the solid material being used as the adsorbing phase e.g., activated carbon. For adsorption in aqueous solution, adsorption is the process of transferring material from a fluid phase to a solid phase. Examples of sorbents are activated carbon, silica gel, alumina, fly ash, zeolite, clay, synthetic resins and biomass. Adsorption is divided into two types according to partitioning force as i) physical adsorption and ii) chemical adsorption **(Table 2.3)**.

2.3.1 Adsorption type

1) Physical adsorption

The physical adsorption involved by relatively weak intermolecular force such as the Van der Waals forces (dispersion-repulsion) and electrostatic interactions. The Van der Waals forces always present while electrostatic interactions is only present with ionic structure molecule such as zeolite. The heat of physical adsorption is actually low (less than 20 kcal/mole) and easy to reverse. The adsorption characteristic is mostly multilayer adsorption which the adsorption space accommodates more than one layer of molecules and not all adsorbed molecules are in contact with the surface layer of the adsorbent. The complex adsorption layers depend on adsorbate concentration. The quantity or accumulation of adsorbate on adsorbent interface or in an interfacial layer are increased while increase adsorbate concentration. Adsorption occurs well in ambient pressure and temperature while adsorbate in gas or solid phase spread to attach on adsorbent surface. But, when decrease pressure and/or temperature the adsorption become reverse as the prominent point for desorption. The physical adsorption is not make change to chemical characteristic both adsorbate and adsorbent.

2) Chemical adsorption

The chemical adsorption occur by chemical binding force which involves a chemical reaction between the surface and the adsorbate such as ion exchange is the strong interaction between the adsorbate and the substrate surface creates new types of electronic bonds. This adsorption type occurs by chemical reaction between adsorbate and adsorbent molecule which make change on adsorbate chemical characteristics. The adsorption destroys existing molecule force and rearrange to the new combined molecule. Chemical adsorption has higher adsorption heat than physical adsorption (40-1,000 Kcal/mole). During adsorption process, the new combined molecule was formed and makes a result of adsorbent decreasing. The chemical adsorption characteristic is monolayer adsorption which is not reversible. The monolayer capacity is defined as the amount of adsorbate which is needed to occupy all adsorption sites as determined by the structure of the adsorbent and by the chemical nature of the adsorptive.

Table 2.3 The general features which distinguish physical adsorption and chemical adsorption (Ruthven, 1984)

Physical adsorption	Chemical adsorption
- Low heat of adsorption	- High heat of adsorption
- < 2 or 3 time latent heat of evaporation	- >2 or 3 time latent heat of evaporation
- Non specific	- Highly specific
- Monolayer or multilayer adsorption	- Monolayer adsorption only
- No dissociation of adsorbed species	- May involved dissociation
- Only significant at relative low temperature	- Possible over a wide range of temperature
- Rapid, non-activated, reversible	- Activated may be slow and irreversible
- No electron transfer, although polarization of sorbate may be occurred	- Electron transfer leading to bond formation between sorbate and sorbent

2.3.2 Factors influencing adsorption

There are various factors influencing adsorption efficiency such as adsorbate and adsorbent type, adsorbate concentration, contact time and agitation rate (Crini and Badot, 2010).

1) Adsorbent type

Adsorbent physical structure such as surface area and porosity play dominant role on adsorption efficiency. The increase of sorbent surface area is increase adsorption efficiency while high porosity makes a result of high surface area. The effective pore size must be bigger than adsorbate molecule or ion for involved effective adsorption into inner zone of adsorbent. Adsorbent chemical structures which consist of specific functional groups on adsorbent surface are important for selected adsorbate species. The increasing of adsorbent will increase adsorption efficiency due to large surface area. Larger size of surface area implies a greater adsorption capacity. The smaller particle sizes reduce internal diffusional and mass transfer limitation to the penetration of the adsorbate.

2) Adsorbate type

In aqueous adsorption condition, adsorbate type play dominant factor for adsorption mechanism. Inorganic hydrophilic adsorbate easy adsorb onto adsorbent surface than organic hydrophobic adsorbate. Weight and size of molecule and electro-negativity of molecule affect to adsorption rate. Therefore, electro-negativity threat of adsorbate can increase hydrophobic property for higher adsorption efficiency.

3) Contact time and agitation rate

Contact time or residence time is important for adsorption efficiency. The longer contact time until equilibrium stage will spent efficiency on adsorbent surface. The system agitation rate is important for reduce internal diffusion of film resistance and penetration of the adsorbate film and increase adsorbate transfer to the inner hole of adsorbent.

4) pH of solution

In aqueous solution, pH is significant to adsorption efficiency. The ionic strength of polar involves chemical adsorption on adsorbent surface. The adsorption on binding site of chemical functional group depend on solution pH which acidic

solution with high H^+ make decrease on adsorption efficiency, while, alkaline solution make chemical coagulation of adsorbate that decrease on adsorption efficiency (Kołodynska et al., 2012; Regmi et al., 2012).

2.3.3 Metals adsorption by biochar

There are various methods for remove heavy metals from aqueous solution i.e. membrane filtration, ion exchange technique, solvent extraction and adsorption (Haris et al., 2011; Tangjuank et al., 2009). These methods are broad used for remove heavy metals from industrial wastewater before discharge to outside environment. Each method has specific and suitable differently depend on wastewater characteristic, budget and human resources. Adsorption is one of the most important physico-chemical methods that is commonly used and applied for heavy metals removal from wastewater and aqueous solutions. Adsorption technique is widely uses because its low cost operation. Adsorbent are ability to remove complex heavy metals can be operated in wide range of pH (Rao et al., 2010). Adsorbent from natural product can be considered as cheap or low-cost if it is abundant in nature which requires little processing before uses. The used adsorbent from natural can be disposal without regenerating. Therefore, the study of enhancement of adsorbent from natural product is considering for decrease amount of adsorbent per each used (Bailey et al., 1999).

Biochars are porous material made from biomass which cheap and easy to find in local area. It is persistent in environment and has high adsorption efficiency (Kołodynska et al., 2012; Regmi et al., 2012; Uchimiya et al., 2011). Biochar has more effective than other natural adsorbent materials from its porosity and high surface area. Biochar can adsorb heavy metals by chemical adsorption which has more stability. Biochar produced from low temperature pyrolysis which caused organic matter left on its surface. These organic matters present as various chemical functional group which has important role on heavy metals adsorption (Trakal et al., 2014). The chemical functional groups on biochar surface such as carboxylic group, hydroxyl group, methylene group and aromatic C–H group have sensitivity for ion adsorption affinity. The biochar properties depend on precursor biomass, pyrolysis condition and post treat after carbonization or activation process. These factors are

important for application of biochar in environmental purpose (Uchimiya, Lima, Klasson, et al., 2010).

Biochar has micro-pore for enhance its surface area for metals adsorption; therefore, most of research focus on improves biochar physico-chemical properties by selecting biomass sources and pyrolysis conditions. The physical properties of each type of biochar are shown in **Table 2.4**. The specific biochar can remove specific metal or cover to several metals in aqueous solution. The adsorption of metals cation to biochar can be grouped into two categories, nonspecific and specific adsorption. Specific adsorption indicates the adsorption of metal in the inner layer forming chemical coordination bonds to biochar surface, while nonspecific adsorption refers to adsorption of metal by simple interaction in the diffuse electric double layer (Park et al., 2011).

Table 2.4 Compare physical property of biochar (Ruthven, 1984)

Characteristics	Micropore	Mesopore or Transitional pore	Macropore
Diameter (A^o)	< 20	20-500	>500
Pore volume (cm^3/g)	0.15-0.5	0.02-0.1	0.2-0.5
Surface area (m^2/g)	100-1,000	10-100	0.5-2

2.3.4 Activation method of biochar

The study on enhancing of biochar for metal removing from aqueous solution is important for reducing amount of biochar for each uses. Basically, the enhance of biochar quality are divided in to two categories as follows: i) physical action process and ii) chemical activation process (Khalkhali and Omidvari, 2005).

1) Physical activation

The physical activation can be done by two steps of heating. First step is pyrolysis at temperature in range of 400-500^{o}C for eliminate volatile matters and follow to heat with high temperature, 800-1,000^{o}C, plus the present with inert gas or stream. The high temperature will oxidize density of carbon molecule to achieve high porosity carbon. The biochar will have the specific physical characteristic such as micro-pore size and density pore generated as internal pore structure. In general, the

physical activation of biochar call activated carbon which has a surface area in excess of 500 m^2.

2) Chemical activation

The chemical activation is the process of enhance biochar chemical properties by using chemical reagent. The carbonization of carbonaceous material at relative low temperature (e.g. 400-700^oC) in the present of a dehydrate agent (e.g. $ZnCl_2$, KOH, H_3PO_4) or alkaline or acid substance. These agents may promote formation of cross-links or formation of matrix legends which important for metals adsorption. The chemical activation process can enhance ionic strength of functional groups matching target metals (Teng et al., 2001). The chemical activation can be done both before carbonization and after carbonization process. More than 60% of biochar from chemical activation are in form of powder.

The physico-chemical activation of biochar with physical and chemical activation can increase both adsorption efficiency and stability of adsorption by combination prominent point of each activation process. Hence, the benefits of activation process are as follow; (Ngah and Hanafiah, 2008)

(1) For decrease pore size and increase surface area of biochar which enhance adsorption efficiency
(2) For increase ion exchange capacity at biochar surface area
(3) For force metal coagulation of metals at the inner pore of biochar

Azargohar and Dalai (2008) studied activation process of activated carbon witch prepared from whitewood by fast pyrolysis. The study was compared between physical and chemical activations. The physical activation process was done by the present of N_2 in the temperature of 600-900ºC while the chemical activation was done by KOH. The activated carbon was suspended and stirred in 1.63 M of KOH for 2 h follow by second pyrolysis at the temperature range of 550-800ºC. The results showed that the BET surface area of activated carbon from chemical activation was 950 m^2/g while activated carbon from physical activation was 836 m^2/g. Regmi et al. (2012) studied on activation of biochar produced from switch grass via hydrothermal carbonization at 300ºC. The cold activation process using KOH at room temperature

was developed to enhance the porous structure and sorption properties of the biochar. The biochar was suspended and stirred in 2N KOH for 1 h before maintained neutral pH by addition of 1N NaOH and/or 1N HCl solution. Experiments conducted with an initial metal concentration of 40 mg/L at pH 5.0 and contact time of 24 h. The result of study on effect of pH ranges showed good adsorption efficiency of biochar on copper (Cu) and Cd at pH 5-7. The adsorption capacity of activated biochar on Cu and Cd removal increased 7.75 and 22.6 folds, respectively. The results also compared with commercial powder activated carbon which gave much lower adsorption capacity than activated biochar for Cu and Cd removal from aqueous solution. Trakal et al. (2014) reported the efficiency of biochar for Cu removal from synthetic and soil solutions. The biochar was produced from brewers draft via pyrolysis at 650ºC and made chemical activation with 2 M KOH to enhance its sorption efficiency to remove Cu. The KOH activation was done by cold condition for 1 h. The results show the biochar surface area was increased from 9.80 to11.6 m^2/g while adsorption capacity increased from 8.77 to 10.3 mg/g.

Ozcimen and Mericboyu (2009) improved the property of raw chestnut shell and grape seed by physically impregnated with $ZnCl_2$ before carbonization with pyrolysis at temperature of 550ºC. The results showed that the adsorption capacity of chestnut shell and grape seed biochar regarding the removal of Cu at pH 5 were 100 and 48.78 mg/g, respectively.

For acidic activation, there are some research focuses on the uses of acid substances for enhancing adsorption efficiency of metals from aqueous solution. Tajar et al. (2009) studied low cost activated carbon derived from nut shells for removing cadmium from aqueous solutions. This study conducted with activated carbons produced from nut shells and commercial activated carbon. The activation process was done with phosphoric acid and heat with the temperature of 475ºC and held for 45 min before flushed with a flow of SO_2 gas for introduced the sulfur on the activated carbon surface. The results showed that the sulfurized nut shell and sulfurized commercial activated carbon can be increased Cd adsorption capacity from 104.17 to 142.86 mg/g and 90.09 to 126.58 mg/g, respectively. The results also found that the activation with phosphoric acid plus SO_2 can increase the activated carbon surface area and carbon-sulfur complexes functional group facilitating the retention of

Cd that strong bond with sulfur. The regeneration process can be done easily with common hydrochloric acid for desorb Cd from the activated carbon. Teng et al. (2001) prepared activated carbon from an Australian bituminous coal. The originated coal was received and made chemical activation by phosphoric acid (H_3PO_4). The mixing of H_3PO_4 and coal was agitated by the time of 1-3 h before heat at 400-600ºC for 1-3 h in the present of N_2. The results reveal that the surface area and pore volume values increased with the chemical ratio of H_3PO_4 and coal. The carbonization temperature of 500ºC in 3 h gave the maximum surface area and pore volume values. Therefore, Cd adsorption capacity of various types of biochar is summarized in **Table 2.5**.

Table 2.5 Cadmium adsorption capacity from biochar produced from various biomass sources and various activation processes

Biochar sources	Activation processes	Pyrolysis temperature (ºC)	Maximum adsorption capacity (mg/g)	References
Swich-grass	KOH	300	34	Regmi et al. (2012)
Pig manure	Non-activated	600	16.6	Kołodynska et al. (2012)
Manure	-	350	51.4	Xu et al. (2013)
Commercial carbon	phosphoric acid + SO_2	475	104.17	Tajar et al. (2009)
Nut Shells	phosphoric acid + SO_2		142.86	
Cashew nut shell	KOH + CO_2	850	14.29	Tangjuank et al. (2009)

Hydari et al. (2012) studied the removal of Cd ion from aqueous solution by commercial activated carbon. The activation process was done by 0.2 M oxalic acid for 4 h before treat with chitosan gel. The result show the effective of amine and hydroxyl group was increase as a result of metals removal efficiency. The optimum condition for Cd (II) ion removal was pH 6 and the Cd adsorption capacity was increased from 10.3 to 52.63 mg/g.

Wang et al. (2013) studied a woody biochar which was the byproduct of gasification of sawdust. The fast pyrolysis temperature was 800ºC. The biochar was activated by biochelating technique and chemical activation by H_2SO_4. The biochelating technique was done by using *Acidithiobacillus ferrooxidans*, isolated from sewage sludge and carried out in deionizer contained with ferric sulfate ($FeSO_4$), Potassium phosphate (K_2HPO_4) and Ammonium sulfate $(NH_4)_2SO_4$. The chemical activation by H_2SO_4, shaking with H_2SO_4 at 200 rpm for 4 h. The adsorption study was conducted with Lead (Pb). The acid treated biochar and biochelated biochar were increased Pb adsorption capacity from 6.18 to 65.82 and 51.34, respectively. The results suggest that biochelating technique was not increased biochar surface area but gave a good result for increased adsorption capacity similar to chemical activation which increased biochar surface area. Hence, the adsorption efficiency not only depended on surface area, but also depended on other chemical properties of biochar surface.

2.4 Remediation of heavy metals contaminated soils

Soil is the important environment surrounding human being surviving and developing. The heavy metals contamination in soil becomes serious issues since the heavy metals pose risks for human health and the environment. The development of industries has sometimes been left behind to pollute surface and ground water. The heavy metals most frequently encountered in this waste include As, Cd, Cr, Cu, Pb, Ni and Zn. On the other hand, the natural contamination of heavy metal was also concerned due to the transport these heavy metals to the surrounding area far away. The heavy metals contaminated in soil are difficult problem to be solved even if few, expensive budget and technologies may be employed. High level of heavy metals contaminated in soil still recognized nowadays because these heavy metals may

transport close to human activities and change to bioavailable form (N. Bolan et al., 2014).

The heavy metals remediation from soil is divided into main three categories; i) physical remediation, ii) chemical remediation and iii) biological remediation (Yao et al., 2013).

2.4.1 Physical remediation

The physical remediation mainly includes soil replacement method and thermal desorption. The soil replacement is using uncontaminated soil to replace the contaminated soil with aim of dilute the pollutant concentration, increase the soil environmental capacity, and thus remediate the soil by the aim of diluting and naturally degrading. This method is suitable for contaminated soil with small area. The limitation of soil replacement is the costly for operate and may lead to second pollution from soil remove to the new places.

The thermal desorption is on the basis of pollutant's volatility and heat the contaminated soil using steam, microwave, infrared radiation to make the pollutant (e.g. mercury, arsenic) volatile. The volatile heavy metals are then collected using the vacuum negative pressure or carrier gas and achieve the aim of removing the heavy metals from soil. However, the limited factors such as the expensive cost and spent long time to desorption.

Electro-kinetic remediation is remediation technology which is mainly applying voltage at the two sides of contaminated soil and then forming electric field gradient. The heavy metals were migrate attach to two poles via electro-migration, and then collected and treated. It has advantages to easily install and operate for low permeable soil. Electro-kinetic remediation method are low cost operate and not destroy the original nature environment. However, the treatment efficiency of the electro-kinetic remediation was almost low.

2.4.2 Chemical remediation

There are various chemical remediation methods to remove heavy metals from soil such as chemical leaching, chemical fixation, electro-kinetic remediation and vitrify technology.

Chemical leaching is using fresh water, reagents, and others fluids or gas wash the contaminated soil that can extract the pollutant from contaminated soil. The leaching technique is follow through the ions exchange, precipitation, adsorption and chelation which transfer heavy metals from soil and dissolve them in a liquid, and then recovered from the leachate. In general, the leaching agents are inorganic eluter, chelation agents, and surfactant, etc. The limited factors for this technique are the expensive cost and the poor degradability of leaching agents in environment.

Chemical fixation is the important method for decrease the migration of heavy metals to water, plant and other environmental media and achieving the remediation of soil. Chemical fixations is employs agents or materials into the contaminated soil and combine covalently with their major chemical constituents to fixing heavy metals to insoluble form or hardly migrate or low toxic form. The heavy metals present in immobile form are less migrating due to its stability and less bioavailability. The amendment materials actually prefer environmental friendly media such as clays, metallic oxides, and biomaterials. These amendment materials should be study before use such as the ability to reduce heavy metal bioavailability and the effect to productivity of the crops.

In situ vitrify technology is heating the soil at high temperature of 1,400~2,000°C, in which process the organic matters volatilize or decompose. The steam produced and pyrolysis product was collected by gas treatment system. A vitrified product is resistant to physical or chemical degradation and makes it lose migration. The vitrify technology is sometime apply for the treatment of radioactively contaminated soil for in situ conditions. This was done to avoid the problems associated with the excavation and transport of radioactively contaminated soils. However, this technology is only suitable for volatile contaminants.

2.4.3 Biological remediation

The biological remediation includes bioremediation and phytoremediation which can make less mobility or remove heavy metals from soil. Bioremediation is a process defined as the use of microorganisms or fungi to neutralize or remove organic and inorganic pollutant from soil. Although, the microorganisms and plant cannot

degrade and destroy the heavy metals, but can affect the migration and transformation through changing their physical and chemical characterizations. The remediation mechanisms include extracellular complexation, precipitation, oxidation-reduction reaction and intracellular accumulation. The advantage of using biological remediation technique is offers a cost effective compared to other remediation methods, because it is a natural process and does not usually produce toxic by-products.

Phytoremediation is an emerging suitable technology that use of various living green plants to remove, destroy, transform or sequester contaminants from soils and waters or to render them harmless (Glick, 2003). Phytoremediation of heavy metals is a cost-effective green and less invasive technology for the environment clean up and some removed metals may be recycled. Phytoremediation of heavy metals involves in several processes including phytoextraction, phytostabilization, phytovolatilization and rhizofiltration (Cunningham et al., 1995).

Phytoextraction or phytoaccumulation is uptake and translocation of metal contaminants in the soil by plant roots within the plants. Contaminants are normally removed by harvesting the plants as hyper-accumulating plants which are absorb unusually large amounts of metals in comparison to other plants. After growing, the plants can be harvested and incinerated or composted to recycle the metals. However, the ash from plants are incinerated must be disposed of in a hazardous waste landfill, but the quantity of them will be less than 10% of original contaminated soil. The process of phytoextraction is illustrated in **Figure 2.2**.

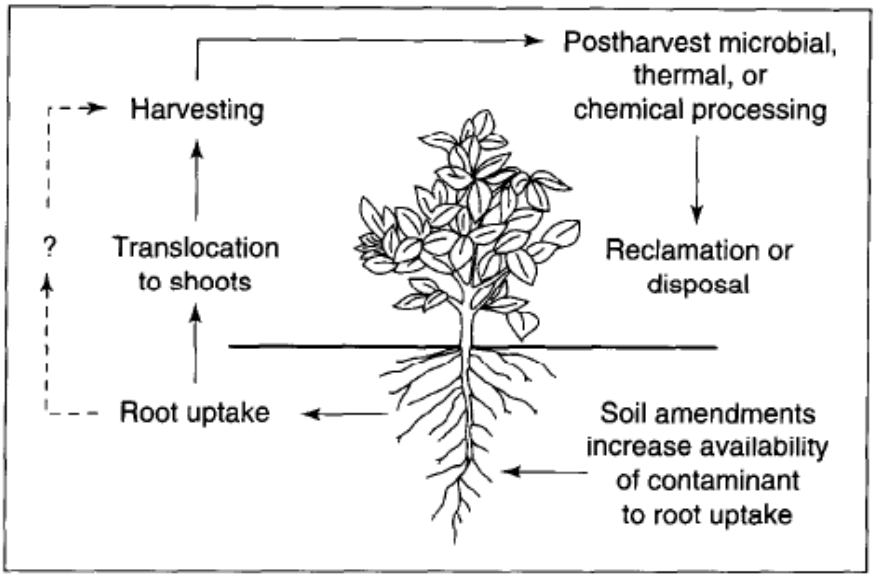


Figure 2.2 Phytoextraction process (Cunningham et al., 1995)

Phytostabilization is the use of plants to reduce the metal bioavailability and prevents migration of metals in the soil and ground water in virtue of absorption and accumulation by roots **(Figure 2.3)** (Glick, 2003; Raskin et al., 1997). Phytostabilization can be used metal-tolerant species restored vegetation to the sites by reestablish a vegetative cover at contaminated area. This technique can change metal solubility and mobility or influence the dissociation of organic compounds. The plants were affected of soil environment can convert metals from a soluble to an insoluble oxidation state. Phytostabilization techniques are suitable for relatively immobile materials and large surface areas. In addition, it may work better with heavier textured soils and soil which are high organic matter content (Cunningham et al., 1995). Lead, arsenic, cadmium, chromium, copper and zinc can be successfully treated by this technique.

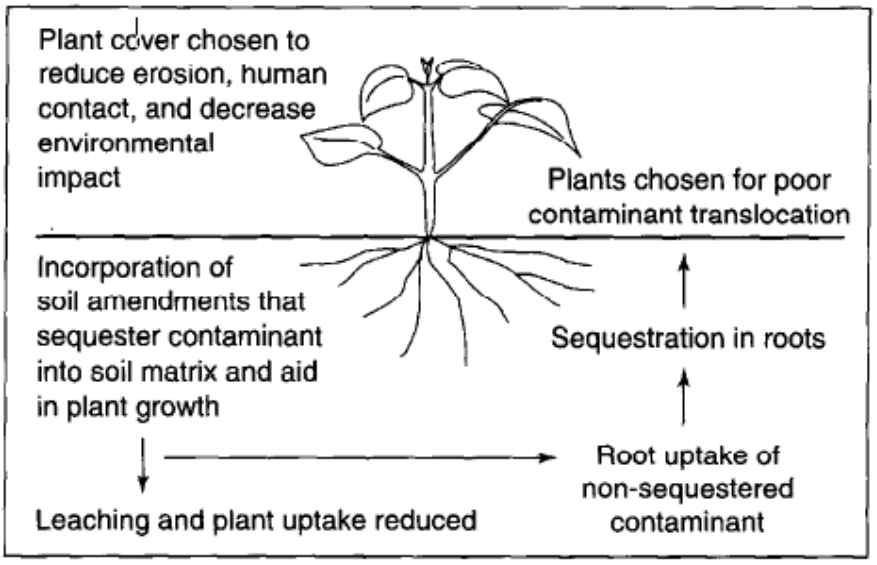


Figure 2.3 Phytostabilization process (Cunningham et al., 1995)

Phytovolatilization is the uptake of volatile metals from soil and water into leaves and release them into the atmosphere. The transformation contaminants that were reduced toxic in the environment. Pollutants in form of volatile molecules were removed by root. Toxic metals such as selenium, arsenic and mercury can be biomethylated to form volatile molecules that can be lost to the atmosphere (Raskin et al., 1997). Rhizofiltration is the use of plant roots to adsorb or precipitate metals from polluted water surrounding the root zone (Glick, 2003).

2.5 The application of biochar for remediation heavy metals contaminated soil

The heavy metals persist in soil with adsorption, coagulation or complex with other substance in soil. Heavy metals enter to the soil environment through both natural earth rock degradation and anthropogenic processes. Most heavy metals occur naturally in soil parent materials, but most of heavy metal forms are not readily bioavailable for plant uptake. Unlike natural earth rock degradation, the anthropogenic activities cause heavy metal contaminations in soil typically have a high bioavailability. Anthropogenic activities such as effluent from industrial processes, manufacturing, the disposal of domestic and industrial waste materials, and

also the application of impurity phosphate fertilizers are the major sources of heavy metals contamination in soils.

The toxicity of heavy metal is not depended on the total concentration of heavy metal, but it depends on the level of heavy metal in bioavailability form. Bioavailability is the proportion of total metal that are available for incorporation into biota (bioaccumulation). Bioavailability is the key factor for determine direction of phytoremediation process. The metals bioavailability does not necessarily correspond with total metal concentration. The less metal bioavailability leads to phytostabilization technique but more metal bioavailability is suitable for phytoextraction. The factors control metal availability are various such as pH, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acid. Plant can uptake heavy metals from soil though plant root by nutrient uptake mechanism. Higher level of metals bioavailability leads to higher uptake by plants.

The application of biochar as soil amendment is one of chemical remediation technique for make complex reaction with heavy metal and immobilizes them in the soil and reduces the potential for adverse effect from the heavy metal. The uses of biochar for adsorb heavy metal is consider for decrease bioavailability of toxic metal. In general, the interaction between biochar and metal are complex and several adsorption mechanisms have been proposed for divalent metal in soil such as ion exchange with negatively charged on biochar surface, complexation with biochar surface functional groups and precipitation or co-precipitation with other organic and inorganic substance on biochar surface (Rees et al., 2014). However, biochar addition did not result in decrease total concentration of heavy metal in soil, but biochar addition result in reduce the bioavailability of heavy metal in soil **(Figure 2.4)** (Park et al., 2011; Paz-Ferreiro et al., 2014). The less bioavailability of heavy metal is mean that the heavy metal is less transport to living organism and surrounding environment.

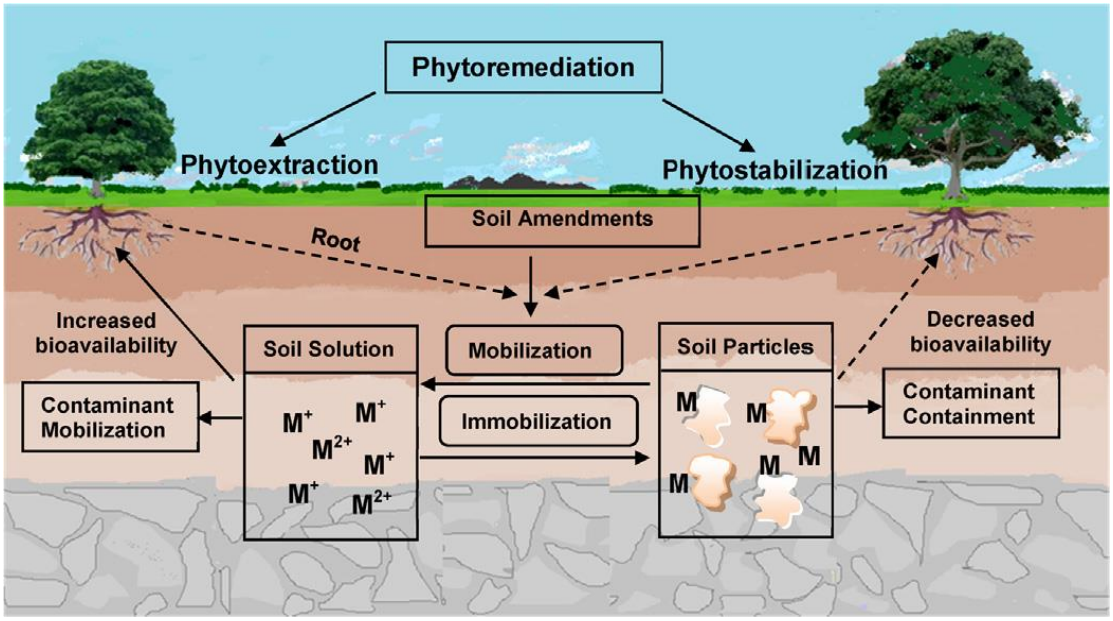


Figure 2.4 Schematic diagram illustrating the link between (im)mobilization, bioavailability and remediation of heavy metals (N. Bolan et al., 2014)

Applications of biochar amendment in soil have been attention in environmental aspects. The benefits of biochar on soil improvement are the cost efficiency method and it can be produced from a wide range of biomass sources. The addition of biochar in soil can improves soil physical property and increase soil nutrient for plant crop (Houben et al., 2013a; Park et al., 2011). Biochar has adsorption property for adsorb various heavy metals from aqueous solution, so the uses of biochar amendment, the biochar can significantly reduce the availability of heavy metal in the soil.

In general, most of biochars amendment are used for immobilize heavy metals in soil. The basic principle involved in the immobilization technique is to release the metals into soil solution and make heavy metals less bioavailability through adsorption, complexation, and precipitation reactions, thereby rendering the heavy metals unavailable for plant uptake and less leaching to groundwater. The biochar properties for addition in soil as heavy metals immobilization are various, it depend on the biochar sources and the pyrolysis condition. The post treatment of biochar is the one of key factors to increase biochar adsorption efficiency for specific the target heavy metals. Therefore, the enhancements of biochar quality are needed to study for specific adsorption mechanism for minimize the biochar portion.

The biochar is one of biomass products which is known as the environmental friendly material. The study of biochar as soil amendment for heavy metals immobility involves many factors such as plant species, soil characteristics and biochar types. Although it found the very low concentration of heavy metals in soil, but, plant has good efficiency for uptake these heavy metals from soil. Plant has potential to form complex chelating agent and transform heavy metals into soluble form and uptake these heavy metals through plant root. Liang et al. (2006) studied the comparative study of biochar and non-biochar containing soil. The presence of biochar in soil led to the higher of soil CEC value. The increase of soil CEC occurred from the result of the high oxidized surface of biochar. Therefore, biochar was more efficient in providing CEC and cation retention than non-biochar in the studied soils.

Uchimiya et al. (2011) produced biochar from cottonseed hulls at pyrolysis temperature of 350, 500, 650, and 800ºC for 4 h under nitrogen flow using a box furnace. The FTIR spectra show the functional groups of biochar surface were decreased when increased pyrolysis temperatures. The study of biochar for retention of Pb, Cu, Ni, and Cd in acidic sandy loam Norfolk Soil by additional 10% of biochar, it was found that electrostatic interactions between cation of metal species and negatively charged surfaces are expected to be significantly greater for the soil amended with biochar produced from 350ºC in comparison to high pyrolysis temperature (500, 650 and 800ºC). The result also suggested that the selection of biochar for soil amendment should be study case-by-case based on the biochar characteristics, soil property, and the target heavy metals. (Uchimiya, Lima, K.T., et al., 2010) prepared biochar from broiler litter manure by pyrolysis at temperature of 350 (350BL) and 700ºC (750BL) under nitrogen flow. The activation biochars (350ABL and 750ABL) were done by stream at 800ºC for 45 min. The stream activation made a result of increase biochar surface area at about 3-5 folds of non-activated biochar. The study of Cd immobilization by 10% addition of biochar in Cd contaminated soil indicates that 350BL and 350ABL have greater Cd immobilization values than 750BL and 750ABL, respectively.

Trakal et al. (2011) reported metals (Cd, Cu, Pb and Zn) sorption behavior after biochar application into a metals contaminated soil from the smelting area. A stem of willow tree was used as biochar source. The pyrolysis condition was set to the

increasing pyrolysis temperature rate of 10 ºC/min until 400ºC. The additional rates of biochar to soil were 1% and 2% (w/w). The results show that biochar application enhanced Cu and Pb sorption in all cases, whereas Cd and Zn sorption efficiency showed no significant changes. The biochar had more selective on Cu and Pb in soil over than Cd and Zn by competitive effects of metals. The result also indicated that the concentration of biochar addition in soil was too low for a negligible effect on metal sorption. Similar to the study of Rees et al. (2014), they reported Short-term effects of woody biochar on soil heavy metals mobility. The biochar was prepared by pyrolysis at the temperature of 450ºC for 36 hr. The contaminated soil was collected near Zinc smelter with record of high concentration of Zn, Cd and Pb. The kinetic result shows the indicated effective metals sorption by biochar affinity of Pb>Cu>Cd>Zn>Ni. The metals adsorption process in soil involved multiple and element-dependent mechanisms. The mechanisms of competition occurred when metals from soil solution contact with biochar surface. Metals sorption may be controlled through the precipitation or co-precipitation with carbonate, phosphate or silicate or by their sorption on newly complex substances formed.

Park et al. (2011) reported that biochar reduces the bioavailability and phytotoxicity of heavy metals. Biochar was prepared from chicken manure and green waste. The results on chicken manure biochar used as soil amendment as promoted plant growth show the increase of plant biomass by 353 and 572% for shoot and root, respectively. The studied of metals immobilization and phytoavailability of Cd, Cu and Pb were examined using naturally contaminated shooting range and spiked soils. The results showed the ability of biochar on reducing NH_4NO_3 extractable Cd, Cu and Pb. The biochar significantly reduced Cd, Cu and Pb accumulation by Indian mustard (*Brassica juncea* L.), and the reduction increased with increasing amount of biochar from 1, 5 and 15% application rates except Cu concentration that show reversed on increasing NH_4NO_3 extractable.

Houben et al. (2013a) studied the beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn by growing rapeseed (*Brassica napus* L.), a bioenergy plant. The contaminated soil was obtained from Cd, Zn and Pb contaminated soil from lead smelters. The commercial biochar was pyrolysis at 600ºC for 30 min and amended with soil for pot experiment. Bioavailable

metal concentrations by $CaCl_2$ extraction decreased with increasing concentrations of biochar amendment. The application of 10% biochar gave the reduction of metal bioavailability reached to 71, 87 and 92% for Cd, Zn and Pb, respectively. Furthermore, the crop biomass production was increased tripled as a result of the soil fertility improvement.

Suppadit et al. (2012) reported the effects of quail litter biochar on the availability of Cd to physic nut (*Jatropha curcas* L.) plants. The apply rate of biochar are four levels, 0, 5, 10, and 15 g/kg soil which contain Cd level at 60.8 mg/kg. The results showed that the soil amended with biochar caused a significant increase in the plant growth and physic nut yield. The biochar amended soil significant decrease in the Cd accumulation in the plant part and significant increase in the soil fertilities. The biochar amended soil also decrease Cd bioavailability to the plants. However, the biochar apply level above 15 g/kg were not advisable because biochar could shift soil pH to exceed the requirements of physic nut production (6.5-7.5) and gave adverse affected to the physic nut.

The application of biochar in soil generally decreases the soil metal bioavailability and decrease metal uptake by plant grow in biochar amended soil. But there are few reports showed the effect of biochar promote plants uptake heavy metal even though the soil metal bioavailability was decreases from biochar amended soil effects. The increases of metals uptake by using biochar was depend on heterogeneity in the response of soil properties and plant types (Paz-Ferreiro et al., 2014). (Fellet et al., 2014) reported the effects of different types of biochar produced from three different feedstocks, pruning residues, fir tree pellets and manure pellets, on changing the substrate conditions to promote plant growth for the phytostabilization of mine tailings. The biochars were applied at three doses, including 0, 1.5 and 3% by dry weight. The biochars induced significant changes of the study soil in terms of pH, EC, CEC and bioavailability of the metals. The biochar from manure pellets and pruning residues reduced shoot Cd and Pb accumulations. In contradictory, fir tree pellets increase Cd accumulation in plant. The results suggest that, biochar has great potential as an amendment for phytoremediation but its effects depend on the type of parent biomass for producing biochar. Therefore, the examination of biochar efficiency on

the mobility/stabilization of heavy metals in real contaminated soils should be done before applying biochar into soil (N. Bolan et al., 2014).

CHAPTER 3
RESEARCH METHODOLOGY

The research methodology was divided into two parts including; (i) efficiency of biochars for Cd removal from aqueous solution and (ii) application of biochar as a soil amendment to enhance phytostabilization of Cd and Zn in *V. radiata* L.: by green-house study.

3.1 Part one: Efficiency of biochars on Cd removal from aqueous solution

3.1.1 Preparation of biochars

In this research, there are two types of biochar, namely cassava stem and rice husk biochar produced from agricultural waste and agricultural by-product, respectively. Raw cassava stem and raw rice husk were collected from crop field and rice mill, oven-dried overnight at 105ºC. Carbonization process was done in the absence of air in laboratory furnace. Pyrolysis temperature was set at the increasing heat rate of 15ºC per min until reaching desired setting temperatures of 300, 400 and 500ºC. The carbonization process was maintained for 60 min (Kwapinski et al., 2010) and made cool in furnace to reach the room temperature. The biochar from each pyrolysis temperature was divided into two types; including (i) non-activated biochar and (ii) activated biochar.

The non-activated biochars, cassava stem (C300, C400 and C500) and rice husk biochar (RH300, RH400 and RH500) were prepared by suspending biochar in deionized water at pH 6, stirred with orbital shaker at 120 rpm for 2 h, filtered and oven-dried overnight at 105ºC. The activated biochars, cassava stem biochars (CA300, CA400 and CA500) and rice husk biochar (RHA300, RHA400 and RHA500) were activated by physico-chemical method. The biochars were pre-activated with 1.63 M KOH solution (Molar of deionized water). The ratio of biochar and 1.63 M KOH was 1 g of biochar to 100 mL of KOH solution according to the optimum conditions reported by Azargohar and Dalai (2008). The biochars were suspended in KOH solution and stirred with orbital shaker at 120 rpm for 2 h, filtered and dried overnight at 105ºC and made second pyrolysis as same as the first pyrolysis

condition. Activated and non-activated biochars were wash with deionized water at pH 6, grounded and sieved to size in the range of 250–500 µm for further use in the experiments.

The biochar yield was influenced by difference pyrolysis temperature and its yield in each preparation at pyrolysis temperature, 300, 400 and 500ºC were studied by dry basis analysis. The dry weight of biochar was determined in each pyrolysis condition compared to dry weight of precursor biomass. The biochar yield was calculated by the dry weight change before and after carbonization as given in equation (1) (Song and Guo, 2012).

$$\% \text{ Yield} = \frac{\text{Weight of final biochar}}{\text{Weight of parent biomass}} \times 100 \qquad \ldots\ldots\ldots\ldots (1)$$

3.1.2 Adsorption study on Cd (II) ion removal efficiency

The series of adsorption experiments were conducted to determine the effect of the pyrolysis temperatures for Cd removal from aqueous solution. The experiments were conducted in the laboratory at room temperature of 25 ± 1ºC. The equilibrium batch static method was used to study the removal efficiency of biochar by removing Cd ion from cadmium nitrate ($Cd(NO_3)_2$) solution. An initial concentration of 10 mg/L Cd $(NO_3)_2$ solution was used as a Cd ion source. For each batch experimental run, 0.10 g of biochar was weighed and packed in a PE filter bag before placing in each 250 mL-polyethylene (PE) bottle, 100 mL of $Cd(NO_3)_2$ was transferred to each PE bottle. An initial pH of $Cd(NO_3)_2$ solution was adjusted to pH 6 with 0.1 M of HCl or 0.1 M of NaOH. Then, all bottles were shaken with orbital shaker at a constant shaking speed of 120 rpm at 30, 60, 90, 180, 300 min and 24 h. At the end of desired time, the PE filter bags were removed. The remaining solution were acidified by conc. HNO_3 to pH less than 2.0 and an amount of Cd (II) ion in solution were analyzed using Flame Atomic Adsorption Spectrophotometer at the wavelength of 326.1 nm (APHA, AWWA and WEF, 2005). The amount of Cd (II) ion adsorbed by biochar was calculated indirectly from the remaining Cd (II) ion concentration in solution. Cd-adsorbed PE filter bags were studied as a control batch to deduct from total adsorption on biochars. The experiment was run with triplicate for each batch. The

removal efficiency of Cd (II) ion [% Cd (II) ion removal] and adsorption capacity (Q_e) were calculated according to the equation (2) and (3), respectively (Tangjuank et al., 2009).

$$\% \text{ Cd (II) ion removal} = \frac{Ci - Ce}{Ci} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

$$Q_e = \frac{(Ci - Ce)}{W} \times V \quad \ldots\ldots\ldots\ldots (3)$$

Where C_i and C_e are the initial metal concentration (mg/L) and the metal concentration at the experimental time (mg/L), respectively, *V* is the volume of solution (L) and *W* is dry weight of biochar (mg).

The activated biochar with the highest Cd (II) ion removal efficiency and non-activated biochar maintained under the same pyrolysis temperature were selected to study the effects of pH, sorption characteristic by isotherm model, adsorbent characterization and adsorbent characterization.

3.1.3 Initial pH on Cd (II) ion removal study

To study the initial effect of pH on Cd (II) ion removal efficiency, the selected biochars from 3.1.2 were studied by the batch equilibrium method. A 10 mg/L of $Cd(NO_3)_2$ solution was adjusted to the pH in the range of 2 to 9 by 0.1 M HCl or 0.1 M NaOH. The biochar was weighed at an amount 0.10 g in PE bag, and 100 mL of $Cd(NO_3)_2$ was transferred to each PE bottle. The batches were stirred at 120 rpm for 60 min of contact time. The condition for analysis of Cd (II) ion remaining in solution was similar to adsorption study in 3.1.2. The results were interpreted for Cd (II) ion removal efficiency.

3.1.4 Isotherm study of selected biochars

In order to investigate adsorption characteristics, batch static experiments were carried out at pH 6. The selected biochars from 3.1.2 were accurately weighed (0.10 g) and filled in PE bag, and 100 mL of $Cd(NO_3)_2$ was transferred to each PE bottle. The initial concentration of $Cd(NO_3)_2$ were varied into a range of 10-50 mg/L. The batches were stirred at 120 rpm until 60 min of contact time. The condition for analysis of Cd remaining in solution was similar to the adsorption study in 3.1.2. The Cd (II) ion sorption capacities were calculated in each final concentration. Adsorption data were interpreted by Langmuir (4) and Freundlich (6) adsorption equations (Kołodynska et al., 2012). The Langmuir isotherm is valid for sorption of metals on specific homogeneous binding site within adsorbent while Freundlich isotherm complies to heterogeneous adsorbent surface area with non-uniform distribution (Gulipalli et al., 2011).

Langmuir equation: $q_{eL} = \frac{q_{max} K_L C_e}{1 + K_L C_e}$ ..... (4)

Where q_{eL} is the Langmuir adsorption capacity at equilibrium (mg/g), C_e is the equilibrium concentration of the metal ion (mg/L), when Ce/Q_e was plotted against Ce, q_{max} is the maximum sorption capacity which was obtained from 1/slope. K_L, the Langmuir adsorption constant, was obtained from equation (5).

$$K_L = \frac{1}{(qmax)(y - intercept)} \quad \text{..... (5)}$$

Freundlich equation: $q_{eF} = K_F C_e^{1/n}$ ..... (6)

Where q_{eF} is the Freundlich adsorption capacity of Cd ion adsorbed (mg/g), C_e is the equilibrium concentration (mg/L), when $\log Q_e$ was plotted against $\log C_e$, n is the Freundlich constant relate to the surface heterogeneity of adsorbing sites was obtained from 1/slope. K_F, Freundlich constant adsorption capacity, was obtained from equation (7).

$$y\text{-}Intercept = \log K_F \quad \text{..... (7)}$$

3.1.5 Adsorbent characterization studies

The physical properties of the biochar relating to specific surface area and total pore volume were obtained by Brunauer–Emmett–Teller (BET) Surface Area and Porosity Analyzer (Micromeritics Asap2020). Biochar surface characteristics of chemical functional groups were determined by FT-IR spectrophotometer (Perkin Elmer Spectrum One). The addition of elements on biochar surface including carbon (C), magnesium (Mg), phosphorous (P), calcium (Ca), potassium (K) and silica (Si) were randomly detected by Scanning Electron Microscope with Energy Dispersive X-ray spectroscopy (SEM/EDX; JSM-6400) which provides a comprehensive picture of the organic and inorganic chemistry on a biochar (Chia et al., 2012). All experimental data were done in three replications.

3.2 Part two: Application of biochar for enhancing cadmium and zinc phytostabilization in *Vigna radiata* L. cultivation : A green-house study

3.2.1 Contaminated soil collection and preparation

The contaminated soil was collected from an agricultural area at Pha Tae village, Mae Sot district, Tak province, Thailand. The sampling site location was N16.673861, E98.626007. Soil was collected at the topsoil layer, at a depth of 0-20 cm. Soil was homogenized, air-dried, ground and passed through a 2-mm sieve.

3.2.2 Soil characteristics analysis

The physical and chemical properties of contaminated soil were collected and analyzed according to Soil Sampling and Methods of Analysis (Carter and Gregorich, 2006) and followed to A handbook of Soil analysis , chemical and physical methods, adopted by Department of Agriculture (DOA, 2010) (**Table 3.1**). Cd and Zn concentrations were also measured in total metal (acid-digested form) and bioavailable forms. To determine total metal concentration, soil samples were acid-digested, using an open tube digestion method, according to standard US.EPA method 3050B (US-EPA, 1996) and analyzed with a flame atomic adsorption

spectrophotometer at the wavelength 326.1 nm (FAAS, Perkin Elmer Analyst 800). The concentrations of bioavailable Cd and Zn forms were extracted from the soil by DTPA, using a soil:extractant ratio of 1:2 (Quevauviller et al., 1998) and analyzed with FAAS.

Table 3.1 Parameters for physical and chemical analysis of Cd contaminated soil

Parameter	Methods	Rfferences
- Soil texture	Hydrometer	(DOA, 2010)
- pH	pH meter (soil : water ; 1 : 2)	(DOA, 2010)
- Cation exchange capacity (CEC)	Ammonium acetate (NH_4OAc)	(DOA, 2010)
- Organic matter	Walkley and Black rapid titration	(DOA, 2010)
- Total nitrogen	Kjeldahl digestion	(USEPA, 1993)
- Available phosphorus	Bray II (NH_4F 0.03N+HCl 0.10N)	(DOA, 2010)
- Extractable potassium	Ammonium acetate (NH_4OAc); Atomic Absorption Spectrophotometer	(DOA, 2010)
- Extractable calcium	Ammonium acetate (NH_4OAc); Atomic Absorption Spectrophotometer	(DOA, 2010)
- Extractable magnesium	Ammonium acetate (NH_4OAc); Atomic Absorption Spectrophotometer	(DOA, 2010)

3.2.3 Biochar preparation and its adsorption efficiency

In the pot experimental study, biochar was produced from biochar kiln made with metal. The kiln size was 90 x 130 cm (diameter x long). For prevent heat loss, kiln wall was coated with cement to prevent heat loss through the wall. Cassava stem, a biochar parent material, was collected from cassava field after harvesting and air-dried for one month before chopping to approximately 30 cm-in-length. The pyrolysis

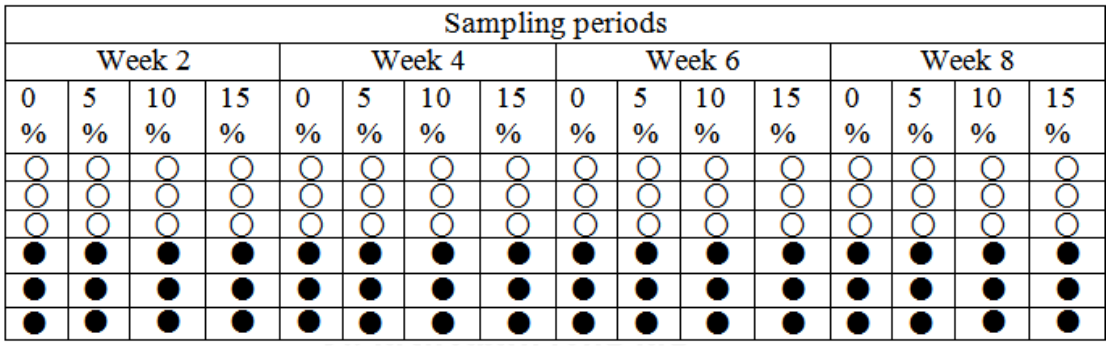
kiln temperature was maintained by cassava stem biomass of self-heating properties about 350^{o}C. Carbonization time was taken about 120 min and left cooling overnight in the stove. For the first step of biochar pyrolysis, out sources heating was used to ignite biomass and after biomass was partial fired, slow down combustion with reducing air supply to kiln. The biochar production stages can be categorized by the smoke emission from smoke stack, drying (white smoke), pyrolysis (yellow smoke) and process complete (blue or clear smoke) (Lehmann and Joseph, 2009). After 120 min, the smoke was clear which show process complete, turn off air inlet both front inlet and smoke stack and leaved it cool down for overnight. The biochar properties of C, H , N, O were analyzed with CHNO analyzer and CEC value by ammonium acetate (NH_4OAc) method.

The benefit of biochar kiln is less energy consumption and can be performed by local community. The initial phase of the pyrolysis process is typically called “endothermic” meaning it requires more energy than it produces. After initial phase, pyrolysis is “exothermic” meaning that it produces more heat than it required to originate the 10% of the final energy produced making it a rather efficient process (Gaunt, 2014). To ensure the biochar efficiency on Cd removal, the Cd (II) ion removal efficiency in aqueous solution of selected biochar produced from biochar kiln was determined in laboratory for Cd (II) ion adsorption capacity from aqueous solution before applied in soil for pot study. Langmuir adsorption isotherm was selected to calculate Cd (II) ion adsorption capacity of biochar in aqueous solution (Kołodynska et al., 2012; Tangjuank et al., 2009; Trakal et al., 2014). To investigate Cd (II) ion adsorption efficiency of biochar, a batch adsorption experiment was performed with adsorption data calculated using an isotherm model. The batch static experiment was conducted in an aqueous solution of Cd $(NO_3)_2$ according to the study in Research Methodology 3.1.4.

3.2.4 Green-house pot trail experiment

To study the effects of biochar on plant growth and metals uptake, a pot experiment was conducted in a green-house. The local green bean (*V. radiata* L.) strain Kamphaeng Saen 1 (KS1) was obtained from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The contaminated soil was mixed

with biochar at three concentrations, including 5, 10 and 15% (w/w) in addition to a control, totally four treatments. Biochar-amended soil was filled into 16 L plastic pots (19 cm in top diameter, 15 cm in bottom diameter and 30 cm in height). Each pot was filled with biochar-amended soil to 90% pot capacity. The testing was carried out according to the OECD Guidelines No.208, OECD Guideline for the testing of chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD, 2006). Each pot was randomly placed in the green-house **(Figure 3.1)** for 2 weeks with daily watering at field capacity before planting. Four green bean seeds were buried in soil at 1 cm depth. Chemical granular fertilizer (NPK: 15-15-15) was applied every pot at week 4 and week 6, or the beginning of the blossom and seeding stages, respectively (Garcia and John, 1976). The fertilizer application rate was 1 g per pot each time.



O= treatment of soil incubation study, ● = treatment of plant study

Figure 3.1 Layout of treatment designed for pot study

3.2.5 Determination of plant growth and soil analysis

Plant samples in each treatment were collected every 2 weeks for 8 weeks, according to the plant growth stages: beginning of growth (week 2), blossom (week 4), seed filling (week 6) and production (week 8). Harvested plants were thoroughly washed with tap water before rinsing with deionized water. For plant growth determination, root maximum length and plant height. Root maximum length was measured from the crown of end of root to the tip of the root and plant height was

measured from the root tip to the longest leaf tip (Shashidhar et al., 2012). Each plant was separated into shoot (leaf and stem) and root and then weighed. Plants were then oven-dried to measured dry weight. Plants' Cd and Zn concentrations were extracted using Acid digestion of sediments, sludge, and soils, method 3050B (US-EPA, 1996) and analyzed with FAAS. At week 8 (seeding stage), the number of bean pods per pot was recorded to determine crop yield. Soil samples were collected every 2 weeks, and soil pH, total concentration and bioavailable forms of Cd and Zn were analyzed. In addition, other soil chemical properties, including CEC, %OM, %N, available P, extractable K, extractable Ca, extractable Mg, and C/N ratio after 8 weeks of plantation were recorded.

To evaluate plants' metals accumulation, the biological concentration factor (BCF) and translocation factor (TF) were calculated. BCF is the metals concentration ratio of metals in plant root to metal concentration in soil as given in equation (8), and TF is described as the ratio of metals concentration in plant shoot to metals concentration in plant root as given in equation (9) (Bech et al., 2012; Malik et al., 2010; Yoon et al., 2006).

$$\text{BCF} = \frac{\text{Metals in } root}{\text{Metals in } soil} \qquad \ldots\ldots\ldots\ldots(8)$$

$$\text{TF} = \frac{\text{Metals in } shoot}{\text{Metals in } root} \qquad \ldots\ldots\ldots\ldots(9)$$

3.2.6 Statistical analysis

Each experiment was performed in triplicate. The mean ($\bar{x}$) and standard deviation (SD) of the plant growth, cadmium concentrations in plants and soil were determined. Data of the plant growth, cadmium contents in plants and soil from different treatments were statistically analyzed using one-way analysis of variance (ANOVA) followed by the Duncan multiple range test at $p < 0.05$. A linear regression analysis was used to find relationship of two affected factors (x, y).

CHAPTER 4
RESULTS AND DISCUSSION OF PART ONE

Efficiency of biochars on Cd removal from aqueous solution

Two types of biochars, including cassava stem and rice husk biochars were prepared using various pyrolysis temperatures. Both biochars were activated by physico-chemical method. The efficiency of non-activated and activated biochars on Cd removal from aqueous solution was investigated.

4.1 Activation of cassava stem biochar by physico-chemical method for stimulating cadmium removal efficiency from aqueous solution

4.1.1 Effect of pyrolysis temperature on cassava stem biochar yields

Pyrolysis process converted raw cassava stem to biochar and made change in weight of about 30-35 % reduction. **Figure 4.1** shows the effect of pyrolysis temperature on biochar yields (% dry weight). The increases of temperature from 300 to 500ºC decreased biochar yields by average in both non-activated biochar and activated biochar. However, the biochar yields after activation was not significantly different from the yields of non-activated biochar ($p<0.05$). Moreover, the biochar yield were not significantly different between three pyrolysis temperatures ($p>0.05$). Linear equations relating the percent of biochar yields and pyrolysis temperature of non-activated biochar and activated biochar were (□) $y = -0.0218x + 41.373$ and (▲) $y = -0.0383x + 45.697$, respectively. From these equations, activated biochar equation shows greater slope than non-activated biochar which reflected to higher decrease on biochar yields at high pyrolysis temperature. These results indicated that carbonization at low temperature produced high biochar yield similar to other studies (Demirbas, 2004; Hassan and Aarts, 2011; Song and Guo, 2012). The biochar yields decrease at high temperature. It was due to both high decomposition of organic content of biomaterials at high temperature and the decomposition of biochar residue to ash content (Sensoz and Angin, 2008). Moreover, high temperature of pyrolysis

could make more dehydration of hydroxyl groups and thermal degradation of ligno-cellulose structures of biochar products causes the decreasing of biochar yields (Novak et al., 2009).

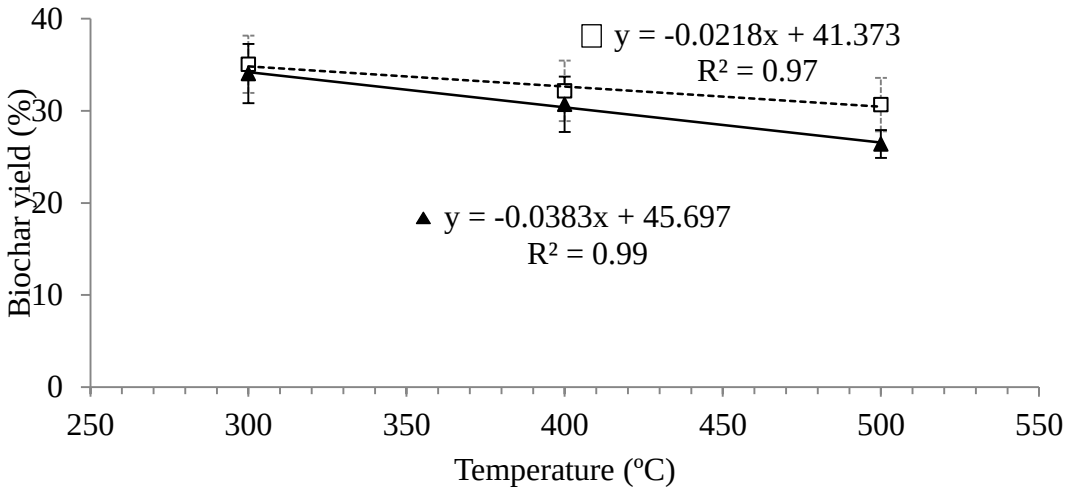


Figure 4.1 Effects of pyrolysis temperatures on yields of non-activated (dot line) and activated cassava stem biochars (solid line)

4.1.2 Cadmium (II) ion removal efficiency

The physical and chemical properties of biochar vary significantly depending on the biomaterial sources, pyrolysis conditions, post and pre-treatments (Song and Guo, 2012). Adsorption efficiency of non-activated biochars (C300, C400 and C500) and activated biochars (CA300, CA400 and CA500) were compared for Cd (II) ion removal efficiency by batch equilibrium method. The results indicated that biochar produced from all pyrolysis temperatures can act as effective sorbent with different sorption efficiencies **(Figure 4.2)**. The results of Cd (II) ion removal efficiency were clearly presented into two data groups; i) low removal efficiency of non-activated biochar and ii) high removal efficiency of activated biochar. The biochar produced from pyrolysis at temperature 300ºC (C300 and CA300) gave the highest Cd (II) ion removal efficiency of each biochar. The high adsorption efficiency of biochar produced at low pyrolysis temperature (300ºC) probably occurred from the presence of complex chemistry on biochar surface. Low pyrolysis temperature could preserve

organic carbon content and aromatic carbon on biochar surface which was important to surface composition of metal adsorption, while the high pyrolysis temperature could leach surface chemistry such as aromatic carbon composition. (Regmi et al., 2012; Song and Guo, 2012)

In addition, the rapid removal rates of Cd (II) ion from aqueous solution were found in all adsorbents within 60 min of contact time **(Figure 4.2)**. These results obviously showed that the large number of free surface sites was available during initial stage of time. The adsorption of Cd (II) ion remains almost constant after 300 min up to 24 h and occupied binding sites on biochar surface. The Cd (II) ion removal efficiency of CA300 was increased up to 1.5 times after activation process compared to non-activated (C300) (increased from 59% to 84%). Therefore, C300 and CA300 were selected to study the adsorption characteristics and biochar properties.

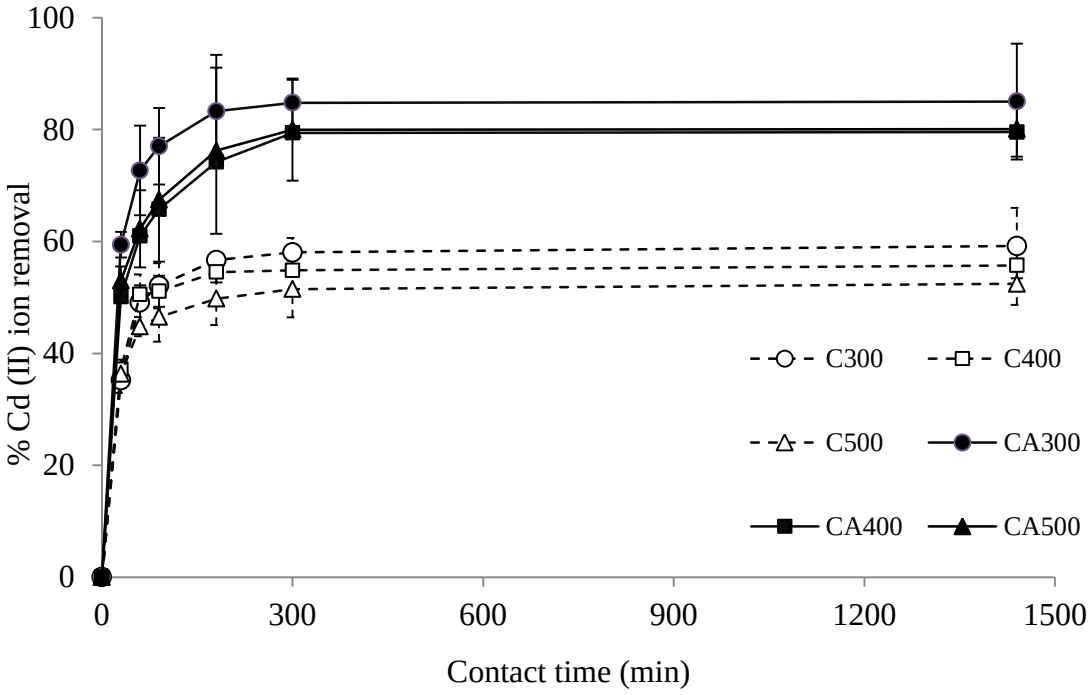


Figure 4.2 Comparison of the Cd (II) ion removal efficiency of non-activated (C) and activated cassava stem biochars (CA) treated at 300, 400 and 500ºC

4.1.3 Effect of pH on Cd (II) ion removal efficiency by activated cassava stem biochar

The pH in aqueous solution affects the sorption process via changing in surface charges and ionic strength in aqueous medium. The results indicated that trend of removal efficiency between C300 and CA300 were similar **(Figure 4.3)**. At pH 2.0, Cd (II) ion adsorption efficiency was very low due to the high potential of H^+ to compete with the metal cations for the adsorption sites on the sorbent surface, meanwhile, the heavy metal cations are released under acidic conditions (Forstner and Wittmann, 1981). The highest percentages of Cd (II) ion removal by both non-activated and activated biochars were found at pH 5.0 to 6.0 and then the percentages of Cd (II) ion removal slightly decreased from pH 7.0 to 9.0. Hence, the activated biochar by alkaline pre-activated could not change on the pH of biochar. KOH might leach out by second pyrolysis. This study gave similar results to the others study of Regmi et al. (2012) and Kołodynska et al. (2012), which indicated that pH 5-6 are the best working solution for Cd (II) ion removal from aqueous solution.

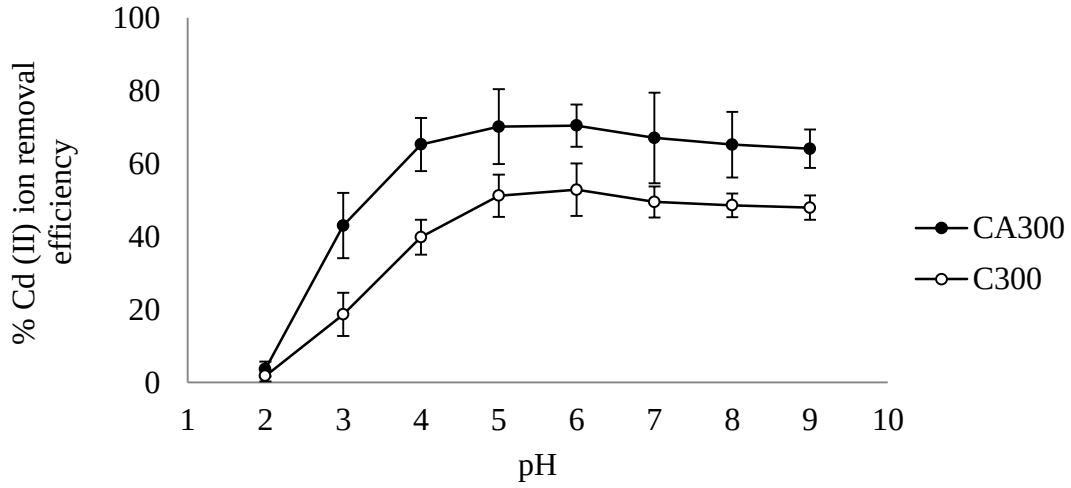
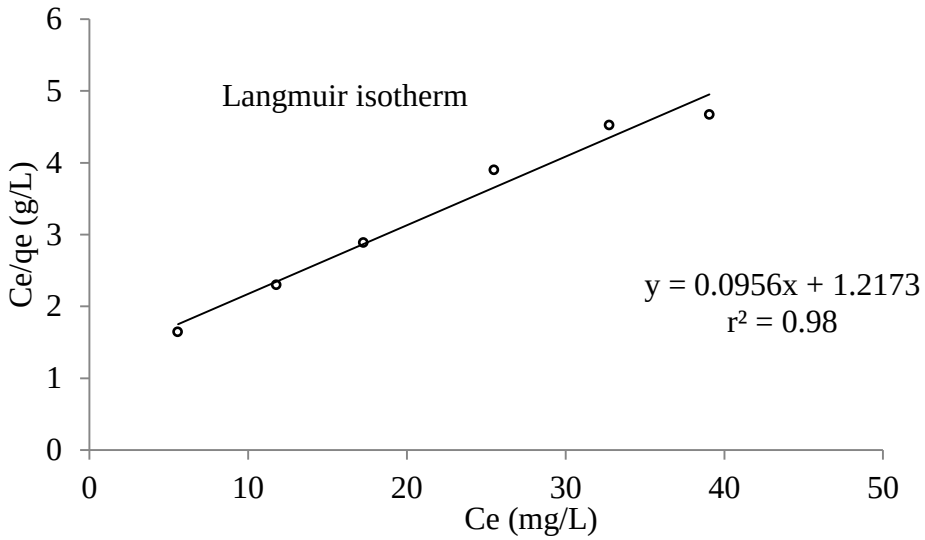


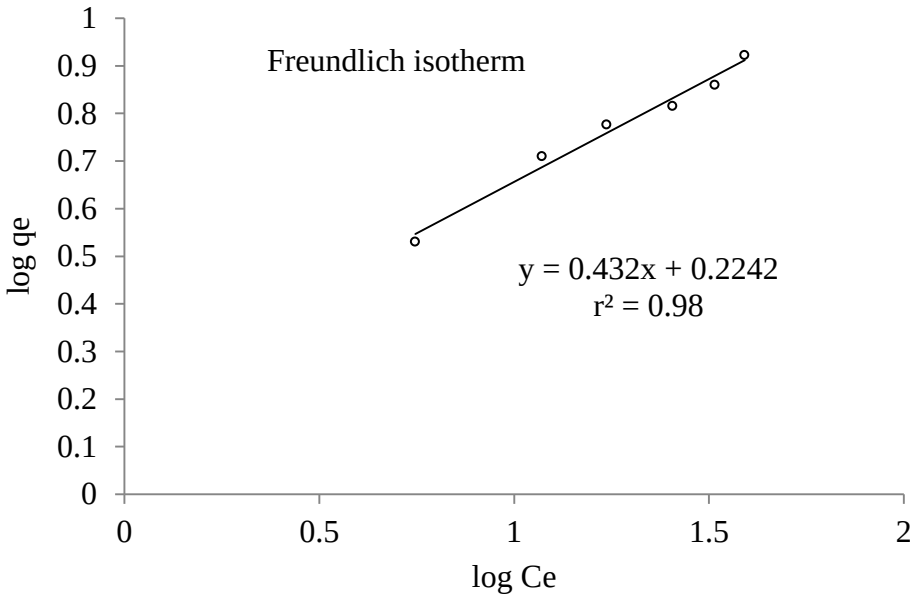
Figure 4.3 Effects of pH on Cd (II) ion removal efficiency by non-activated (C300) and activated (CA300) cassava stem biochars

4.1.4 Adsorption isotherms

Adsorption isotherm was studied for understanding sorption efficiency and sorption characteristics of Cd (II) ion on biochar surface. The results showed that, at equilibrium conditions, the Cd (II) ion removal rate increased with increasing the initial concentration of $Cd(NO_3)_2$. **Figure 4.4** and **Figure 4.5** show the linear isotherm plot of non-activated and activated biochar (C300 and CA300). To determine adsorption characteristic, on the basis of coefficient of correlation (r^2), r^2 was determined infinitively between biosorbent and metal ion. For non-activated biochar, the linear plot of Langmuir and Freundlich isotherm show r^2 equal to 0.98. It means that the sorption characteristics of non-activated biochar were fitted to both isotherm models at equilibrium concentration. The sorption mechanism of Cd (II) ion on biochar surface was not only strictly to mono layer with limited binding site adsorption, but also have some complex mechanism with physical interactions on biochar surface and lead to involved multilayer adsorption (Liu et al., 2012). For the isotherm of biochar after activation, the r^2 of isotherm plots were higher than 0.95 both from Langmuir and Freundlich, but Langmuir r^2 fitted better than the Freundlich isotherm at r^2 value of 0.98. It probably means that adsorption characteristics of Cd (II) ion on biochar surface after activation tended to form monolayer adsorption with chemical adsorption. However, adsorption characteristics of multilayer can be occurred on a heterogeneous surface by others physical forces. As similar to the study of Kołodynska et al. (2012), studied of Cd (II) ion removal by manure biochar which presented r^2 values above 0.9 both Langmuir and Freundlich isotherm.



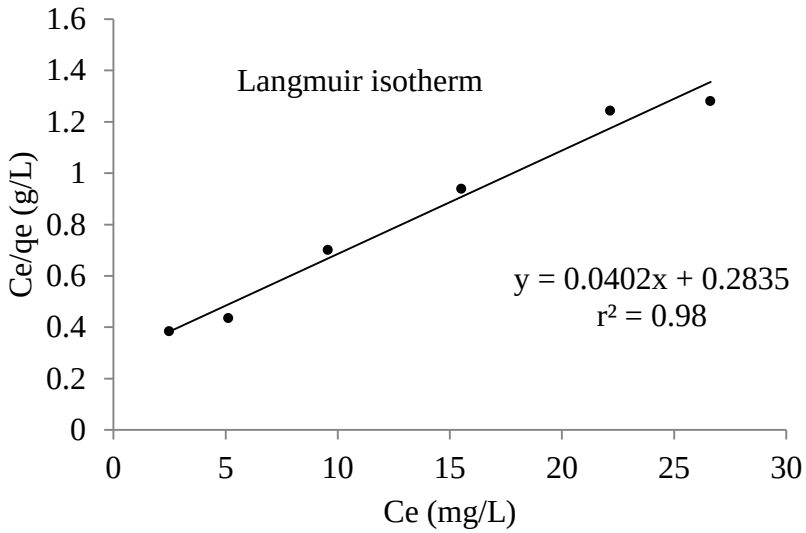
(A)



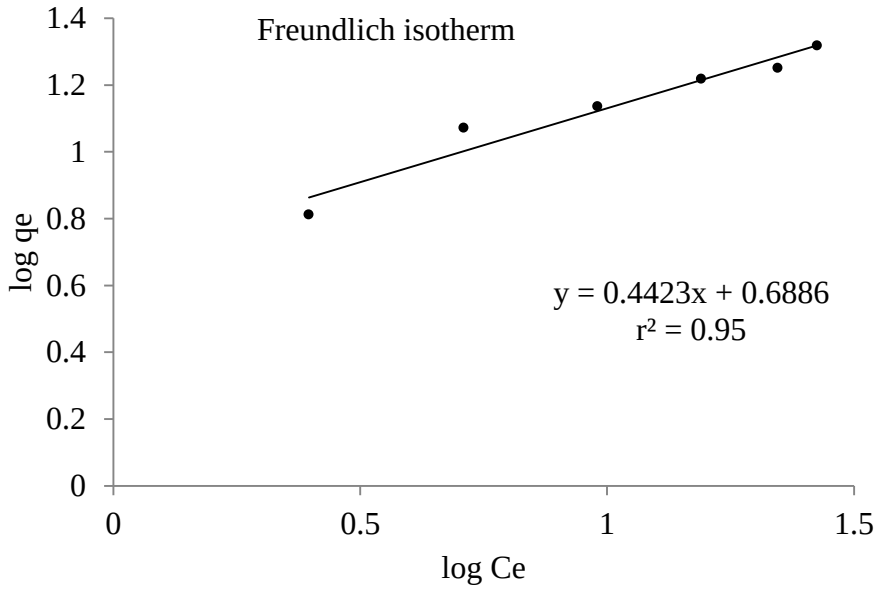
(B)

Figure 4.4 Adsorption isotherms of Cd (II) ion on non-activated cassava stem biochar (C300)

(A) Langmuir isotherm (B) Freundlich isotherm



(A)



(B)

Figure 4.5 Adsorption isotherms of Cd (II) ion on activated cassava stem biochar (CA300)

(A) Langmuir isotherm (B) Freundlich isotherm

Adsorption isotherm parameters are shown in **Table 4.1**. Langmuir equation, the value of q_{max} and K_L were determined from the slope and intercepts of the straight-line plots by equation (4) and (5). By comparisons of sorption capacity from the values of slope, non-activated biochar showed greater slope than activated biochar at about 2.4 times which corresponded to maximum adsorption capacity of activated biochar from calculation. Hence, maximum adsorption capacity of non-activated biochar and activated biochar illustrated from Langmuir equation were 10.46 and 24.88 mg/g, respectively. For Freundlich constant, the values of $0.1<1/n<1$ showed favorable adsorption of Cd (II) ions onto adsorbents. The greater of sorption for activated biochar was approximately three times higher than that of non-activated biochar as inferred from the values of K_F, 4.88 and 1.67, respectively. From the presented factors, it can be concluded that the sorption of Cd (II) ion on surface of activated biochar is known to be a homogenous surface by monolayer adsorption which had some interaction of multilayer adsorption by complexities of biomaterials surface.

Table 4.1 Isotherm parameters of Cd (II) ion adsorption by non-activated (C300) and activated (CA300) cassava stem biochars

Biochar	Langmuir isotherm			Freundlich isotherm		
	K_L(L/mg)	q_{max} (mg/g)	r^2	K_F (L/g)	1/n	r^2
C300	0.078	10.46	0.98	1.67	0.43	0.98
CA300	0.014	24.88	0.98	4.88	0.44	0.95

4.1.5 Adsorbent characterization

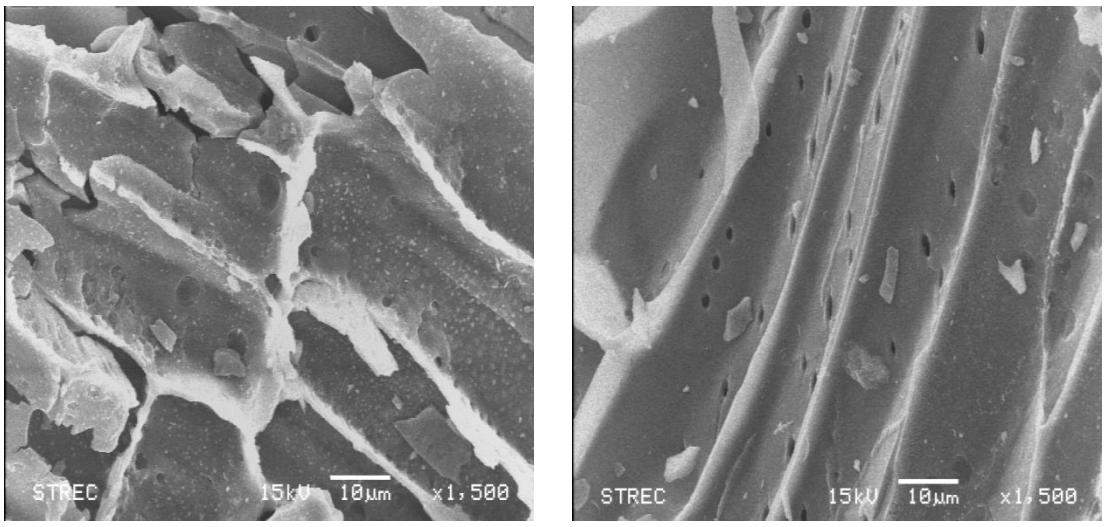
Biochar structure is not a homogeneous material, but it is carbon rich, porous, various functional group and relatively pollution-free material which can be used as a favorable non-biodegradable adsorbent for industrial wastewater treatment (Liu et al., 2012). Surface area, element composition and functional groups on surface area of non-activated (C300) and activated (CA300) biochars are presented as follows:

1) Surface area

The surface area of C300 and CA300 were analyzed by BET method. The results in **Table 4.2** showed that CA300 exhibited higher surface area than C300 at about 1.3 times (6.88 to 9.49 m^2/g), while CA300maintained pore volume and average pore size. It can be seen that the activation can increased surface area due to the KOH adsorbed onto biochar surface, burn out by second pyrolysis and leaved micro-pore on biochar surface (Azargohar and Dalai, 2008). **Figure 4.6** shows SEM images of non-activated and activated cassava stem biochars. Activation did not make change on the major structure morphology. Only micro-pores on activated biochar surface were observed. These micro-pores occurred from KOH burn out by second pyrolysis and resulted in the connection of meso-pores and made accessibility to the inner closed macro-pores to exhibit larger surface area.

Table 4.2 Physical characteristics of cassava stem biochar under non-activated and activated conditions

Biochar	BET(m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
C300	6.88	0.002373	1.379
CA300	9.49	0.002468	1.395



(a) (b)

Figure 4.6 SEM images of (a) non-activated and (b) activated cassava stem biochars

2) Element composition

Proximate analysis by random spot of SEM/EDX showed similar element compositions of biochar surface both non-activated and activated. The carbon and oxygen contents were not significance change, $p<0.05$ after activation **(Table 4.3)**. Hence, Carbon and oxygen were major elements for carbonyl/carboxyl group (C=O) which was in agreement with the FT-IR results **(Figure 4.7)**.

Table 4.3 Element compositions of non-activated (C300) and activated (CA300) cassava stem biochars

Biochar	C (%)	O (%)	Mg (%)	P (%)	Ca (%)	K (%)
C300	70.64±4.56	28.12±3.82	0.31±0.12	0.35±0.37	0.01±0.06	0.55±0.31
CA300	73.78±3.69	24.66±2.84	0.11±0.54	0.17±0.16	0.15±0.15	1.11±0.67

3) Functional groups on biochar surface

The present of functional groups involve interact of pollution by various of chemical force such as electrostatic interaction, ion-exchange, chelation/complexion, hydrogen bonding and weak force of van der Waal forces, are importance for bio-sorption process (Crini and Badot, 2009). The adsorption efficiency of biochar is mainly influence by chemical structure on surface of biochar. The low pyrolysis temperature could preserve surface functional group and lead to combined chemical and physical adsorption (Trakal et al., 2014). Biochar surface functional group was analyzed by FT-IR technique. **Figure 4.7** shows the functional groups on surface of non-activated (C300) and activated (CA300) biochars in range of spectra between 4000 cm^{-1} to 400 cm^{-1}. The peaks of functional groups of C300 and CA300 were quite similar but CA300 showed slightly larger area than that of C300. The larger functional groups are hydroxyl group, methylene group and aromatic C–H components. The band at about 3434 cm^{-1} showed vibration of hydroxyl group (OH) which is presented in all of the fiber sources and was mainly functional group of metal adsorption form aqueous solution (Haris et al., 2011; Rao et al., 2010). The bands locate between 2960-2924 cm^{-1} corresponded to vibration of methyl and methylene groups (C-H) (Tangjuank et al., 2009). The peak at 1696 cm^{-1} indicates aromatic carbonyl/carboxyl group (C=O) (Regmi et al., 2012; Yongbin et al., 2010) and the peak at 1595 cm^{-1} indicates the presence of aromatic rings vibration (C=C) (Regmi et al., 2012). Broad peaks between 1439 cm^{-1} to 1233 cm^{-1} present methylene group (CH_2) (Bardakci and Bahceli, 2010; Regmi et al., 2012). The peaks at 874–780 cm^{-1} and 572 cm^{-1} are assigned to the aromatic C–H components (Chen et al., 2011; Haberhauer et al., 1998).

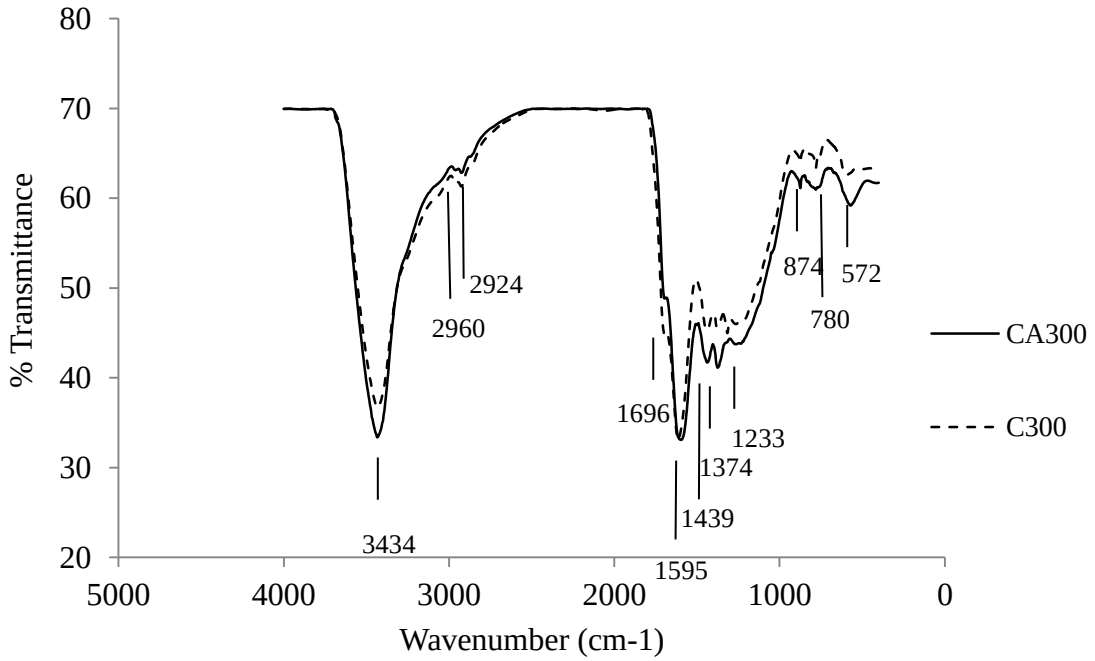


Figure 4.7 FT-IR spectra of non-activated biochar (C300) and activated cassava stem biochar (CA300)

This study had complied with the studies of Regmi et al. (2012) and Kumar et al. (2011). They confirmed the presence of several oxygen functional groups (carboxylic, hydroxyl/phenolic, carbonyl) on the surface of the biochars. Functional groups such as (C=O) demonstrated high coordination with heavy metals. These findings suggested that both non-activated and activated cassava stem biochar have similar functional groups which mostly remained intact during activation with KOH and the second pyrolysis at 300 ºC.

4.1.6 Conclusions of cassava stem biochar study

The pyrolysis temperatures were the major factor affected to adsorption efficiency of Cd (II) ion on biochar surface. Cassava stem biochar which was produced from the pyrolysis temperature at 300 ºC had higher Cd (II) ion removal efficiency than those pyrolysis temperatures at 400ºC and 500ºC. Activation of biochar with physico-chemical method could increase Cd (II) ion removal efficiency for all pyrolysis temperatures. The highest Cd (II) ion removal efficiencies were found at initial pH in range of 5-6 and tended to be decreased when pH solutions was decreased. The activation could increase the BET surface area as a result of increased Cd (II) ion removal efficiency from 59 to 84%. Nevertheless, FT-IR results showed the results of unchanged surface functional group by second pyrolysis. The adsorption isotherms of both non-activated and activated biochars were fitted well to Langmuir and Freundlich isotherms (r^2>0.95) and indicated that adsorption characteristics occurred with mono layer adsorption and have some complex of multilayer adsorption behavior. The major conclusion of this study is that activated cassava stem biochar have to be consideration as cost efficiency adsorbent for Cd (II) ion removal from wastewater.

4.2 Physico-chemical activation on rice husk biochar for enhancing of cadmium removal from aqueous solution

4.2.1 Effect of pyrolysis temperatures on rice husk biochar yield

The results found that the trend of the biochar yield was significantly decreased when pyrolysis temperatures increased. Figure 4.8 shows the effect of the different pyrolysis temperatures on biochar yields. A pyrolysis temperature at 300ºC gave the highest biochar yield in both non-activated and activated biochar. Biochar yields at a pyrolysis temperature of 300, 400 and 500ºC were significantly different ($p<0.05$). Biochar yields after activation was slightly decreased at all pyrolysis temperatures; however, they were not significantly different ($p>0.05$). It complies with other studies of biochar carbonization from biomass at difference pyrolysis temperature. Biochar yields are expected to increase when the pyrolysis temperature becomes lower (Demirbas, 2004; Hossain et al., 2011; Song and Guo, 2012; Sukiran et al., 2011). The decrease of biochar yields at higher temperature was probably caused by both high decomposition of organic content of biomaterials and the decomposition of the biochar residue to ash content (Sensoz and Angin, 2008). High temperature of pyrolysis could make more dehydration of hydroxyl groups and thermal degradation of ligno-cellulose structures of biochar products cause the decreasing of biochar yields (Novak et al., 2009).

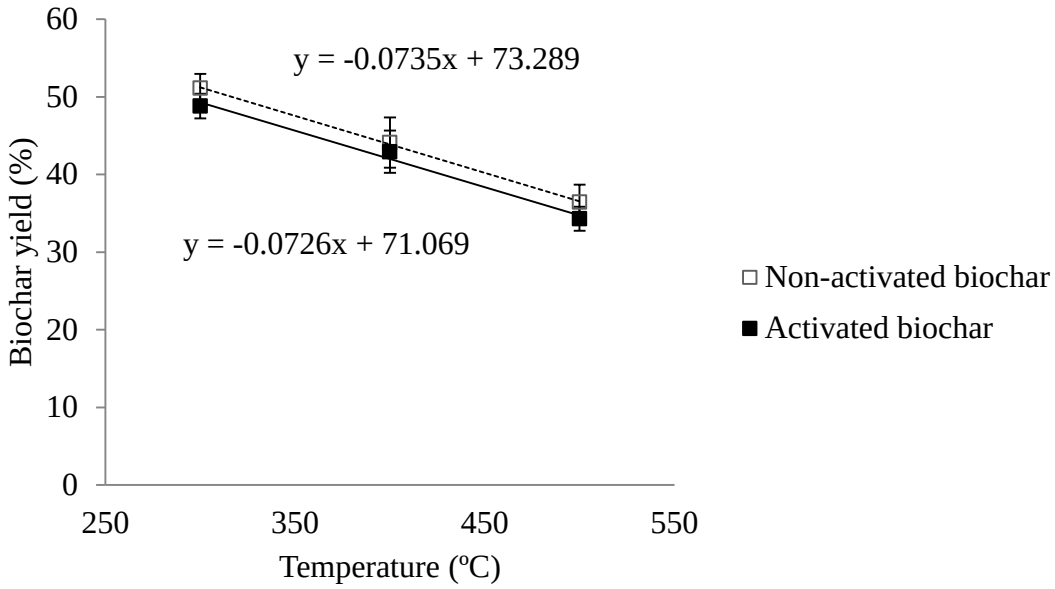


Figure 4.8 Rice husk biochar yields at different pyrolysis temperatures

4.2.2 Cadmium removal efficiency of rice husk biochars

The comparison between difference biochar treatments for Cd (II) ion removal efficiency at equilibrium time are shown in **Figure 4.9**. It is clear that all activated biochars (RHA300, RHA400 and RHA500) had much higher Cd (II) ion removal efficiency than those of non-activated biochars (RH300, RH400 and RH500). The Cd (II) ion removal efficiencies of all activated biochars were higher than those of non-activated biochar about 8-9 times at 180 min, hence, RHA300 gave the highest Cd (II) ion removal efficiency of 97%.

Time profiles of liquid phase concentration (C_t/C_0) for the adsorption of Cd (II) ion from $Cd(NO_3)_2$ solution within 180 min are shown in **Figure 4.10**. The results show the different adsorption ratio of all biochars. Activated biochars showed much higher adsorption rates than those of non-activated biochars. The adsorption rate of activated biochars, RHA300, RHA400 and RHA500, were divided into three stages of adsorption. The first stage occurred very fast at first 30 min since high concentration of Cd (II) ion and large free binding sites on biochars surface which is dominant for adsorption capacity; the second stage was slower adsorption rate due to binding site of surface area which occurred between 30 and 180 min, and the last third stage was very slow which occurred between 180 min and 24 hr as a result of occupied binding

site. For non-activated biochar, RH300, RH400 and RH500, the adsorption rates were much slower than activated biochar at same contact times. Hence, the biochar produced from pyrolysis temperature at 300ºC (RH300 and RHA300) were selected to study the pH influence, adsorption characteristic and composition analysis.

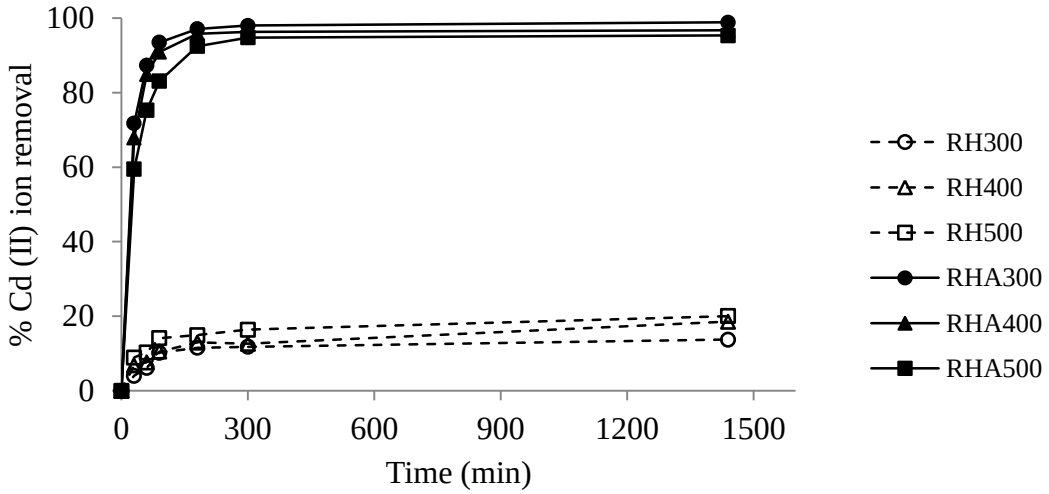


Figure 4.9 Effect of contact time on removal efficiency of non-activated and activated biochars treated at 300, 400 and 500 ºC

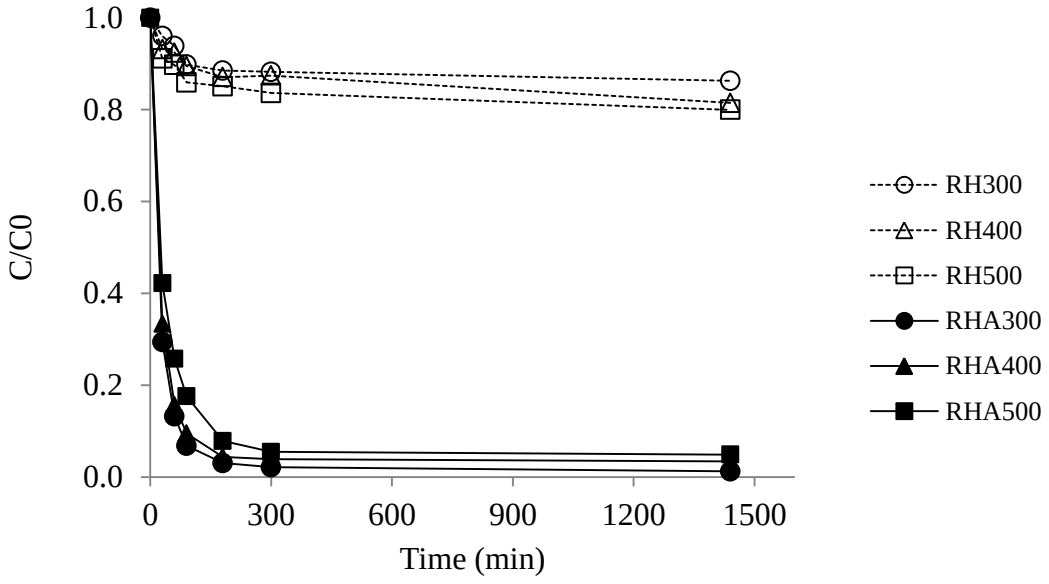


Figure 4.10 Time profiles of Cd ion in liquid phase concentration of non-activated (RH) and activated rice husk (RHA) biochars treated at 300, 400 and 500 ºC

4.2.3 Effect of pH on Cd removal efficiency

The pH is one of the most important factors influencing the metal sorption onto biomaterials (Bilal et al., 2013). In this study, to study effect of pH change on solubility of Cd (II) ion, the $Cd(NO_3)_2$ was used to study as Cd (II) ion source. The pH of $Cd(NO_3)_2$ solution was adjusted from pH 2 to 10 by NaOH and let the interval time of reaction for 30 min. The supernatant was taken for analyses of Cd (II) ion by AAS. The results are shown in **Figure 4.11**. As seen, the percentage of Cd (II) ion were very high (98.6-99.5%) between pH 2 to 5 and slightly decrease at pH 6 to 8 (92.4-95.0%). At pH 8 to 10, Cd (II) ion was dramatically decreased until remaining at 2.7%. Since pH is one of the most important parameters affecting the adsorption process, higher pH causes precipitation of Cd (II) ion in form of Cadmium hydroxide ($Cd(OH)_2$) by the reaction of $2\ NaOH + Cd(NO_3)_2 = Cd(OH)_2 + 2\ Na(NO)_3$. Similar to Blazquez et al. (2005), they reported the effect of pH change on Cd precipitation by alkaline solution where NaOH is used for pH adjustment, the result showed the precipitation of $Cd(OH)_2$ at pH10 and 11. Charerntanyarak (1999) reported the optimum pH for chemical coagulation and precipitation by lime treatment was more than 9.5.

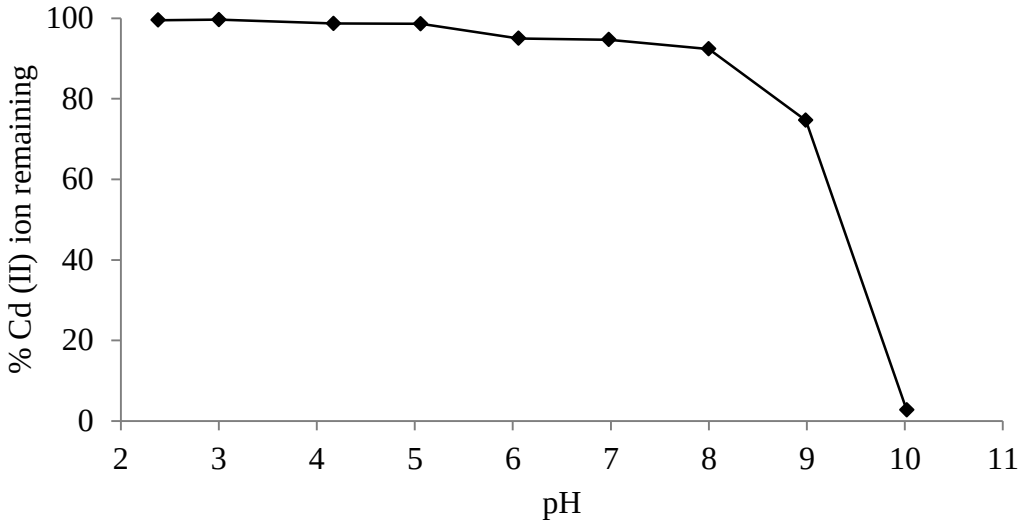


Figure 4.11 The effect of pH solution on the precipitation of Cd (II) ion against pH change

The results of initial pH on Cd (II) ion adsorption of RH300 and RHA300 are shown in **Figure 4.12**. At pH 2, Cd (II) ion removal efficiency was extremely low due to the acidic condition with high potential of H^+ ion (Forstner and Wittmann, 1981). At pH 2 to 4, Cd removal efficiency was significantly increased. At pH 5-8, the result shows an increase in Cd (II) ion adsorption with high removal efficiency and at pH 8-9, the percentages of Cd (II) ion removal efficiency slightly decreased. The negative charge on the biochar surface was the major adsorption method through electrostatic attraction and the adsorption efficiency was increased due to a possible association from the anions of oxygen-containing functional groups that can form surface complexes with Cd (II) ion on the biochar surface (Pan et al., 2013). The increase of pH makes biochars surface charge becomes more negative. Therefore, metal ion can be adsorbed through electrostatic attraction at the cation exchange sites on the biochars' surface, while negative charge on the biochars will increase the adsorption capacity (Pan et al., 2013). Our findings are supported by several metal adsorption investigations on other biochar surfaces (Kołodynska et al., 2012; Low et al., 2000; Regmi et al., 2012; Srivastava and Thakur, 2006). They reported that working pH solution for metal removal from aqueous solution should be slightly acidic.

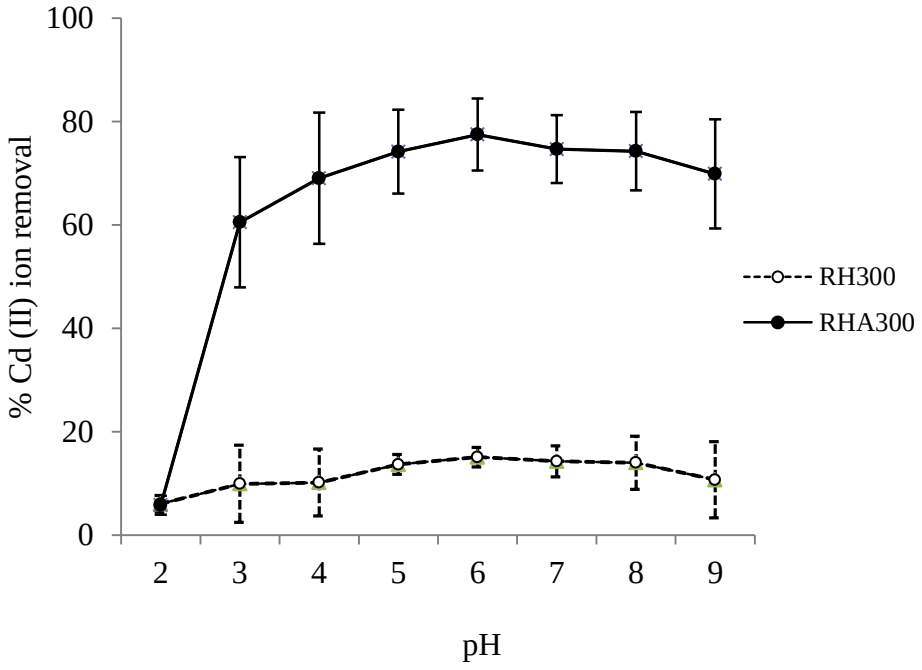


Figure 4.12 Effects of pH on Cd removal efficiency at 60 min of contact time by non-activated (RH300) and activated (RHA300) rice husk biochars

4.2.4 Adsorption characteristics by isotherm study

Sorption characteristics of Cd ion on biochars surface were determined by isotherm study. The isotherm parameters are listed in **Table 4.4** and adsorption isotherms representing linearized plots are shown in **Figures 4.13** and **4.14.** The adsorption data of RH300 and RHA300 were fitted to Langmuir and Freundlich isotherm with correlation coefficient value (r^2) greater than 0.90 (0.94 and 0.93). For RHA300, after activation, r^2 was shifted higher and fitted well to both Langmuir and Freundlich with the value of 0.98 and 0.99, respectively. To interpret Langmuir isotherm line, activated biochar showing low Ce value versus low Ce/qe ratio was presented with high Cd (II) ion adsorption capacity. Activated biochar exhibited a higher adsorption capacity (Q_{max}, 45.87 mg/g) more than 6 times over non-activated biochar (7.76 mg/g). To interpret Freundlich isotherm line, the Freundlich constant, K_F value of activated biochar showed greater value than non-activated biochar at about 3.5 times which presents higher adsorption capacity after activation. Hence, Q_{max} and K_F were used as the adsorption factors for grading the adsorption efficiency of adsorbents (Okeola and Odebunmi, 2010). It can be concluded that adsorption characteristic of Cd ion on activated biochar surface is not restricted to mono layer adsorption with the finite binding site, but have characteristic of multi-layer adsorption with non-uniform distribution of chemical and physical forces (Gulipalli et al., 2011).

Table 4.4 Isotherm parameters of Cd (II) ion adsorption by non-activated (RH300) and activated rice husk biochars (RHA300)

Biochar	Langmuir isotherm			Freundlich isotherm		
	K_L(L/mg)	Q_{max} (mg/g)	r^2	K_F (L/g)	1/n	r^2
RH300	0.047	7.76	0.94	0.69	0.549	0.93
RHA300	0.040	45.87	0.98	2.40	0.717	0.99

The results of isotherm study found that the higher maximum sorption capacity of activated biochar may be understood by the fact that activated biochar exhibits higher BET surface area than non-activated biochar. Kumar et al. (2011) reported that the adsorption capacity of the alkaline-treated raw rice husk for Cd (II) ion was 8.58 mg/g to 20.24 mg/g. While El-Shafey (2007) reported that acid treated raw rice husk had the adsorption capacity between 32.05 and 41.15 mg/g. This study found that the higher adsorption capacities of activated rice husk biochar occurred from a high porous surface area of biochar which could provide more binding sites for Cd (II) ion adsorption.

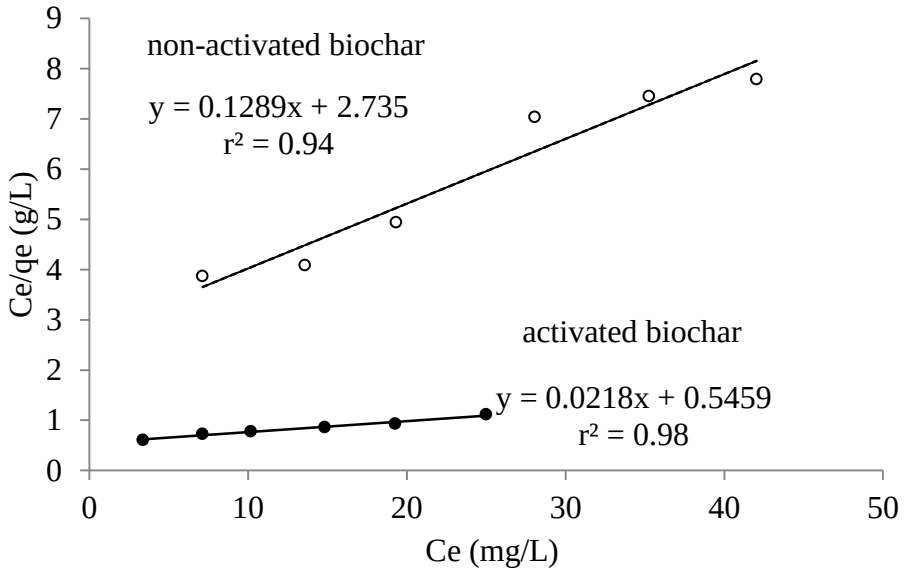


Figure 4.13 Langmuir isotherm of non-activated (RH300) and activated rice husk (RHA300) biochars

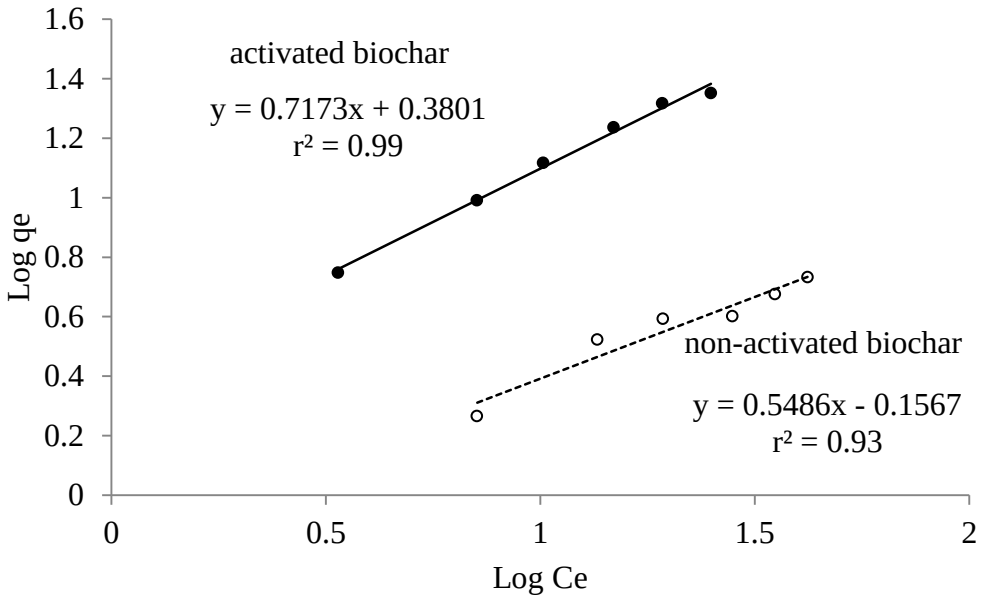


Figure 4.14 Freundlich isotherm of non-activated (RH300) and activated rice husk (RHA) biochars

4.2.5 Physical and chemical characteristics of biochar

1) Surface area

The surface area and porosity of non-activated (RH300) and activated (RHA300) biochars were analyzed by BET method. **Table 4.5** shows that the physico-chemical activation increased the rice husk biochar surface area 1.5 times higher than non-activated biochar (7.71 to 11.57 m^2/g). This larger surface area of activated biochar was supported by the results of higher Cd removal efficiency. Furthermore, the activation could decrease about 0.5 times of pore volume and 2.5 times of average pore size which are the important factors for increasing surface area. The preparation of biochar at the low temperature pyrolysis provided the high carbon content and had more surface area than that of pyrolysis at the high temperature (Daffalla et al., 2010). The surface area of biochar is generally lower than activated carbon produced from high temperature; ranging from 5 m^2/g to around 65 m^2/g depending on the sources of biomass and carbonization conditions (Bhandari et al., 2014).

Table 4.5 Characteristics of rice husks surface area under non-activated (RH300) and activated rice husk biochar (RHA300)

Biochar	BET(m^2/g)	Pore volume (cm^3/g)	Average pore size (nm)
RH300	7.71	0.009046	46.9181
RHA300	11.57	0.005484	18.9573

Martinez-Escandell and Castro (2013) reported the mechanism of KOH activated to carbon material. The KOH attached to carbon surface and reacted with biochar carbon released hydrogen originates from KOH and formed potassium carbonate (K_2CO_3) as shown in reaction (10). The pyrolysis process with limited oxygen also evolved CO and CO_2 as described in reactions (11, 12). The decomposition of K_2CO_3 was carbon consumption, which is associated with the further development of porosity by the decomposition of carbon as described in reaction (10).

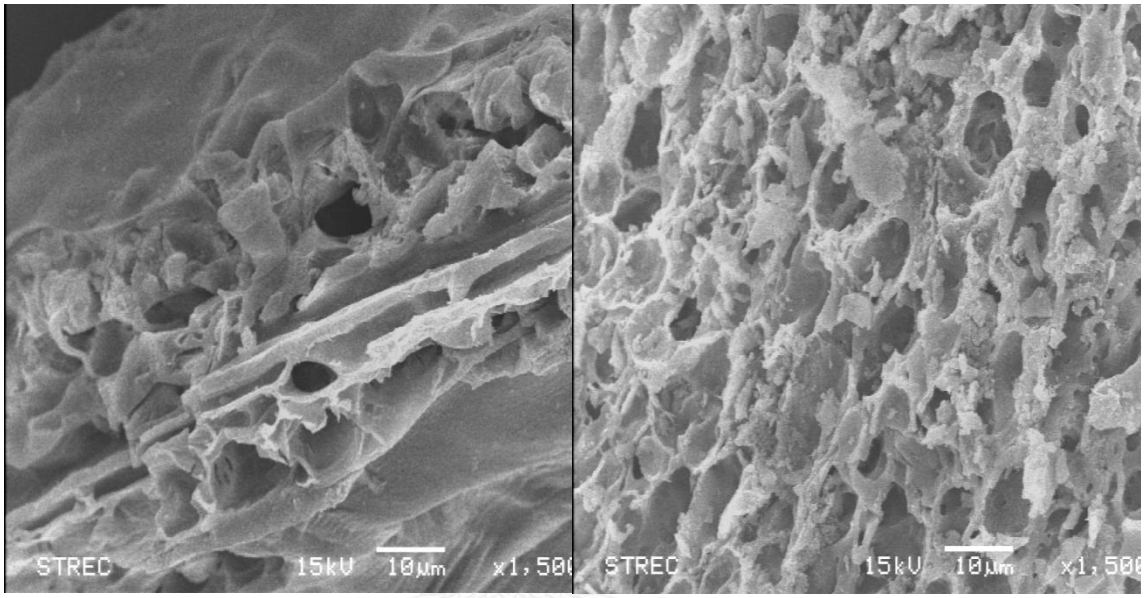
$$6\,KOH + 2\,C \longrightarrow 2\,K_2CO_3 + 2K + 3H_2 \quad \ldots\ldots\ldots (10)$$

$$4\,KOH + C \longrightarrow 4\,K + CO_2 + 2H_2O \quad \ldots\ldots\ldots (11)$$

$$K_2CO_3 + 2\,C \longrightarrow 2\,K + 3CO \quad \ldots\ldots\ldots (12)$$

These micro-pores play an important role on larger binding site for metals adsorption (Regmi et al., 2012; Song and Guo, 2012). Trakal et al. (2011) reported that cold activation of brewers draft biochar with 2M KOH leads to an enhanced surface area from 9.8 to 11.6 m^2/g. The cold activation was activated by clean up blocked pores mainly by tar particles to enhance the surface area. However, activated biochar by KOH solution plus second pyrolysis could make 1.5 time of surface area and decrease average pore size of biochar by modified carbon surface structure which results in high adsorption capacity.

Scanning electron micrographs of rice husk biochar surface structure between non-activated (RH300) and activated (RHA300) biochars are shown in **Figure 4.1**5. Activation made a change in biochar surface. These can clearly be observed from the images of activated biochar, which show a high porosity correlation to a BET surface area.



(a) (b)

Figure 4.15 SEM images of (a) non-activated (RH300) and (b) activated (RHA300) rice husk biochars

2) Elemental composition on biochar surface

The elemental compositions on the biochar surface were randomly spot analyzed by SEM/EDX technique. **Table 4.6** shows the different element compositions on the biochar surface of non-activated (RH300) and activated (RHA300) biochars. After activation, the element contents except oxygen were not significant changed ($p<0.05$). The low temperature pyrolysis at 300ºC might preserve the content of biochar while oxygen content was increasing, resulting from KOH burning as seen in the reaction (10–12).

Table 4.6 Percent of element compositions of non-activated and activated biochars

Biochars	C	O	Mg	P
RH300	39.61±6.27	44.42±1.21[a]	0.14±0.05	0.14±0.08
RHA300	46.00±0.94	50.86±2.21[b]	0.17±0.05	0.13±0.15
Biochars	Ca	K	Si	
RH300	0.14±0.09	N.D.	14.28±2.64	
RHA300	0.18±0.01	1.29±0.83	12.56±4.93	

Note: Means with different super scripts are significant difference ($p<0.05$).

N.D. = non-detected

3) Surface functional group study

Rice husk contains cellulose, hemicelluloses and lignin as main components (El-Shafey, 2007). The carbonization process converts these carbonaceous materials to biochars and resulting remain functional groups on the biochar surface. The chemical functional groups on the biochar surface are the important factors for heavy metal adsorption. FT-IR technique was used to study the chemical functional group of biochar surface before and after activation. **Figure 4.16** shows the vibration frequency of functional groups on non-activated (RH300) and activated (RHA300) biochar surfaces. The spectra were in a range between 4,000 cm^{-1} and 400 cm^{-1}. The peaks of functional groups of RH300 and RHA300 were similar dominant spectra, but RHA300 showed a larger area of two major peaks, 3,411 $cm^{-1}$and 1,605 cm^{-1}. The broad band at about 3411 cm^{-1} showed vibration of a hydroxyl group (OH). The peak at 2,930 cm^{-1} corresponded to the vibration of methyl and methylene groups (C-H) (Tangjuank et al., 2009), peak at 1605 cm^{-1} indicated aromatic carbonyl/carboxyl group (C=O) (Regmi et al., 2012; Yongbin et al., 2010), which peak at 1088 cm^{-1} showed the silica function group of Si-O-Si, (Daffalla et al., 2010) and the sharp peak at 461 cm^{-1} showed metal-halogen bond (Daifullah et al., 2003). The weak peaks at 1,366 cm^{-1} appeared after activation might be alkyne or isopropyl group and the peak at 797 cm^{-1} assigned to Si-H (Daifullah et al., 2003).

FT-IR results showed the difference of intensity peak of two groups, a hydroxyl group and carbonyl/carboxyl group. The activation makes change to enhance the quantity of these two groups that are mainly a functional group of metal adsorption form aqueous solution (Haris et al., 2011; Rao et al., 2010). Hence, the activation was not made to silica groups, which are the major functional groups of rice husk biochar as same as the study of Daifullah et al. (2003). These findings concluded that the activation of rice husk biochar could increase Cd (II) ion adsorption capacity by increasing functional group as Cd binding sites on the biochar surface.

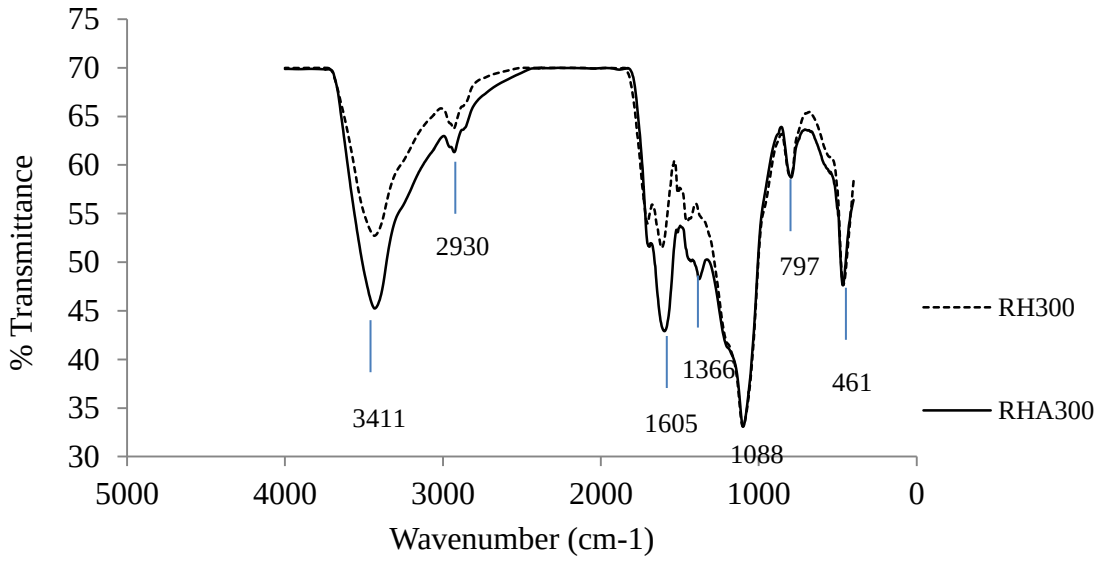


Figure 4.16 FTIR spectra of non-activated (RH300) and activated (RHA300) rice husk biochars

4.2.6 Conclusions of rice husk biochar study

Rice husk biochar, derived from an agricultural by-product, could be attractive alternative sorbent for Cd (II) ion removal from aqueous solution. Physico-chemical activation technique can stimulate rice husk biochar for high Cd (II) ion removal efficiency. The use of KOH, common chemical reagent plus low temperature pyrolysis make changes on rice husk biochar both physical and chemical properties. The increase of the BET surface area and specific chemical functional groups could

be synergism for enhanced Cd (II) ion removal efficiency. Therefore, advantages of cost effective adsorbent and easily available make it an efficient alternative biosorbent for Cd (II) ion removal from aqueous solution.

CHAPTER 5
RESULTS AND DISCUSSION OF PART TWO

Application of biochar for enhancing cadmium and zinc phytostabilization in *Vigna radiata* L. cultivation: A green-house study

The selected biochar was used to study the effect of biochar as soil amendment for enhancing phytostabilization of Cd in plant. The Cd (II) ion adsorption efficiency of selected biochar was determined in laboratory before applying to Cd contaminated soil for pot trial experiments.

5.1 Criteria to select biochar for pot study

The factors for evaluation of biochar to use as soil amendment in this pot experiment were carefully selected. The criteria to select biochar were consist of its Cd (II) ion removal capacity from aqueous solution, available in mass production, no effect to plant growth and practical for preparation in mass production. The large amounts of biochar (30 kg) were needed for each crop in the pot study. The various reasons for decision making of biochar selection are follows;

5.1.1 Data from the result of this study in part one, rice husk biochar produced from physico-chemical activation at 300°C gave the highest Cd (II) ion removal efficiency from aqueous solution, but, it could not prepared in mass production with general biochar kiln. Because the rice husk was very small, the carbonization in mass production should prepare in fluidized bed process which consume high energy and not practical for preparation and application.

5.1.2 For activated cassava stem biochar (CA300) which provided the second highest Cd (II) ion removal efficiency from aqueous solution, the second pyrolysis after KOH pre-activation was not completed success because the biochar from the first pyrolysis was almost full carbon and it could not made self-fuelling process like raw biomass. Moreover, the preliminary study of activated cassava stem biochar applying in soil lead to soil pH shift and not suitable for green bean planting. Even though the cassava stem biochar was washed with water for several times. The suitable soil pH for green bean was 6.5-7.0 and should not be above 7.5. The

preliminary study of activated cassava stem biochar for green bean plant lead to green bean died in first two week with yellow leaves and die early, moreover, soil pH was higher than 8.

5.1.3 The non-activated cassava stem biochar was selected for this pot study because it was the third highest Cd (II) ion removal efficiency and the physico-chemical properties such as porosity and surface functional group similar to activated cassava stem biochar (Table 4.2 and Figure 4.6), moreover, it could be prepared in mass production with biochar kiln as previously described in Research Methodology 3.2.3.

5.2 Soil and biochar characteristics

Cadmium contaminated soil was air-dried, ground and sieved through a 2-mm sieve before analysis of physical and chemical properties. Physical and chemical characteristics of soil are shown in **Table 5.1**. The cassava stem biochar had the following physical and chemical properties: surface area of 6.88 m^2 g^{-1}, Cation Exchange Capacity (CEC) of 93.57 cmol kg^{-1}, 66.7±0.12 % carbon, 2.78±0.16 % hydrogen, 0.22±0.08 % nitrogen, 30.29±0.34 % oxygen (by difference). Cadmium was not detected in the biochar. The biochar was ground and sieved to obtain particle size between 250 -500 μm for batch static adsorption study.

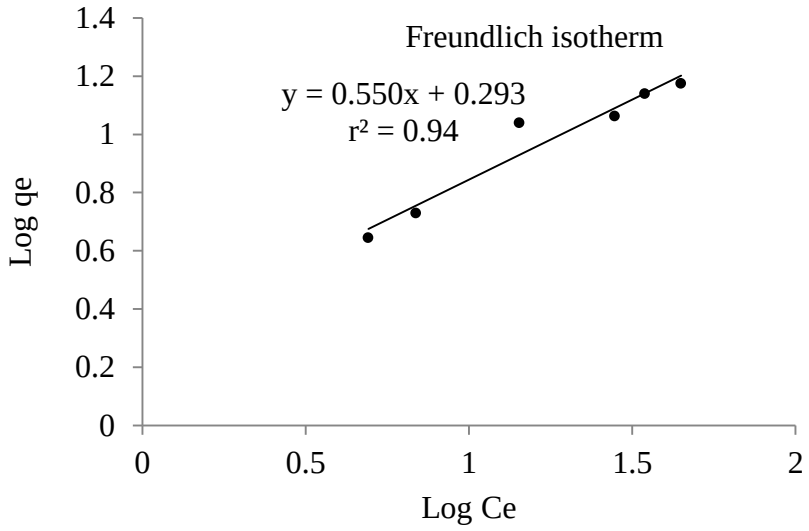
Table 5.1 Physical and chemical characteristics of Cd contaminated soil

Parameters		Values
Particle size distribution	% Sand	48
	% Silt	28
	% Clay	24
Texture class		Silty clay loam
pH (H_2O : Soil, 1:1)		7.9
CEC (cmol/kg)		13
Organic matter (%)		2.20
Total nitrogen (%)		0.12
Carbon to nitrogen ratio (C/N ratio)		10.05
Available phosphorus (Bray II, mg/kg)		24
Extractable potassium (NH_4OAc, mg/kg)		180
Extractable calcium (NH_4OAc, mg/kg)		6,256
Extractable magnesium (NH_4OAc, mg/kg)		353
Total Cd (mg/kg)		58
Total Zn (mg/kg)		1,220
Bioavailable Cd (mg/kg)		11
Bioavailable Zn (mg/kg)		28

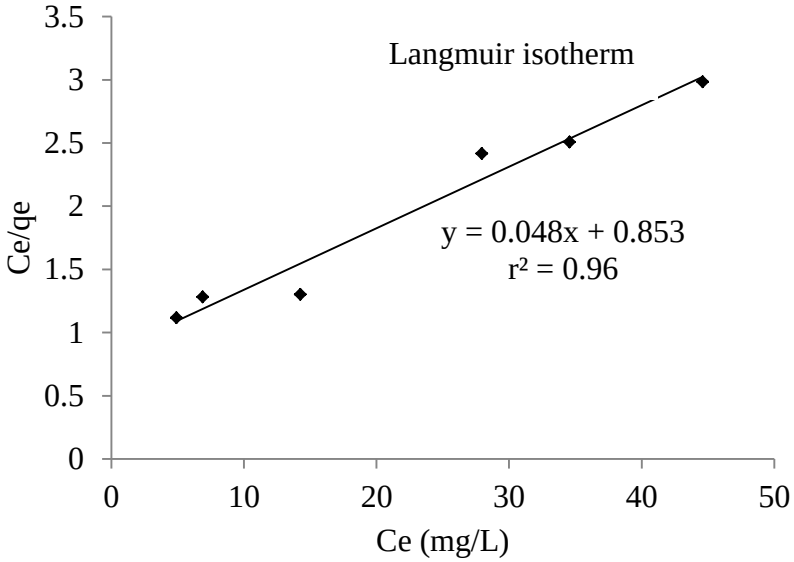
5.3 Adsorption efficiency of Cd ion onto biochar

The Langmuir isotherm is useful for grading the adsorption efficiency of adsorbents (Ozcimen and Mericboyu, 2009). Langmuir isotherm can be used for describing Cd (II) ion adsorption characteristics on biochars (Tangjuank et al., 2009). **Figure 5.1** shows the adsorption data plotted with the trend line correlation coefficient (r^2). It shows that the Cd (II) ion adsorption characteristic on biochar surface fit well with both Langmuir and Freundlich isotherm. The values of r^2 in Langmuir and Freundlich were 0.96 and 0.94, respectively. The adsorption capacity of Langmuir isotherm illustrates the maximum adsorption capacity of biochar to be 20.53 mg/g, a positive result for Cd (II) ion adsorption.

The various biochar adsorption capabilities produced from different materials, pyrolysis conditions and activation methods are shown in **Table 5.2**. In this study, cassava stem biochar showed good adsorption efficiency without activation.



(A)



(B)

Figure 5.1 Freundlich and Langmuir isotherms of cassava stem biochar

(A) Langmuir isotherm (B) Freundlich isotherm

Table 5.2 Cadmium adsorption capability of biochars produced from different sources of biomass

Biochar sources	Activation processes	Carbonization temperatures (ºC)	Q_{max} (mg/g)	Sources
Swichgrass	KOH	300	34	Regmi et al. (2012)
Pig manure	Non-activated	600	16.6	Kołodynska et al. (2012)
Manure	-	350	51.4	Xu et al. (2013)
Nut Shells	Phosphoric acid	475	104.17	Tajar et al. (2009)
	Phosphoric acid + SO_2		142.86	
Cashew nut shell	KOH + CO_2	850	14.29	Tangjuank et al. (2009)
Cassava stem	Non-activated	350	20.53	This study (on 5.3)

5.4 Promoting plant growth in contaminated soil by biochar addition

Soil amendment with biochar resulted in an increase in nutrients for plant growth (Park et al., 2011). The results of this pot trial experiments showed that the seed germination rate was 100% of all treatments. **Table 5.3** shows plant growth with different biochar concentrations. During weeks 2-6, plant growth increased by increasing biochar concentrations. At week 8, 10% biochar enhanced plant growth enormously in length and fresh weight of shoot and root. At 15% biochar, plant growth decreased significantly compared to all treatments. Moreover, plants showed early mortality symptoms with yellow leaves falling from the plants. This could be indicated that high biochar concentration causes an imbalance of nutrients in the soil. The biochar adsorbed some micronutrients from soil and reduced nutrients' bioavailability. The biochar in soil amendment had a positive impact as it decreased soil bulk density with enhanced soil porosity. Moreover, biochar can increase soil respiration, thus improving nutrient flow for plant growth (Park et al., 2011). This study's findings indicate that biochar at concentrations of 10% is the optimum concentration for promoting plant growth.

The effect of biochar on plant growth varied with the type of biochar and amount of application rates (Park et al., 2011). In general, the biochar application rate ranged between 0.2 and 20 % biochar and depended on plant types and biochar properties (Mukherjee and Lal, 2014). High concentration of biochar could improve soil pH in acidic soil, but too much biochar could lead to adverse impact on plant growth due to high ash content lead to high soil's alkalinity and caused plants' micronutrients deficiency. The beneficial impact of biochar was reported by Zheng et al. (2013), showing that 5% biochar concentration was the optimum concentration for maize growth, and Park et al. (2011) reported that a 15% biochar concentration delivered through green waste on Indian mustard, promoted the highest plant growth. On the other hand, Upadhyay et al. (2014) reported that the optimum green-waste biochar application rate to lettuce growth was 30 ton/ha, but when the biochar application rate was increased to 100 ton/ha, plant growth decreased. The results also similar to the study of Suppadit et al. (2012), they report that the 15% of quail litter biochar led to soil pH shift and not suitable for physic nut growth.

Table 5.3 Shoot and root growth of *V. radiata* L. at different biochar concentrations

Plant parts	Treatments (%)	Cultivating periods			
		Week 2	Week 4	Week 6	Week 8
Shoot height (cm)	0	13.79±1.41 [a]	20.22±2.02 [a]	27.61±3.75 [a]	39.35±1.44 [b]
	5	14.92±1.26 [a]	23.04±1.26 [a]	35.42±3.86 [b]	41.58±2.77 [c]
	10	18.42±2.47 [b]	26.21±2.47 [b]	36.21±3.55 [b]	41.63±2.68 [c]
	15	13.33±1.34 [a]	21.25±3.41 [a]	28.83±2.60 [a]	34.08±4.09 [a]
Shoot fresh weight (g)	0	2.30±0.17 [a]	9.64±0.89 [a]	37.57±2.29 [a]	37.91±2.02 [a]
	5	2.64±0.23 [ab]	11.52±2.42 [a]	41.17±1.62 [ab]	41.48±1.10 [b]
	10	2.99±1.42 [b]	14.80±2.18 [b]	42.98±2.18 [b]	41.97±2.27 [b]
	15	2.45±1.38 [ab]	16.52±1.38 [b]	39.27±1.06 [a]	36.84±1.86 [a]
Root length (cm)	0	8.67±2.46 [ns]	12.14±3.00 [a]	16.17±3.54 [a]	18.00±3.34 [a]
	5	7.58±1.16 [ns]	12.58±3.26 [a]	18.67±3.36 [a]	20.33±0.82 [ab]
	10	7.58±2.43 [ns]	15.13±2.65 [b]	22.83±2.44 [b]	23.17±1.48 [b]
	15	8.81±1.48 [ns]	18.75±1.48 [c]	22.50±2.55 [b]	22.67±1.83 [b]
Root fresh weight (g)	0	0.56±0.08 [ns]	1.25±0.30 [a]	2.40±0.43 [a]	3.09±0.16 [a]
	5	0.60±0.07 [ns]	1.64±0.06 [b]	3.22±0.23 [b]	3.69±0.19 [b]
	10	0.64±0.10 [ns]	1.83±0.08 [b]	4.63±0.11 [c]	4.46±0.22 [c]
	15	0.58±0.04 [ns]	1.93±0.10 [b]	5.04±0.71 [c]	4.31±0.07 [c]

Plant weight, presented as total weight per pot, values are means of: plant part in the pot (n=3), mean ($\bar{x}$ ±SE) with different superscripts are significantly different among treatment (column) in each plant growing part.

The number of total bean pods and amount of ripen beans at week 8 is shown in **Figure 5.2**. The treatment of 10 % biochar achieved the highest number of bean pods with a significant difference to other treatments that complied with plant growth measurement. Meanwhile, 15% biochar had no significant difference in the amount of

beans pods at 0% and 5% of biochar at $p<0.05$. Thus, it can be concluded that 10% biochar, not only is the optimum concentration to promote plant growth, but it is also the optimum concentration to promote plant production.

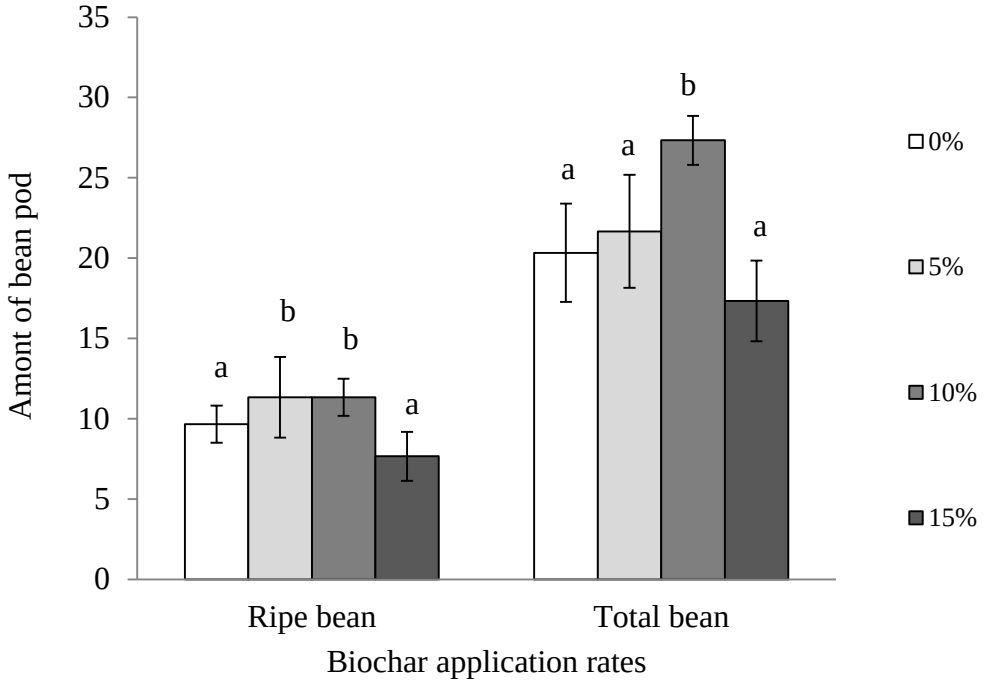


Figure 5.2 Number of bean pods after plantation in Cd contaminated soil amended with different biochar concentrations

5.5 Effect of biochar on Cd and Zn plant uptake and accumulation

Cd and Zn concentrations in plant parts over a growing period are presented in **Table 5.4 and 5.5**. At the beginning of the blossom stage (week 4), Cd concentrations in shoots and roots were nearly three times and two times higher than that of week 2 respectively, due to a high uptake of nutrients. Cd concentration of roots increased significantly with biochar addition. At week 6, the beginning of the seed filling stage, the plants showed maximum uptake of nutrients for developing seeds, which could be combined with Cd and Zn uptake. The highest Cd concentration was found in the roots of *V. radiata* L. with 10% biochar addition. The combined approach of plant and

biochar addition increased Cd uptake by increasing plant growth with biochar addition in soil. For all treatments of week 8, Cd uptake to plant shoot tended to decrease by about 35%. Furthermore, alkaline soil pH effected the bioavailability of Cd due to strong binding of Cd with soil (Chaney, 2010). Similar to the study of Hooda (1996), who reported that the liming soil to higher pH lead to reduced Cd and Zn concentration in carrot and spinach.

There was no significant difference between all treatments with biochar amended soil. Cd accumulation in bean seed and shell was equal. Cd concentrations in bean seed and shell of all treatments ranged between 5.6 and 8.5 mg/kg, which are much higher than the Codex standard for polished rice (0.4 mg/kg). Cd concentration in bean seed and shell showed significant increases with concentrations of 10% and 15%. For Zn concentration in plant parts, Zn showed continuous increase throughout the crop period. Zn accumulation in roots was higher than shoots. At week 8, biochar at 5, 10 and 15% concentrations significantly decreased Zn in shoots, but not in roots and bean pods. Several reports cover numbers of species showing about 500 mg/kg of Zn concentration in plant reduced 25% of yield reduction and some reports suggest that 290 mg/kg of Zn concentration make dry leave associated with reduce yield (Chaney, 2010). Hence, in this study, the green bean was not showing any effects from Zn accumulation level.

Table 5.4 Cadmium concentrations in *V. radiata* L. at different growth periods

Plant parts	Treatment	Cd concentration (mg/kg)			
	%	Week 2*	Week 4	Week 6	Week 8
Shoot	0	3.60	14.2±1.94 ^{ns}	17.13±1.07 ^{a}	11.5±0.55 ^{ns}
	5	3.90	13.8±1.56 ^{ns}	18.80±0.61 ^{b}	12.7±1.22 ^{ns}
	10	4.80	15.4±0.25 ^{ns}	18.13±1.00 ^{ab}	12.7±0.57 ^{ns}
	15	5.90	15.4±0.75 ^{ns}	18.37±0.32 ^{ab}	12.4±1.69 ^{ns}
Root	0	23.4	35.8±2.39 ^{a}	56.56±7.18 ^{a}	25.4±0.41 ^{ns}
	5	34.5	75.2±5.29 ^{b}	59.44±6.47 ^{a}	25.1±5.68 ^{ns}
	10	34.3	76.2±4.54 ^{b}	74.81±5.74 ^{b}	32.5±7.66 ^{ns}
	15	40.8	66.9±8.47 ^{b}	69.78±10.47 ^{ab}	28.0±1.10 ^{ns}
Bean seed	0				5.8±0.62 ^{a}
	5				6.4±0.23 ^{a}
	10				8.5±0.68 ^{b}
	15				7.8±0.10 ^{b}
Bean shell	0				5.6±0.47 ^{a}
	5				6.6±0.99 ^{a}
	10				8.3±0.46 ^{b}
	15				8.3±0.18 ^{b}

* One combined replicated due to less plant dry weight,

The means and the S.E. (n = 3) followed by the different superscript letters within column are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Table 5.5 Zinc concentrations in *V. radiata* L. at different growth periods

Plant parts	Treatment %	Zn concentration (mg/kg) Week 2*	Week 4	Week 6	Week 8
Shoot	0	45.2	64.5±2.27 ^{ns}	71.77±8.77 ^{b}	95.6±14.85 ^{a}
	5	51.5	61.0±9.98 ^{ns}	67.03±6.54 ^{ab}	76.5±3.89 ^{b}
	10	52.0	66.2±3.90 ^{ns}	74.00±10.74 ^{b}	77.2±2.12 ^{b}
	15	52.5	66.3±3.35 ^{ns}	53.37±5.94 ^{a}	69.4±6.23 ^{b}
Root	0	54.4	356.3±26.66 ^{b}	322.77±40.46 ^{ns}	357.9±15.26 ^{ns}
	5	34.6	234.6±35.00 ^{a}	274.00±22.08 ^{ns}	323.6±49.39 ^{ns}
	10	34.3	245.1±32.96 ^{a}	274.50±19.59 ^{ns}	331.3±18.27 ^{ns}
	15	40.8	236.3±17.39 ^{a}	268.00±32.58 ^{ns}	295.4±51.51 ^{ns}
Bean seed	0				44.1±0.58 ^{ns}
	5				41.1±2.12 ^{ns}
	10				40.6±4.08 ^{ns}
	15				39.3±3.07 ^{ns}
Bean shell	0				33.5±2.27 ^{ns}
	5				28.6±3.70 ^{ns}
	10				29.7±1.18 ^{ns}
	15				29.0±2.35 ^{ns}

* One combined replicated due to less plant dry weight,

The means and the S.E. (n = 3) followed by the different superscript letters within column are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Plant uptake Cd and Zn depended on soil properties, plant species, cultivation practice, fertilizer and property of metals sources (Chaney, 2010). The effect of Zn on Cd uptake and accumulation by plant are not consistent (Adiloglu et al., 2005). The relationship of Cd and Zn were shown in characteristic, antagonistic or synergistic effect (Alloway, 1995). The interaction of Cd and Zn were not exactly followed to one direction depended on many factors such as uptake, transport and accumulation process. It may be illustrated that the ratio of Cd to Zn in plant media controls the characteristic of synergistic and antagonistic between these two cations (Kabata-Pendias, 2000). Hocine-Benabid et al. (2007) reported the interaction of Zn and Cd in beans (*Phaseolus vulgaris*), the results showed antagonistic relation of Cd at difference concentration of Zn in plant. Therefore, many factors affected to Cd and Zn uptake by plant. Xian (1989) reported that the proportion of metals uptake to metal amounts for plants in soil polluted by a zinc smelter showed greater Cd uptake affinities than Zn. Cd does not have a known transfer function in plants and exhibits similar chemical properties to Zn (Pence et al., 2000; Tan et al., 2011). The increased Cd uptake could be the result of increasing plant growth from biochar addition that enhances overall nutrients and Cd uptake. Sessitsch et al. (2013) studied the role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils, the results indicated that plant bacterial communities in rhizosphere zone associated with plant growth and plant's uptake heavy metals performance. Bacterial populations associated with plants growth lead to enhanced plants accumulation of heavy metals which enhance the efficiency and rate of phytoextraction. Nitrogen-fixing bacteria produce molybdate binding tetra-dentate catecholates, which is the Chelating agent for Cd binding. Croes et al. (2013) reported the majority of Cd/Zn tolerant strains, *Brassica napus* L., had capable of phosphorus solubilization and nitrogen fixation. This plant execrates phytohormones, 1-aminocyclopropane-1-carboxylate deaminase and siderophores, by nitrogen fixation and phosphates solubilization. Plant-associated rhizobacteria that produce siderophores and/or organic acids can also enhance toxic trace elements availability in soils and by consequence their uptake by plants. Similar to the study of Ma et al. (2014), who reports mycorrhizas nitrogen fixing bacteria which increase Cd (II) ion uptake by *Populus* × *Canescens*.

Although several studies report on the use of biochar for heavy metals immobilization, in this work, the biochar stimulated Cd plant uptake, but tended to reduce Zn uptake in shoots. The increase of metals uptake using biochar can depend on heterogeneity in response to soil and plant types (Paz-Ferreiro et al., 2014). Fellet et al. (2014) used three feed stock biochar, namely orchards' pruning residues, fir tree pellets and manure pellets for soil amendment, and they found that fir tree pellet biochar-amended soil increased Cd accumulation in three plants species.

To understand the role of biochar in soil, Cd and Zn adsorbed by biochar were analyzed. After harvesting plants, biochar in soil (only in the pot of 15% biochar) was separated from soil and analyzed for Cd and Zn levels by acid digestion. Results showed that Cd and Zn amounts adsorbed on biochar were 15.43 ± 0.06 and 173.33 ± 23.60 mg/kg respectively. Zn adsorption capacity was 11 times higher than that of Cd, indicating that biochar plays an important role in Zn adsorption in high Zn contaminated soil. Although biochar adsorbed Zn at higher levels than Cd, a comparison of Zn (1,220 mg/kg) and Cd (58 mg/kg) soil concentrations showed the Zn level to be about 21 times higher than that of Cd, indicating that Cd has a high adsorption affinity than Zn. Similar to the study of Rees et al. (2014), indicated effective metals sorption by biochar affinity of Pb>Cu>Cd>Zn>Ni.

5.6 Reducing Cd and Zn bioavailability in soil amendment with biochar

The bioavailability of Cd and Zn extracted by DTPA is shown in **Table 5.6**. The results indicate that biochar could efficiently reduce Cd and Zn bioavailability. Increased biochar concentrations resulted in decreased Cd and Zn bioavailability. Zn bioavailability was 2-3 times higher than Cd bioavailability for all treatments, indicating that each biochar and soil in pots when well mixed maintains their adsorption mechanism up to the time of planting. At week 8 of cultivation, Cd bioavailability of all biochar concentrations was not significantly different, but it significantly decreased when compared to the control treatment. For Zn bioavailability, the 15% biochar showed a significant decrease in Zn bioavailability when compared to 5 % and 10% biochar. These results justify that 5% biochar is a cost effective biochar concentration to reduce Cd bioavailability.

The immobilized mechanism of heavy metals in contaminated soil when varied according to soil and amendment properties and the presence of organic matter and other metals in soil affect metals bioavailability (Romkens et al., 2009). Furthermore, soil organic matter increases competitive adsorption mechanisms of organic matter and metal ion onto a biochar surface (Chorom et al., 2013). The role of biochars on reducing bioavailable forms of metals in soil mostly occur from adsorption of metal ions onto biochar surfaces, and specific biochar properties perform specific adsorption efficiency, which is induced by different metals' immobilization (Park et al., 2011; Uchimiya, Lima, Klasson, et al., 2010). In this study, **Figure 5.4** shows the increase of available phosphorous (P), which is important for plant growth during blossom seed filling stage. This available P could increase Cd concentration in green beans, similar to the study of Panwar et al. (1999), which reported that the increasing P concentration led to an increased Cd concentration in green beans.

Table 5.6 Concentrations of Cd and Zn bioavailable forms in soil during cultivation

Metals	Treatment %	Metal concentration (mg/kg)			
		Week 2	Week 4	Week 6	Week 8
Cd	0	10.72±0.28 ^{c}	10.82±0.26 ^{c}	10.66±0.29 ^{c}	10.70±0.81 ^{b}
	5	9.52±0.21 ^{b}	9.49±1.12 ^{b}	8.56±0.48 ^{b}	8.67±1.26 ^{a}
	10	9.01±0.32 ^{ab}	9.10±0.70 ^{ab}	8.77±0.16 ^{b}	7.72±1.06 ^{a}
	15	8.39±0.80 ^{a}	8.11±0.23 ^{a}	7.53±0.45 ^{a}	7.50±0.22 ^{a}
Zn	0	27.44±0.81 ^{ns}	27.06±1.68 ^{b}	26.07±1.73 ^{b}	26.28±1.04 ^{c}
	5	27.63±0.02 ^{ns}	24.18±1.12 ^{a}	22.99±0.72 ^{a}	21.64±0.49 ^{b}
	10	27.63±0.02 ^{ns}	23.41±0.19 ^{a}	23.03±0.16 ^{a}	21.18±0.79 ^{b}
	15	26.62±0.89 ^{ns}	22.35±0.95 ^{a}	22.19±0.71 ^{a}	19.11±0.94 ^{a}

The means and the S.E. (n = 3) followed by the different superscript letters within column are significantly different ($p < 0.05$) according to Duncan's multiple range test.

5.6 Changes of chemical properties in biochar-amended soil during cultivation

The changes of soil pH during plant growth are shown in **Figure 5.3(a)**. The results indicate that soil pH increased after biochar addition but then tended to decrease and stabilize after week 2. At week 8, the soil pH of all treatments showed no significant difference. However, **Figure 5.3(b)**, which presents biochar-amended soil incubation studied parallel to green bean plantation, shows less pH decrease than those in plant pots. Hence, it can be concluded that plants do have an effect on soil pH. Soil with fresh biochar amendment usually affects soil pH. Thomson et al. (1993) reported that soil pH change during a bean crop and soil pH in their pot experiment decreased due to acidification of the rhizosphere for N_2 fixation. Plant nitrogen uptake is found in three main forms, including i) NO_3^- anion, ii) NH_4^+ cation and iii) nitrogen fixation from N_2 in the air. It has been shown that plant NH_4^+ uptake and N_2 fixation result in the release of H^+ and lead to acidic soil surrounding rhizosphere (N. S. Bolan et al., 1991). Biochar addition has oxidation and adsorption properties of organic acids as a result of soil pH shift (Houben et al., 2013b). In general, biochars can lead to increased soil pH and maintain stable soil pH levels with the presence of biochar carbonate contents that have chemical buffering capacity (Cao et al., 2009; Yuan et al., 2011). In this study, the presence of rich concentrations of Zn ion and Cd ion also presented an adsorption mechanism for metals to be adsorbed onto the carbonate contents, which correlates with the presence of hydroxyl, carboxylate and carbonyl groups (Lee et al., 2010). Therefore, metal adsorption from soil on to those functional groups results in less alkaline biochar amendment soil and alkaline soil property.

Hong et al. (2014) reported soil pH condition that allow Cd precipitate as Cd minerals in phosphate amended soil. Soil pH might not affect Cd precipitation as $Cd_3(PO_4)_2$ by direct reaction of Cd and phosphate in the studied soil even though when soil pH shifted up to 9.0. However, Cd might precipitate as $CdCO_3$ with increasing soil pH up to 9.0 in phosphate untreated soil. The solid solution formation can be occurred by substitution of Cd for calcium in calcium carbonate. Cadmium and calcium have almost identical ionic radii so that Cd can readily substitute of calcium in this carbonate mineral (McLean and Bledsoe, 1992). Hence, in this study, soil pH was not increase more than 8, therefore, Cd (II) ion in soil probably less precipitate to other Cd forms.

Soil properties are important factors that affect metal uptake from plants (Tangahu et al., 2011). A low pyrolysis temperature results in high biochar yields and low ash content, which also leads to lower alkaline pH (Cao et al., 2009; Hassan and Aarts, 2011; Singh et al., 2010; Song and Guo, 2012; Thomson et al., 1993). **Figure 5.4** shows the changes of soil chemical characteristics of each treatment after 8 weeks of cultivation. Soil particle sizes of all treatments were analyzed. The soil, a silty clay loam, has a moderately fine texture soil. The soil pH of biochar amendments of 5, 10 and 15% biochar was slightly alkaline. It was observed that increased biochar concentration trended to increase all nutrients values except extractable calcium (Ca). Soil organic matter (OM) increased by increasing biochar concentration. In the soil microbial habitat, biochar mobilized and released organic matter, nitrogen, phosphorous and soluble potassium (Ameloot et al., 2013; Mukherjee and Zimmerman, 2013). OM also has an important role in the nutrients reservoir through microbial degradation (Mclatchey and Reddy, 1998). In this study, the increase of other soil nutrients such as, total N, extractable K, extractable Mg, from moderate (0 % biochar) to high nutrient level (15% biochar) resulted from an increase of OM. Since CEC correlated with increased O and C contents (Lee et al., 2010), CEC of biochar-amended soil increased by increasing biochar concentration, similar to the earlier findings of Liang et al. (2006) and Houben et al. (2013a). Therefore, the overall findings of biochar amendment in soil show an increase in soil fertilities.

The increased of microbial population result to decomposition of organic matter by biological action, nevertheless, the increase of microbial did not results in biotransformation process of valency change, since Cd exists in nature only valence state of +2, microbial oxidation or reduction of Cd is improbable. (Riser-Roberts, 1998)

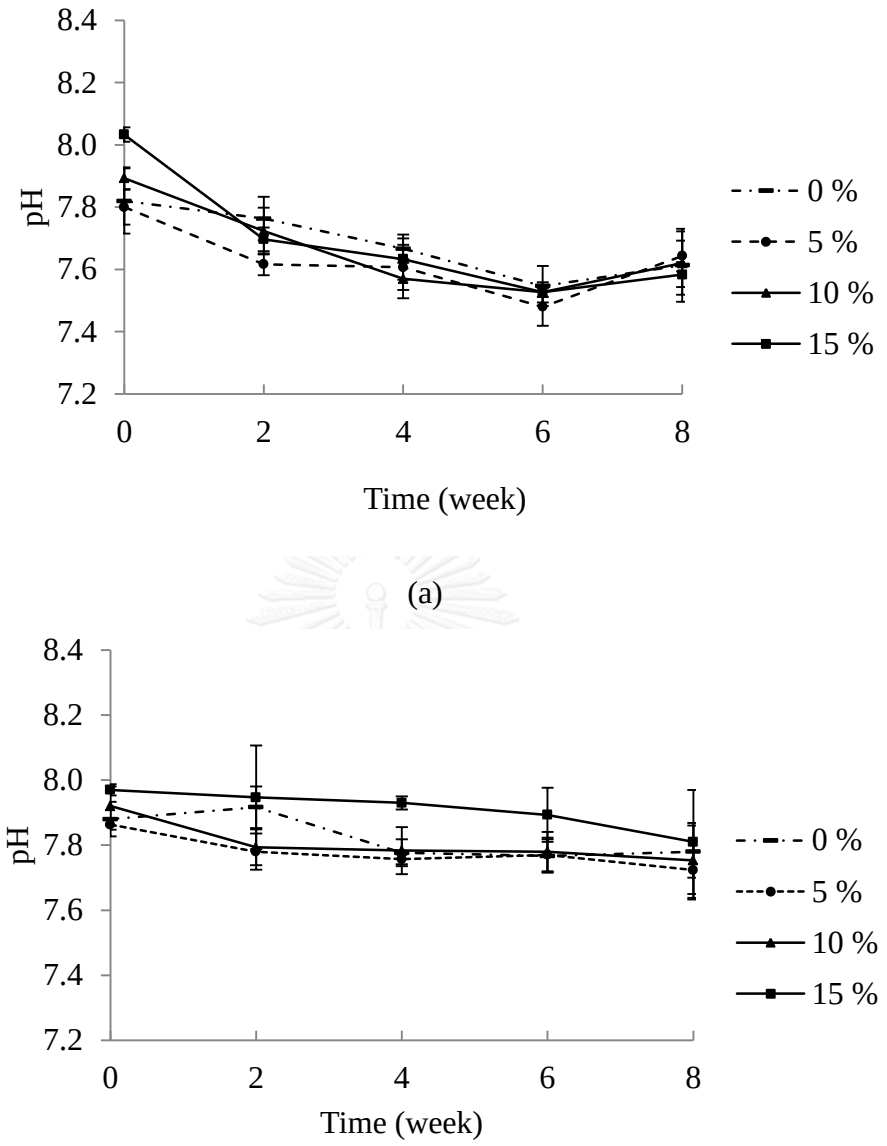


Figure 5.3 Soil pH change during *V. radiata* L. cultivation, (a) pot with plant and (b) pot without plant

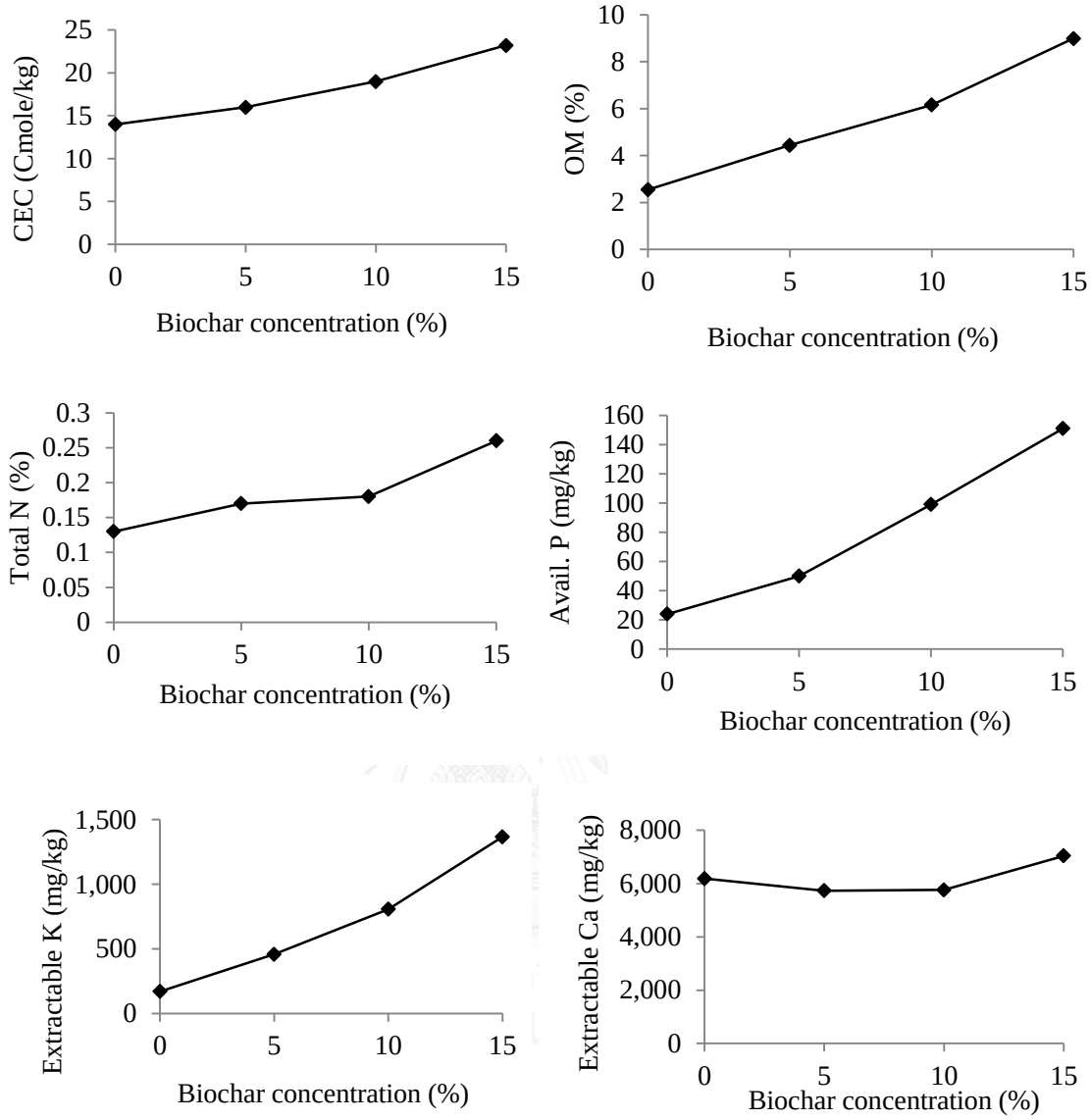


Figure 5.4 Trend lines of soil chemical characteristics of each treatment after 8 weeks of cultivation

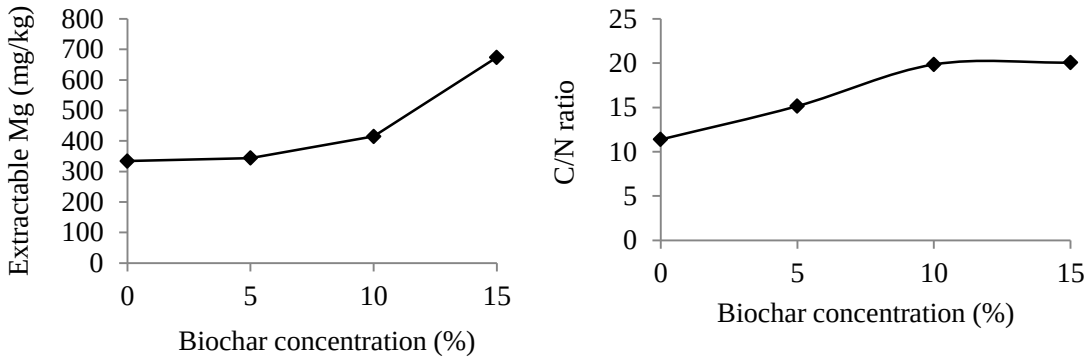


Figure 5.4 Trend lines of soil chemical characteristics of each treatment after 8 weeks of cultivation (cont.)

5.7 Mobility and competitive sorption of metals

Hydrated ionic size in water solution is an important factor for determine movement capability of each metal pass through specific pore size. In aqueous solution, the biochar pore sizes are varying in diameter sizes which consist of macro-pore on outer surface area and micro-pore in the inner area. The mean bond distance, ionic radius, and configuration of the hydrated metal ions are important for movement of metal ions pass through outer surface area both until reach the inner pore. Persson (2010) reported Hydrated metal ions size in aqueous solution. The water molecule bind to metal ions through ion-dipole bonds of mainly electrostatic character. The results indicated that, $Cd(H_2O)_6^{2+}$ and $Zn(H_2O)_6^{2+}$ aqua complex size were 0.23 and 0.208 nm, respectively. Configuration of hydrated Cd and Zn ions in aqueous solution were octahedron shape and with similar size. The comparison of hydrated Cd and Zn ions size to the average pore size of cassava stem biochar of 1.3 nm, it is implied that hydrated Cd and Zn ions can mobile pass through effective average pore size of biochar. Hence, Cd had higher sorption affinity than Zn due to it lower value of hydrate metals radius (Zhang and Luo, 2014).

In this study, the study of metals competition was conducted in chelating agent extracted from soil (DTPA). The biochar was agitated in DTPA soil solution at 120 rpm for 2 h and supernatant were filled and analyzed for remaining Cd, Zn and Pb by AAS. The results are shown in **Table 5.7**. It was shown that, biochar had good

adsorption to Cd than Zn and Pb, even though in the present of chelating agent. However, the result of Cd uptake by green bean still high by other reasons. Rees et al. (2014) reported the effect of biochar on the mobility of complex metal ions in soil. The results indicated that the sorption kinetic of metals onto biochar surface follow a decreasing order of Pb>Cu>Cd>Zn>Ni. Moreover, the result with the multi-elements sorption show sorption affinity of Cd slightly higher than Zn in the same trend direction, but Zn show higher desorption rate than Cd. Similar to the study of Trakal et al. (2011), they report the sorption efficiency of biochar on multi-element (Cd, Cu, Pb and Zn) in solution and biochar amended soil. The result indicated that Cu and Pb had higher sorption than Cd and Zn, while Cd and Zn had similar sorption efficiency onto biochar. It was founded in this study that the Zn/Cd ratio content of biochar separated and extracted from soil, was two times higher than Zn/Cd ratio in contaminated soil without biochar.

Table 5.7 Amount of metals adsorb onto biochar in DTPA soil solution

Treatments	Metals concentration (mg/kg)		
	Cd	Zn	Pb
Soil solution	12.08±1.09	30.23±1.89	10.32±1.78
Soil DTPA+BC	7.12±0.73	28.98±0.83	10.75±2.09

The ratio of Cd and Zn bioavailability to Cd and Zn concentration are shown in **Table 5.8**. The percentages of Cd and Zn bioavailability tended to decrease after increased biochar application rates. The biochar play adsorption mechanism role on these metals bioavailability similar to that reported by Suppadit et al. (2012). They reported the positive effect of quail litter biochar apply in Cd contaminated site at Mae Sot (same area of this study). The quail litter biochar were applied in contaminated soil contained 60.8 mg/kg of Cd. The quail litter significantly decreases the bioavailability of Cd to physic nut plants.

Table 5.8 Percentage of bioavailable metals at various growth periods

Metals	Biochar application rates (%)	Percentage of bioavailable metals compared to total metal concentration				
		Week0	Week2	Week4	Week6	Week8
Cd	0	19.22	18.89	19.15	18.34	19.33
	5	18.69	17.71	17.71	15.88	16.29
	10	19.39	18.14	18.79	17.37	15.55
	15	20.59	18.26	17.78	16.06	16.61
Zn	0	2.34	2.40	2.45	2.46	2.78
	5	2.38	2.43	2.22	2.14	2.30
	10	2.38	2.46	2.20	2.20	2.24
	15	2.39	2.41	2.12	2.14	2.05

The metals sequential fractions in soil indicate the available exchangeable form of heavy metals and they can be transformed into an un-exchangeable form (Qiu and Guo, 2010; Tessier et al., 1979). Sriprachote (2006) reported Cd fractions by a BCR Sequential Extraction Procedure of soil samples from lowland area beside Mae Tow stream (near area of this study). The results are shown in **Table 5.9**. It can be seen that, the percentage of exchangeable Cd fraction form soil (water soluble + exchangeable) was 17.14% and illustrated to the total Cd concentration in this study (58 mg/kg), the exchangeable Cd was about 9.94 mg/kg. Hence, the exchangeable Cd was similar to Cd bioavailable form (DTPA) of this study (11 mg/kg). Different soil characteristics lead to different soil mobility (Matos et al., 2001). However, considering that the cost of DTPA extraction is lower than that of sequential extraction, therefore, it would be convenient to know whether simple extraction would be enough to decide on the application of amendment (Mendoza et al., 2006).

Table 5.9 Percent of metals fraction of soil samples from lowland area beside Mae Tow stream (Sriprachote, 2006)

Metals	Fractionation (%)					
	Water soluble	Exchangeable	Carbonate	Fe-Mn oxide	Organic	Residual
Cd	0.40	16.74	55.14	17.70	5.92	4.10

5.8 Combined approach of biochar-amended soil on plant growth and Cd phytostabilization

To assess plant ability for Cd and Zn accumulation and translocation, BCF and TF for each treatment were calculated. BCF expresses the capacity of a plant to transfer metals from surrounding soil uptake to plant roots (Yoon et al., 2006). **Table 5.10** shows BCF and TF of Cd and Zn during cultivation. Results show that BCF_{Cd} increases with plant growth, except at week 8 when plant growth drops due to a plant's declining phase. BCF_{Cd} values greater than 1 were observed in soil amended with biochar at week 4 and 6 and increased with increasing biochar concentration. These findings suggest that biochar addition increases BCF_{Cd} and Cd hyperaccumulation in green bean during the blossom and seed filling stages. BCF_{Zn} was lower than BCF_{Cd}, while biochar addition had no effect on BCF_{Zn}. Only at week 4, BCF_{Zn} significantly decreased in the treatment of biochar addition when compared to the treatment without biochar addition.

Translocation factor value reflects plant ability to translocation metals from plant roots to shoots as shown for TF_{Cd} and TF_{Zn} in **Table 5.10**. The results show that TF_{Cd} increased with plant growth throughout the growth period. However, TF_{Cd} was less than one at all sampling periods. In addition, TF_{Cd} was greater than TF_{Zn} at all growth periods. Due to the toxic effects of Cd and Zn, green beans had limited Cd and Zn transfer from roots to shoots, considered low TF. However, plants with high BCF and low TF have good phytostabilization potential (Cui et al., 2007; Malik et al., 2010; Yoon et al., 2006). This study's results indicate that *V. radiata* L. has the ability

to retain Cd in roots and limit Cd translocation from roots to shoots, making this plant type suitable for Cd phytostabilization in Cd and Zn contaminated soil.

Table 5.10 BCF and TF of Cd and Zn in green beans planted in biochar-amended soil

Metals	Factor	Biochar apply rates (%)	Week 2	Week 4	Week 6	Week 8
Cd	BCF	0	0.41	0.96±0.07 ^{a}	0.97±0.08 ^{a}	0.46±0.02 ^{a}
		5	0.64	1.40±0.09 ^{b}	1.10±0.11 ^{a}	0.47±0.11 ^{ab}
		10	0.69	1.57±0.09 ^{b}	1.48±0.09 ^{b}	0.65±0.14 ^{ab}
		15	0.89	1.47±0.21 ^{b}	1.49±0.25 ^{b}	0.62±0.05 ^{b}
	TF	0	0.15	0.26±0.05 ^{b}	0.31±0.05 ^{ns}	0.45±0.03 ^{ns}
		5	0.11	0.19±0.03 ^{a}	0.32±0.04 ^{ns}	0.52±0.11 ^{ns}
		10	0.14	0.20±0.02 ^{a}	0.24±0.03 ^{ns}	0.41±0.09 ^{ns}
		15	0.14	0.23±0.02 ^{a}	0.27±0.04 ^{ns}	0.44±0.07 ^{ns}
Zn	BCF	0%	0.02	0.32±0.07 ^{b}	0.30±0.04 ^{ns}	0.38±0.02 ^{ns}
		5	0.03	0.22±0.03 ^{a}	0.26±0.01 ^{ns}	0.34±0.05 ^{ns}
		10	0.03	0.23±0.05 ^{a}	0.26±0.02 ^{ns}	0.35±0.02 ^{ns}
		15	0.04	0.22±0.02 ^{a}	0.26±0.03 ^{ns}	0.32±0.06 ^{ns}
	TF	0%	0.15	0.19±0.05 ^{ns}	0.23±0.05 ^{ns}	0.27±0.03 ^{ns}
		5 %	0.11	0.26±0.05 ^{ns}	0.25±0.04 ^{ns}	0.24±0.05 ^{ns}
		10 %	0.14	0.28±0.07 ^{ns}	0.27±0.04 ^{ns}	0.23±0.01 ^{ns}
		15 %	0.14	0.28±.02 ^{ns}	0.20±0.04 ^{ns}	0.24±0.06 ^{ns}

The means and the S.E. (n = 3) followed by the different superscript letters within column are significantly different ($p < 0.05$) according to Duncan's multiple range test.

5.9 Mass balances of metal distribution

Mass balances of metal distribution in soil and plant were illustrated to know the distribution of Cd and Zn ion in the contaminated soil of study area. **Table 5.11** shows the summary of metals balance in assumption that the Cd and Zn adsorbed on biochar at 15.43 and 173.33 mg/kg, respectively (from biochar leaching study). The calculation was performed at week 8 with dry weight of total plant parts. The Cd bioavailable phase consisted of the Cd (II) ion in soil solution which was exchangeable fraction and decrease when increase biochar concentration. In this study, the mass of Cd in plant was very low because plant weight was less than one gram. However, the mass balance shows metals distribution among soil and biochar. It was clearly understood that, metals were increased in biochar when increased biochar concentration and percent of available Cd were decreased. Percent of Cd in biochar was greater than Zn due to its high adsorption ability.

Table 5.11 Percentages of mass balances of metals distribution in studied soil at week 8

Metals	Biochar application rates (%)	Total metal in soil (%)		Total metal in plant (%)	Adsorbed in Biochar (%)
		Non- available metals	Available metals		
Cd	0	80.67	19.32	<1	0.00
	5	82.44	16.05	<1	1.50
	10	81.62	15.03	<1	3.34
	15	78.64	15.66	<1	5.69
Zn	0	97.21	2.78	<1	0.00
	5	96.77	2.27	<1	0.96
	10	95.80	2.20	<1	2.00
	15	94.84	1.98	<1	3.17

5.10 Conclusions

A 10% biochar is the optimum concentration to promote growth and increased bean pod yield of plant. However, 15% biochar shows an adverse effect on plant growth. Cadmium concentrations in roots of *V. radiata* L. planted in soil with biochar addition were higher than that of without biochar addition. In particular, *V. radiata* L. cultivated in Cd contaminated soil with 10% biochar showed the highest Cd accumulation in the root tissue. Conversely, Zn concentrations in plant roots treated with biochar were decrease compared to those without biochar addition. The use of biochar to immobilize Cd in contaminated soil with high Zn levels should be considered because metals have a competitive property on biochar adsorption. The *V. radiata* L. offers a high potential to accumulate Cd in its root but less Cd translocation to its shoot. The positive effects of biochar on plant growth, high Cd uptake by plant roots and limited metal mobility by plant shoots indicates that Cd is the target of biochar on Cd phytostabilization. These findings recommend the use of cassava stem biochar for soil amendment during *V. radiata* L. cultivation to enhance Cd phytostabilization. *V. radiata* L. also has a good affinity to Cd uptake from soil as well as high levels of Cd accumulated in bean seed. Therefore, *V. radiata* L. seed is not suitable for consumption due to high Cd content in seed. Cd contamination seems to occur mostly with high Zn levels at a wide variety contaminated sites. Thus, these findings suggest that the application of biochar for the immobilization and phytostabilization of Cd in presence of high Zn level in soil must be considered by a case-by-case basis.

CHAPTER 6
SUMMARIES AND RECOMMENDATION

6.1 Summaries

This research proposed the uses of biochars produced from agricultural by-products as an alternative biosorbents. Thus, the prominent points of these findings could be concluded as follows:

1) The yields of both cassava stem and rice husk biochars decreased at high pyrolysis temperature and physico-chemical activation was not affected to biochar production yields.

2) Cassava stem and rice husk biochars which was produced from the pyrolysis temperature at 300ºC had higher Cd (II) ion removal efficiency from aqueous solution than those of pyrolysis temperatures at 400 and 500ºC.

3) Physico-chemical activation of cassava stem and rice husk biochars increased the efficiency of Cd (II) ion removal from aqueous solution. The percentages of Cd (II) ion removal of activated cassava stem and rice husk biochars were higher than those of non-activated cassava stem and rice husk biochars. The use of alkaline-treatment (KOH) followed by low temperature pyrolysis as the physico-chemical activation caused the changes of physical and chemical properties of biochar. The activated cassava stem and rice husk biochars prepared at pyrolysis temperature of 300ºC had the highest Cd (II) ion removal efficiency by 84 and 97%, respectively.

4) The rapid removal rates of Cd (II) ion from aqueous solution by non-activated and activated cassava stem and rice husk biochars were found within 60 min. The highest Cd removal efficiencies by both biochars were found at initial pH in range of 5-6.

5) The Langmuir and Freundlich isotherms indicated that the sorption characteristics of non-activated (C300) and activated (CA300) cassava stem biochar were fitted to both isotherm models at equilibrium concentration ($r^2 > 0.95$). The maximum adsorption capacity of non-activated (C300) and activated (CA300) cassava stem biochars calculated from Langmuir equation were 10.46 and 24.88 mg/g, respectively. For Freundlich isotherm, the sorption capacity as inferred from the

values of K_F of non-activated (C300) and activated (CA300) cassava stem biochars were 1.67 and 4.88, respectively. From Langmuir isotherm, the maximum adsorption capacity of non-activated (RH300) and activated (RHA300) rice husk biochars were 7.76 and 45.87 mg/g, respectively. For Freundlich isotherm, the sorption capacity (K_F) of non-activated (RH300) and activated (RHA300) rice husk biochars were 0.69 and 2.40, respectively.

6) The results of the green-house study about the effects of cassava stem biochar produced from 350 ºC by local biochar kiln as soil amendment on the growth of *V. radiata* L. can be concluded that 10% cassava stem biochar is the optimum concentration to promote the plant growth. The highest shoot height, root length, shoot fresh weight and root fresh weight were found in the soil supplemented with 10% cassava stem biochar.

7) After plantation of *V. radiata* L. in Cd contaminated soil with different biochar rates for 4 weeks, Cd concentrations in root tissues of *V. radiata* L. increased significantly with biochar addition. In particular, the highest Cd concentration in root was found in plant cultivated in soil with 10% biochar. In contrast to Zn accumulation, Zn concentration in plant roots was decreased with biochar addition. However, the higher Cd concentrations were found in bean seeds and bean shells in the treatments of 10 and 15% biochar addition.

8) Concentrations of Cd and Zn bioavailable forms in soil were significantly decreased with the increases of biochar concentration in soil. Biochar could reduce the Cd and Zn bioavailability in soil during the cultivation period.

9) BCF_{Cd} values of *V. radiata* L. planted in soil with biochar addition for 4 weeks were significantly higher than those of without biochar addition. Contradictorily, BCF_{Zn} values of *V. radiata* L. decreased with biochar addition. TF_{Cd} values of *V. radiata* L. decreased with biochar addition at the 4-week of plant growth and no significant different in TF_{Zn} of *V. radiata* L. in all treatments. *V. radiata* L. has the ability to retain Cd in roots and limit Cd translocation from roots to shoots. It can be concluded that a combined approach using biochar and *V. radiata* L could enhance plant growth and Cd phytostabilization.

6.2 Recommendation

According to the results from this study, the recommendation for further study could be drawn as follows:

1) This research was conducted with Cd (II) ion as single heavy metal in aqueous solution with great Cd (II) ion removal efficiency. These biochars probably have adequate adsorption efficiency to other heavy metals; therefore, the study of remove complex heavy metals from aqueous solution should be performed for further study.

2) The application of biochar for Cd biostabilization or Cd phytoremediation in other plant species should be studied because the other plant species might show difference results from *V. radiata* L. In addition, the over application of biochar caused reduction of plant growth. The optimum concentration of biochar for applying to other plants should be studied.

3) The use of biochar for stimulating phytoremediation of other heavy metals, e.g. Pb, Cu should be studied.

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APPENDIX

Appendix A: biochar yield

A1: Cassava stem biochar

Samples	Pyrolysis temperature (^{o}C)								
	300			400			500		
	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)
1	22.36	7.92	7.74	20.03	6.23	6.05	23.14	6.46	5.91
2	47.23	17.93	17.46	18.21	6.53	6.18	25.65	8.65	6.55
3	16.37	5.20	5.01	55.61	16.43	15.56	61.12	18.58	17.21

A2: Rice husk biochar

Samples	Pyrolysis temperature (^{o}C)								
	300			400			500		
	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)	Biomass weight	Biochar weight (1 st pyrolysis)	Biochar weight (2 st pyrolysis)
1	20.00	10.65	9.86	20.10	9.34	9.01	20.76	7.22	6.89
2	30.46	15.23	15.26	25.31	11.43	11.1	10.21	3.98	3.68
3	8.46	4.24	3.98	45.16	18.28	18.01	61.12	21.7	20.55

Appendix B: Cadmium removal against pH change

B1: Remaining of Cd (II) ion against pH change of cassava stem biochar (mg/L)

pH	C300			CA300		
2	8.406	8.494	8.242	8.414	8.132	8.118
3	6.694	6.462	7.266	4.462	4.740	5.318
4	4.826	5.294	5.176	2.704	3.114	3.024
5	4.042	4.426	3.984	2.404	2.844	2.380
6	4.314	4.002	3.736	2.352	2.582	2.624
7	4.398	4.324	4.054	2.456	3.142	2.744
8	4.482	4.308	4.202	2.708	2.862	3.222
9	5.246	4.916	5.022	3.282	3.618	3.582

B2: Remaining of Cd (II) ion against pH change of rice husk biochar (mg/L)

pH	RH300			RHA300		
2	9.316	9.110	9.402	9.178	9.042	9.376
3	8.450	9.756	9.306	4.376	3.994	3.404
4	8.024	9.084	8.876	2.502	3.212	3.044
5	8.422	8.202	8.116	2.376	2.348	2.710
6	8.188	8.050	8.362	2.308	2.204	2.010
7	8.636	8.220	8.182	2.492	2.504	2.224
8	8.552	7.716	8.130	2.608	2.444	2.240
9	7.096	7.082	6.224	2.896	3.120	2.524

Appendix C: Adsorption data

C1: Cadmium adsorption by cassava stem biochar (non-activated)

Contact times (min)	Types of cassava stem biochar					
	C300			C400		
0	9.15	9.392	9.158	9.15	9.392	9.158
30	5.952	5.871	6.134	5.878	5.687	5.84
60	4.637	4.247	4.2687	4.532	4.52	4.654
90	4.235	4.589	4.45	4.42	4.471	4.658
180	4.05	4.09	4.124	4.081	4.234	4.152
300	3.842	3.985	3.792	4.156	4.157	4.163
1440	3.962	3.477	3.863	4.165	3.981	4.112

Contact times (min)	Types of cassava stem biochar		
	C500		
0	9.15	9.392	9.158
30	5.985	5.863	5.791
60	5.008	5.182	5.102
90	4.704	4.982	5.132
180	4.392	4.722	4.806
300	4.226	4.549	4.664
1440	4.219	4.412	4.547

Remaining Cd (II) ion concentration in solution (mg/L)

C2: Cadmium adsorption by cassava stem biochar (Activated)

Contact times (min)	Types of cassava stem biochar					
	CA300			CA400		
0	8.812	8.832	8.978	8.812	8.832	8.978
30	3.754	3.828	3.658	4.588	4.728	4.496
60	2.312	2.716	2.534	3.78	3.704	3.318
90	1.978	2.122	2.268	3.528	3.134	2.828
180	1.382	1.556	1.692	2.646	2.516	1.98
300	1.394	1.468	1.354	2.068	1.722	1.912
1440	1.254	1.358	1.537	1.983	1.798	1.883

Contact times (min)	Types of cassava stem biochar		
	CA500		
0	8.812	8.832	8.978
30	4.023	4.352	4.651
60	3.531	3.432	3.493
90	2.982	2.878	3.152
180	2.132	2.224	2.231
300	1.765	1.952	1.825
1440	1.92	1.897	1.698

Remaining Cd (II) ion concentration in solution (mg/L)

C3: Cadmium adsorption by rice husk biochar (non-activated)

Contact times (min)	Types of cassava stem biochar					
	RH300			RH400		
0	9.15	9.392	9.158	9.15	9.392	9.158
30	8.806	9.082	8.7	8.646	8.596	8.558
60	8.696	8.64	8.664	8.55	8.52	8.52
90	8.312	8.232	8.324	8.084	8.566	8.134
180	8.232	8.152	8.122	8.062	8.006	8.046
300	8.088	8.18	8.16	7.978	7.986	8.245
1440	7.954	7.954	7.986	7.524	7.502	7.534

Contact times (min)	Types of cassava stem biochar		
	RH500		
0	9.15	9.392	9.158
30	8.38	8.398	8.452
60	8.324	8.218	8.308
90	7.872	7.99	7.928
180	7.796	7.918	7.852
300	8.058	7.4	7.7
1440	7.402	7.318	7.422

C4: Cadmium adsorption by rice husk biochar (activated)

Contact times (min)	Types of cassava stem biochar					
	RHA300			RHA400		
0	8.812	8.832	8.978	8.812	8.832	8.978
30	2.75	2.214	2.858	2.632	2.998	3.245
60	1.582	0.834	1.098	1.102	1.32	1.754
90	0.786	0.434	0.584	0.628	0.79	1.1
180	0.3	0.252	0.25	0.304	0.41	0.45
300	0.172	0.216	0.17	0.308	0.364	0.352
1440	0.076	0.13	0.112	0.328	0.356	0.216

Contact times (min)	Types of cassava stem biochar		
	RHA500		
0	8.812	8.832	8.978
30	4.892	3.552	2.778
60	3.434	2.078	1.336
90	2.61	1.33	0.736
180	1.312	0.416	0.358
300	0.722	0.378	0.348
1440	0.426	0.414	0.452

Appendix D: Biochar element compositions

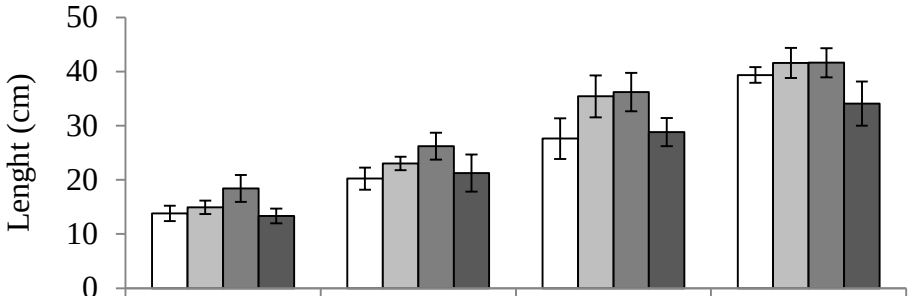
D1: Cassava stem biochar

Samples	Elements					
	C	O	Mg	P	Ca	K
C300 (1)	69.85	28.9	0.35	0.11	-	0.8
C300 (2)	75.92	23.58	0.19	0.04	-	0.27
C300 (3)	64.94	32.77	0.45	0.87	0.14	0.83
C300 (4)	71.84	27.21	0.24	0.37	0.05	0.28
AVE	70.64	28.12	0.31	0.35	0.10	0.55
STDEV	4.56	3.82	0.12	0.37	0.06	0.31
CA300 (1)	70.48	27.11	0.19	0.41	0.37	1.43
CA300 (2)	76.91	22.21	0.1	0.17	0.12	0.48
CA300 (3)	70.67	27.13	0.11	0.09	0.08	1.91
CA300 (4)	77.03	22.2	0.06	0.04	0.03	0.64
AVE	73.78	24.66	0.11	0.17	0.16	1.11
STDEV	3.69	2.84	0.054	0.16	0.15	0.67

D2: Rice husk biochar

Samples	Elements						
	C	O	Si	Mg	P	Ca	K
RH300 (1)	35.17	52.42	12.41	0.18	0.19	0.19	-
RH300 (2)	44.05	49.3	16.14	0.1	0.08	0.06	-
AVE	39.61	50.86	14.28	0.14	0.14	0.14	
STDEV	6.27	2.21	2.64	0.05	0.08	0.09	
RHA300 (1)	45.34	45.28	16.23	0.2	0.14	0.25	1.88
RHA300 (2)	46.67	43.57	8.89	0.13	0.12	0.12	0.7
AVE	46.00	44.42	12.56	0.17	0.13	0.18	1.29
STDEV	0.94	1.21	4.93	0.05	0.01	0.09	0.83

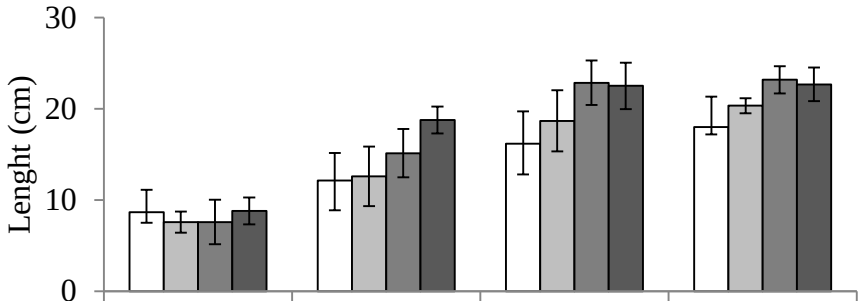
Appendix E: Plant growth data



	Week 2	Week 4	Week 6	Week 8
0%	13.79	20.22	27.61	39.35
5%	14.92	23.04	35.42	41.58
10%	18.42	26.21	36.21	41.63
15%	13.33	21.25	28.83	34.08

Cultivating periods

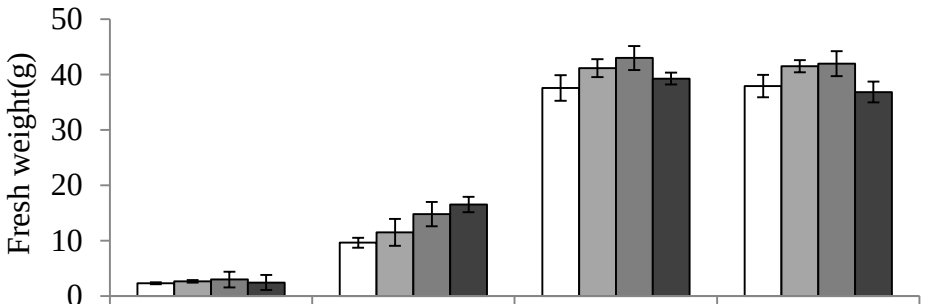
E: 1 Plant's shoot length during cultivating periods



	Week 2	Week 4	Week 6	Week 8
0%	8.67	12.14	16.17	18.00
5%	7.58	12.58	18.67	20.33
10%	7.58	15.13	22.83	23.17
15%	8.81	18.75	22.50	22.67

Cultivating periods

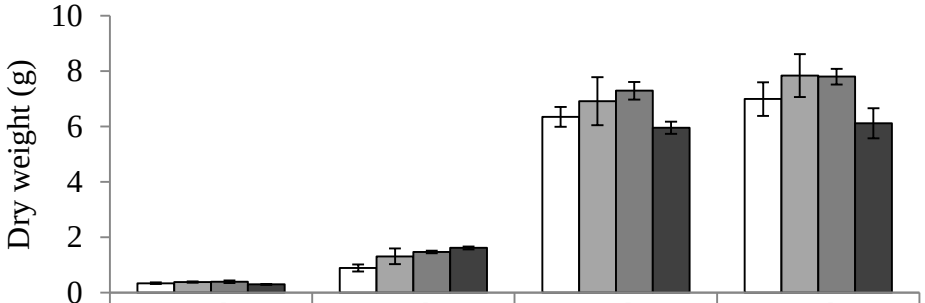
E: 2 Plant's root length during cultivating periods



	Week 2	Week 4	Week 6	Week 8
0%	2.30	9.64	37.57	37.91
5%	2.64	11.52	41.17	41.48
10%	2.99	14.80	42.98	41.97
15%	2.45	16.52	39.27	36.84

Cultivating periods

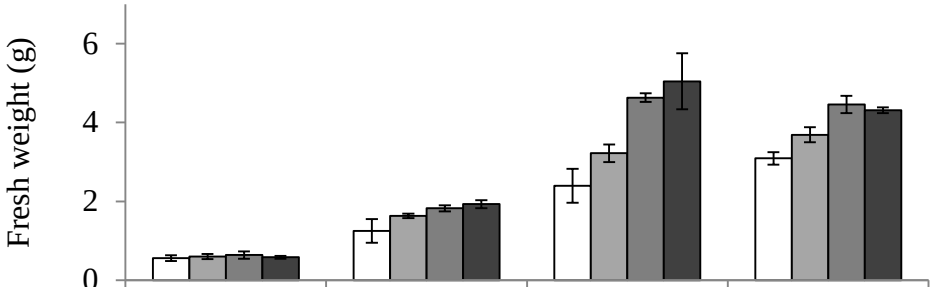
E: 3 Plant's shoot fresh weight



	Week 2	Week 4	Week 6	Week 8
0%	0.34	0.89	6.35	6.99
5%	0.38	1.31	6.92	7.84
10%	0.39	1.47	7.29	7.80
15%	0.29	1.61	5.96	6.12

Cultivating periods

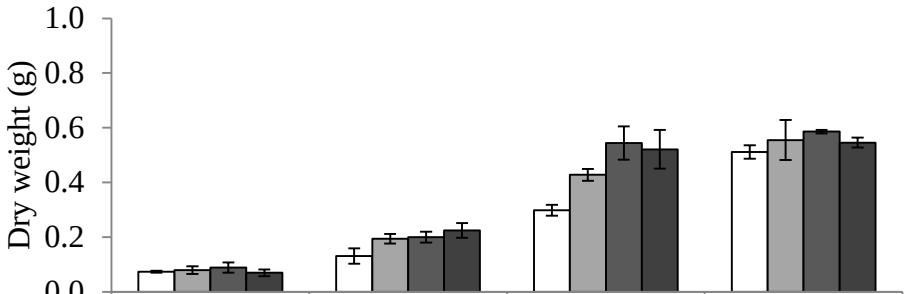
E: 4 Plant's shoot dry weight



	Week 2	Week 4	Week 6	Week 8
0%	0.56	1.25	2.40	3.09
5%	0.60	1.64	3.22	3.69
10%	0.64	1.83	4.63	4.46
15%	0.58	1.93	5.04	4.31

Cultivating periods

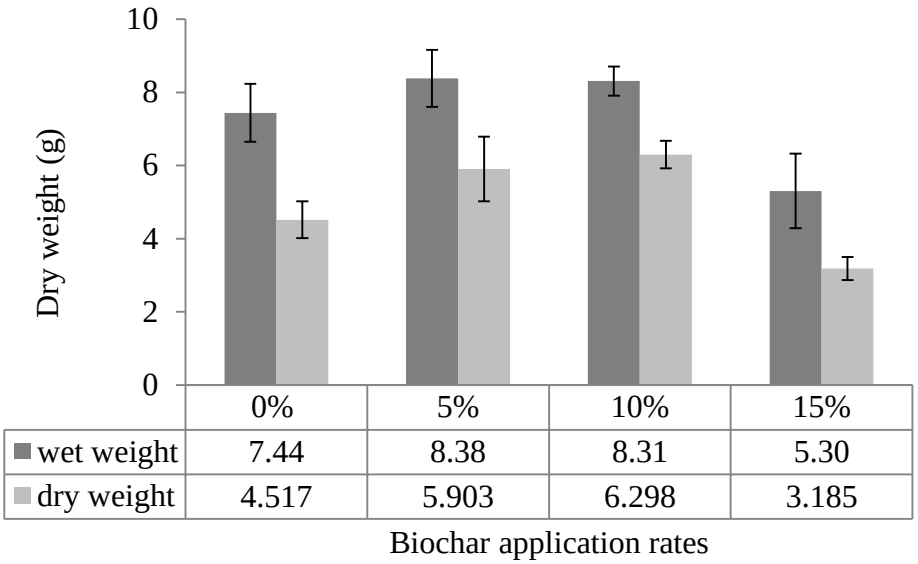
E: 5 Plant's root fresh weight



	Week 2	Week 4	Week 6	Week 8
0%	0.07	0.13	0.30	0.51
5%	0.08	0.19	0.43	0.56
10%	0.09	0.20	0.54	0.59
15%	0.07	0.23	0.52	0.55

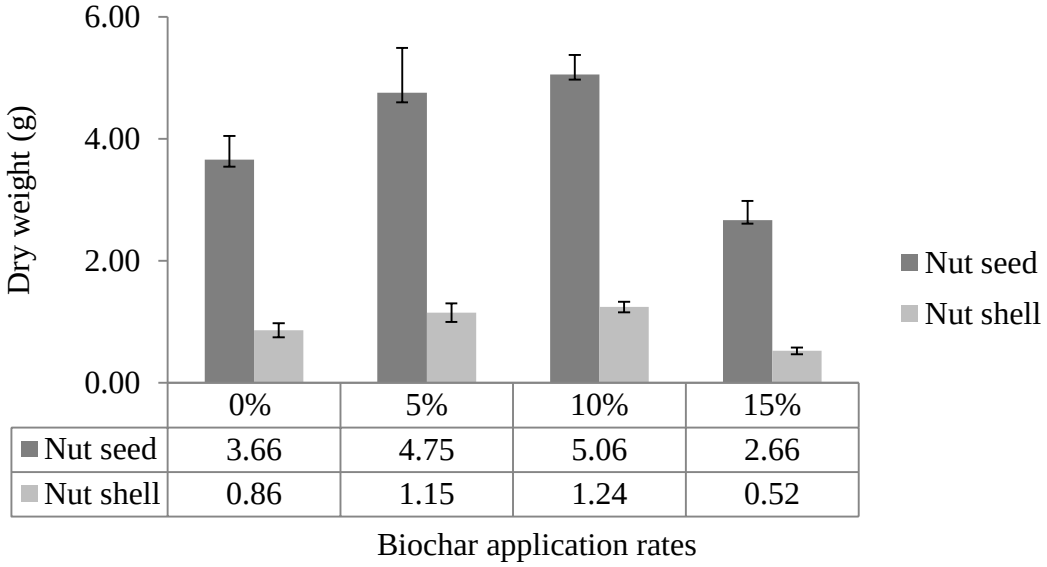
Cultivating periods

E: 6 Plant's root dry weight



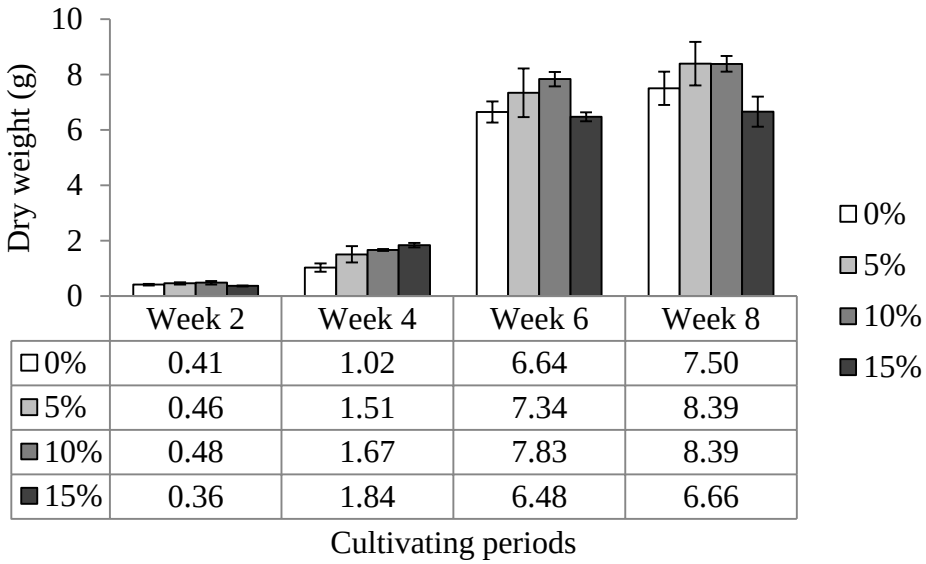
	0%	5%	10%	15%
wet weight	7.44	8.38	8.31	5.30
dry weight	4.517	5.903	6.298	3.185

E: 7 Comparation of green bean's fresh weight and dry weight (included nut shell)



	0%	5%	10%	15%
Nut seed	3.66	4.75	5.06	2.66
Nut shell	0.86	1.15	1.24	0.52

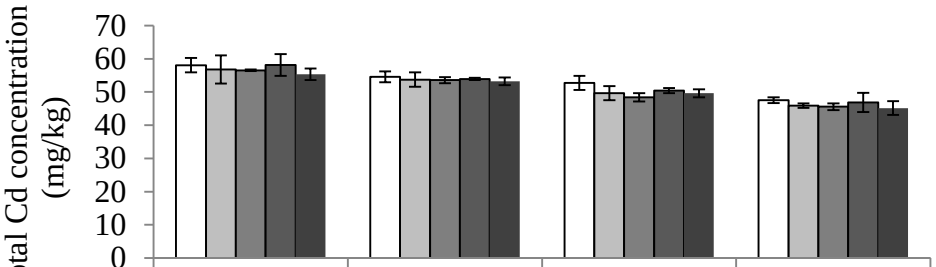
E: 8 Comparation of nut seed and nut shell weight



	Week 2	Week 4	Week 6	Week 8
0%	0.41	1.02	6.64	7.50
5%	0.46	1.51	7.34	8.39
10%	0.48	1.67	7.83	8.39
15%	0.36	1.84	6.48	6.66

E: 9 Plant’s total dry weight during cultivating periods

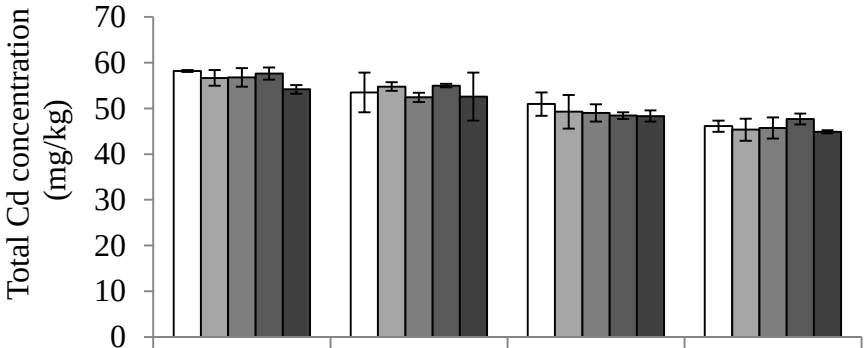
Appendix F: Metals concentration in soil



	0 %	5%	10%	15%
week 0	58.09	54.59	52.74	47.54
week 2	56.77	53.75	49.66	45.92
week 4	56.53	53.59	48.44	45.58
week 6	58.12	53.92	50.45	46.90
week 8	55.36	53.21	49.65	45.16

Biochar application rates

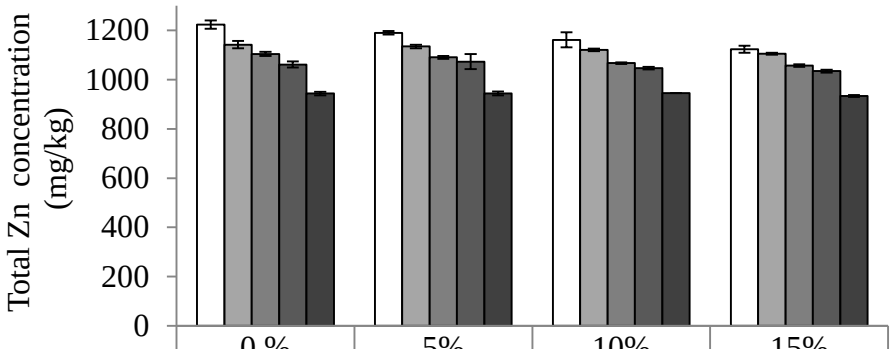
F1: Total Cd concentration of pot soil (with plant)



	0 %	5%	10%	15%
week 0	58.18	53.48	50.96	46.12
week 2	56.65	54.79	49.27	45.34
week 4	56.78	52.42	48.99	45.71
week 6	57.64	54.99	48.44	47.70
week 8	54.17	52.59	48.32	44.89

Biochar application rates

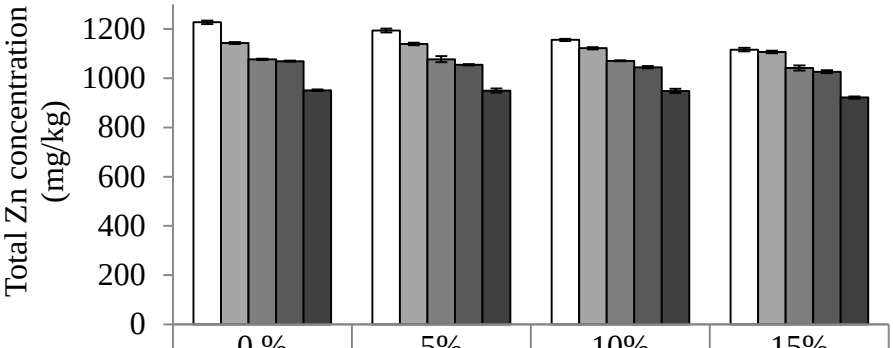
F2: Total Cd concentration of incubated soil (without plant)



	0 %	5%	10%	15%
week 0	1,223.86	1,190.24	1,161.65	1,123.40
week 2	1,142.33	1,134.67	1,121.00	1,105.33
week 4	1,104.33	1,090.67	1,067.00	1,056.67
week 6	1,061.67	1,073.33	1,046.67	1,034.67
week 8	943.63	944.17	945.37	933.93

Biochar application rates

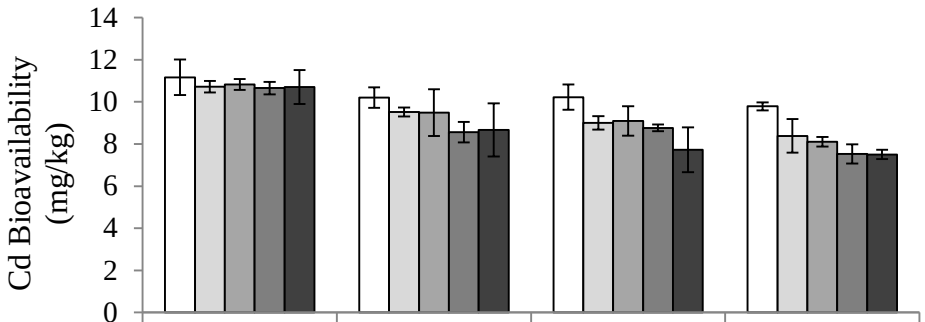
F3: Total Zn concentration of pot soil (with plant)



	0 %	5%	10%	15%
week 0	1,227.00	1,193.67	1,155.67	1,116.00
week 2	1,143.33	1,139.33	1,122.00	1,106.67
week 4	1,077.00	1,077.33	1,070.67	1,041.33
week 6	1,069.33	1,054.33	1,044.67	1,026.67
week 7	951.00	949.90	948.53	921.60

Biochar application rates

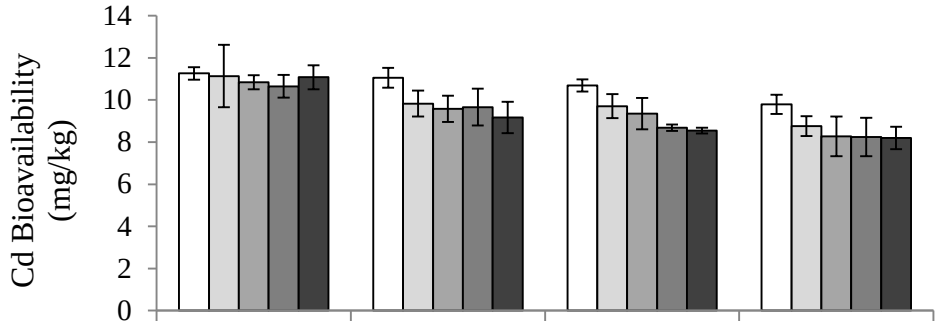
F4: Total Zn concentration of incubated soil (without plant)



	0%	5 %	10 %	15 %
week 0	11.17	10.21	10.23	9.79
week 2	10.72	9.52	9.01	8.39
week 4	10.82	9.49	9.10	8.11
week 6	10.66	8.56	8.77	7.53
week 8	10.70	8.67	7.72	7.50

Biochar application rates

F5: Cadmium bioavailability concentration of pot soil (with plant)

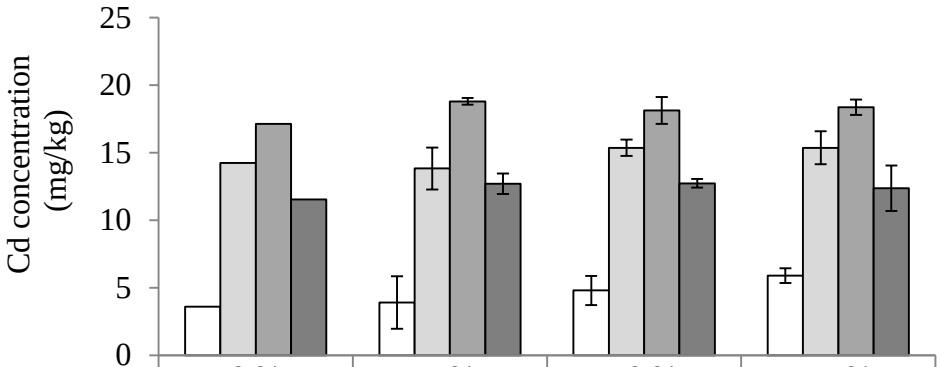


	0%	5 %	10 %	15 %
week 0	11.26	11.06	10.69	9.80
week 2	11.14	9.83	9.71	8.76
week 4	10.84	9.58	9.35	8.27
week 6	10.65	9.66	8.69	8.24
week 8	11.08	9.18	8.55	8.20

Biochar application rates

F6: Cadmium bioavailability concentration of incubated soil (without plant)

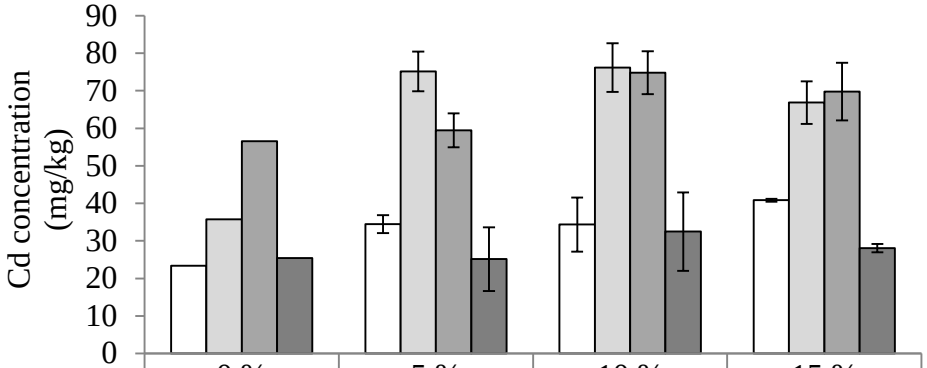
Appendix G: Metals concentration in plant parts



	0 %	5 %	10 %	15 %
week 2	3.60	3.90	4.80	5.90
week 4	14.2	13.8	15.4	15.4
week 6	17.13	18.80	18.13	18.37
week 8	11.5	12.7	12.7	12.4

Biochar application rates

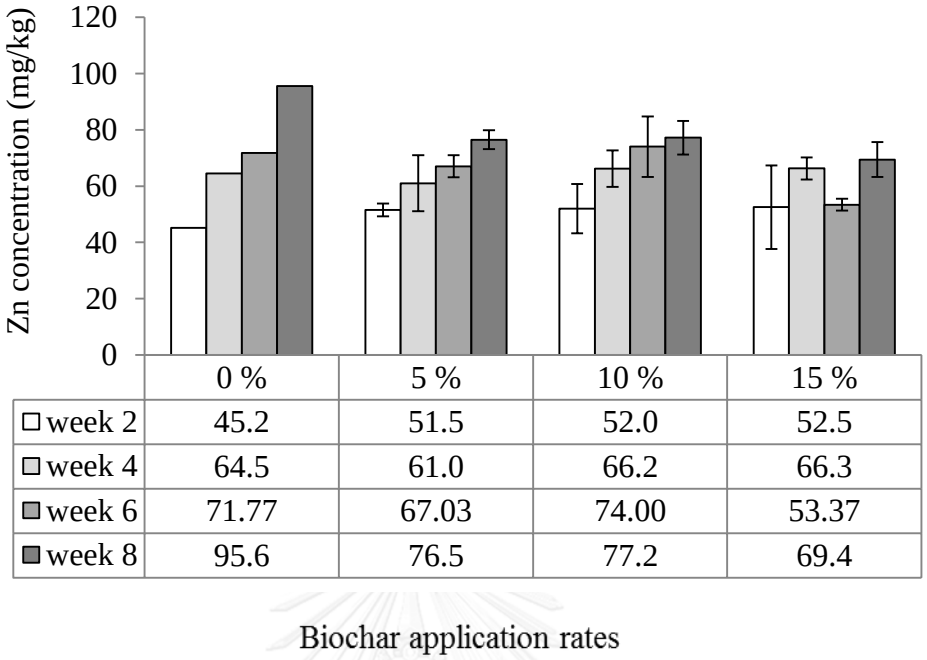
G1: Cadmium concentration in shoot



	0 %	5 %	10 %	15 %
week 2	23.4	34.5	34.3	40.8
week 4	35.8	75.2	76.2	66.9
week 6	56.56	59.44	74.81	69.78
week 8	25.4	25.1	32.5	28.0

Biochar application rates

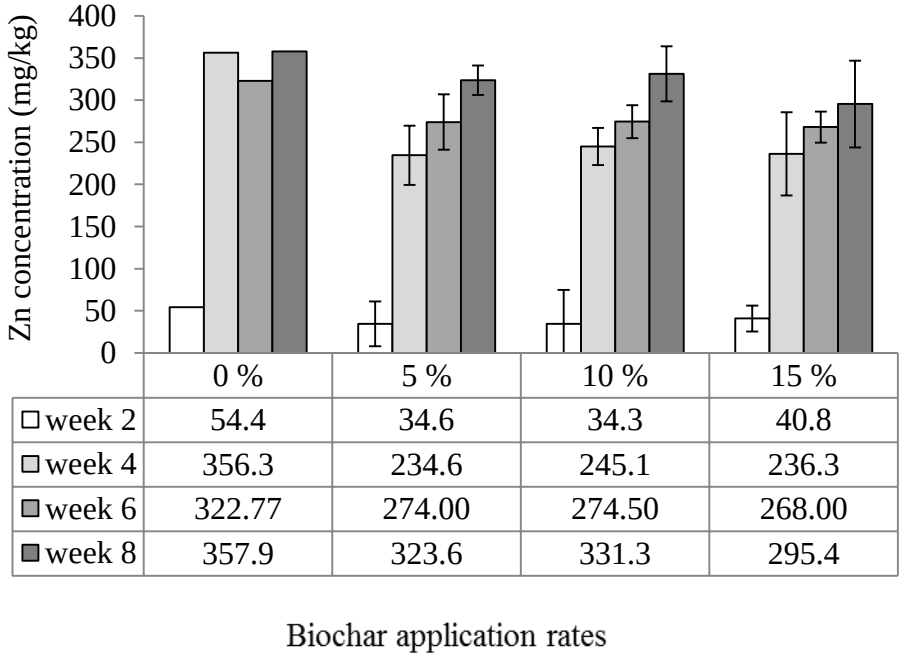
G2: Cadmium concentration in root



	0 %	5 %	10 %	15 %
week 2	45.2	51.5	52.0	52.5
week 4	64.5	61.0	66.2	66.3
week 6	71.77	67.03	74.00	53.37
week 8	95.6	76.5	77.2	69.4

Biochar application rates

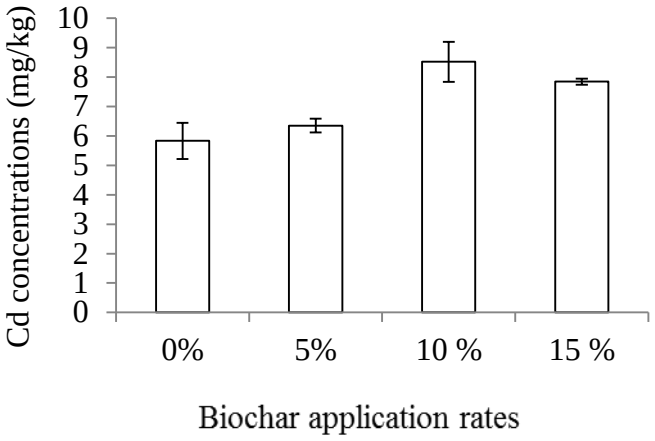
G3: Zinc concentration in shoot



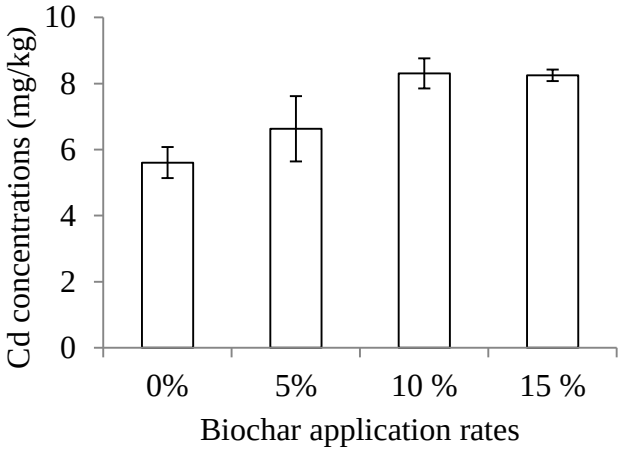
	0 %	5 %	10 %	15 %
week 2	54.4	34.6	34.3	40.8
week 4	356.3	234.6	245.1	236.3
week 6	322.77	274.00	274.50	268.00
week 8	357.9	323.6	331.3	295.4

Biochar application rates

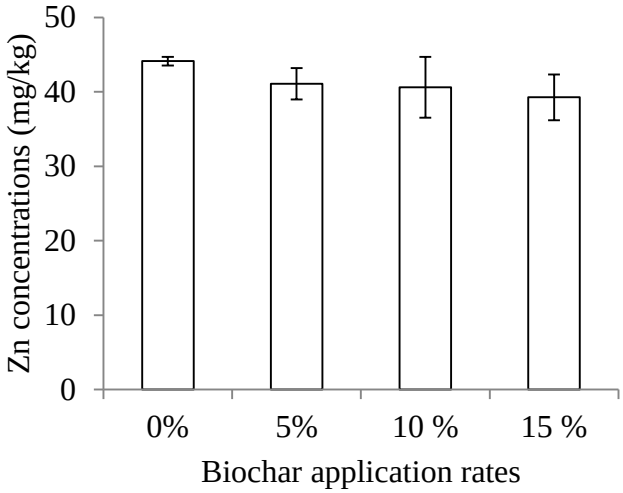
G4: Zinc concentration in root



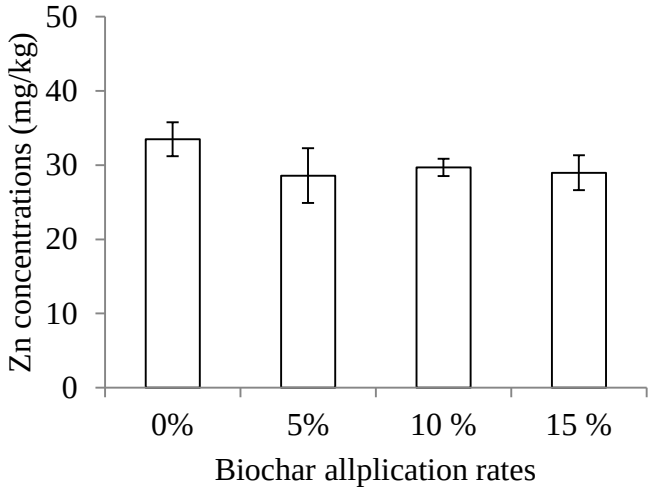
G5: Cadmium concentration in nut seed



G6: Cadmium concentration in nut shell



G7: Zinc concentration in nut seed



G8: Zinc concentration in nut shell

Appendix H: Comparison of Green bean root morphology

H1: Green bean root of 0% biochar amended soil (Week 2)

H2: Green bean root of 10% biochar amended soil (Week 2)

H3: Green bean root of 0% biochar amended soil (Week 4)

H4: Green bean root of 10% biochar amended soil (Week 4)

H5: Green bean root of 0% biochar amended soil (Week 6)

H6: Green bean root of 10% biochar amended soil (Week 6)

H7: Green bean root of 0% biochar amended soil (Week 8)

H8: Green bean root of 10% biochar amended soil (Week 8)

Appendix I: Statistical analysis (SPSS Statistic 17.0)

I1: Plant growth analysis (shoot height)

GET FILE='H:\Paper 3 plant\Cd DTPA.sav'. SAVE OUTFILE='H:\Paper 3 plant\Plant shoot growth.sav' /COMPRESSED. DATASET CLOSE DataSet4. UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 1 นาที
Comments		
Input	Data	H:\Paper 3 plant\Plant shoot growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.047

[DataSet5] H:\Paper 3 plant\Plant shoot growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47.883[a]	3	15.961	9.858	.005
Intercept	2742.163	1	2742.163	1693.565	.000
Treatment	47.883	3	15.961	9.858	.005
Error	12.953	8	1.619		
Total	2803.000	12			
Corrected Total	60.837	11			

a. R Squared = .787 (Adjusted R Squared = .707)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	13.3333	
0	3	13.8000	
5	3	14.9000	
10	3		18.4333
Sig.		.186	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.619.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 1 นาที
Comments		
Input	Data	H:\Paper 3 plant\Plant shoot growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.047

[DataSet5] H:\Paper 3 plant\Plant shoot growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	61.713[a]	3	20.571	7.338	.011
Intercept	6183.480	1	6183.480	2205.760	.000
Treatment	61.713	3	20.571	7.338	.011
Error	22.427	8	2.803		
Total	6267.620	12			
Corrected Total	84.140	11			

a. R Squared = .733 (Adjusted R Squared = .634)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	20.2667	
15	3	21.2667	
5	3	23.0333	
10	3		26.2333
Sig.		.088	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.803.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 1 นาที
Comments		
Input	Data	H:\Paper 3 plant\Plant shoot growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.062
	Elapsed Time	0:00:00.063

[DataSet5] H:\Paper 3 plant\Plant shoot growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	175.763[a]	3	58.588	5.836	.021
Intercept	12320.021	1	12320.021	1227.297	.000
Treatment	175.762	3	58.587	5.836	.021
Error	80.307	8	10.038		
Total	12576.090	12			
Corrected Total	256.069	11			

a. R Squared = .686 (Adjusted R Squared = .569)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	27.6333	
15	3	28.8667	
5	3		35.4333
10	3		36.2333
Sig.		.646	.765

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10.038.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 1 นาที
Comments		
Input	Data	H:\Paper 3 plant\Plant shoot growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.048

[DataSet5] H:\Paper 3 plant\Plant shoot growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	113.149[a]	3	37.716	49.035	.000
Intercept	18416.168	1	18416.168	23943.013	.000
Treatment	113.149	3	37.716	49.035	.000
Error	6.153	8	.769		
Total	18535.470	12			
Corrected Total	119.302	11			

a. R Squared = .948 (Adjusted R Squared = .929)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
15	3	34.1000		
0	3		39.3667	
5	3			41.6000
10	3			41.6333
Sig.		1.000	1.000	.964

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .769.

a. Uses Harmonic Mean Sample Size = 3.000.

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
15	3	34.1000		
0	3		39.3667	
5	3			41.6000
10	3			41.6333
Sig.		1.000	1.000	.964

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .769.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I2: Plant growth1analysis (shoot fresh weight)

GET FILE='F:\Paper\Paper 3 plant\Stat\Plant shoot growth.sav'. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav' /COMPRESSED. UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 11 นาฬิกา 8 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.126

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.795[a]	3	.265	3.226	.082
Intercept	80.653	1	80.653	981.176	.000
Treatment	.795	3	.265	3.226	.082
Error	.658	8	.082		
Total	82.106	12			
Corrected Total	1.453	11			

a. R Squared = .547 (Adjusted R Squared = .378)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

		Subset	
Treatment	N	1	2
0	3	2.2967	
15	3	2.4500	2.4500
5	3	2.6367	2.6367
10	3		2.9867
Sig.		.201	.059

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .082.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 11 นาฬิกา 11 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.139

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	87.258[a]	3	29.086	11.007	.003
Intercept	2065.613	1	2065.613	781.662	.000
Treatment	87.258	3	29.086	11.007	.003
Error	21.141	8	2.643		
Total	2174.012	12			
Corrected Total	108.399	11			

a. R Squared = .805 (Adjusted R Squared = .732)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	9.6400	
5	3	11.5167	
10	3		14.8000
15	3		16.5233
Sig.		.195	.230

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.643.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 11 นาฬิกา 11 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.110
	Elapsed Time	0:00:00.146

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	49.420[a]	3	16.473	4.784	.034
Intercept	19437.530	1	19437.530	5645.084	.000
Treatment	49.420	3	16.473	4.784	.034
Error	27.546	8	3.443		
Total	19514.496	12			
Corrected Total	76.966	11			

a. R Squared = .642 (Adjusted R Squared = .508)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	37.5700	
15	3	39.2633	
5	3	41.1700	41.1700
10	3		42.9833
Sig.		.052	.266

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3.443.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 11 นาฬิกา 12 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.063
	Elapsed Time	0:00:00.194

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	58.824[a]	3	19.608	5.655	.022
Intercept	18770.430	1	18770.430	5413.337	.000
Treatment	58.824	3	19.608	5.655	.022
Error	27.740	8	3.467		
Total	18856.993	12			
Corrected Total	86.563	11			

a. R Squared = .680 (Adjusted R Squared = .559)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	36.8433	
0	3	37.9067	
5	3		41.4800
10	3		41.9700
Sig.		.504	.755

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3.467.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I3: Plant growth analysis (root length)

SAVE OUTFILE='H:\Paper 3 plant\plant root growth.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\plant root growth.sav' /COMPRESSED. UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 7 นาที
Comments		
Input	Data	H:\Paper 3 plant\plant root growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.031

[DataSet5] H:\Paper 3 plant\plant root growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.110[a]	3	1.370	1.150	.386
Intercept	803.603	1	803.603	674.824	.000
Treatment	4.110	3	1.370	1.150	.386
Error	9.527	8	1.191		
Total	817.240	12			
Corrected Total	13.637	11			

a. R Squared = .301 (Adjusted R Squared = .039)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset 1
5	3	7.6000
10	3	7.6000
0	3	8.7000
15	3	8.8333
Sig.		.229

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.191.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 7 นาที
Comments		
Input	Data	H:\Paper 3 plant\plant root growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.048

[DataSet5] H:\Paper 3 plant\plant root growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	82.647[a]	3	27.549	18.520	.001
Intercept	2581.333	1	2581.333	1735.350	.000
Treatment	82.647	3	27.549	18.520	.001
Error	11.900	8	1.487		
Total	2675.880	12			
Corrected Total	94.547	11			

a. R Squared = .874 (Adjusted R Squared = .827)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

		Subset		
Treatment	N	1	2	3
0	3	12.1667		
5	3	12.6000		
10	3		15.1333	
15	3			18.7667
Sig.		.675	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.488.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 7 นาที
Comments		
Input	Data	H:\Paper 3 plant\plant root growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.047

[DataSet5] H:\Paper 3 plant\plant root growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	92.229[a]	3	30.743	7.568	.010
Intercept	4820.021	1	4820.021	1186.467	.000
Treatment	92.229	3	30.743	7.568	.010
Error	32.500	8	4.063		
Total	4944.750	12			
Corrected Total	124.729	11			

a. R Squared = .739 (Adjusted R Squared = .642)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	16.1667	
5	3	18.6667	
15	3		22.5000
10	3		22.8333
Sig.		.167	.845

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4.063.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 11 นาฬิกา 7 นาที
Comments		
Input	Data	H:\Paper 3 plant\plant root growth.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.046
	Elapsed Time	0:00:00.063

[DataSet5] H:\Paper 3 plant\plant root growth.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	50.729[a]	3	16.910	7.958	.009
Intercept	5313.021	1	5313.021	2500.245	.000
Treatment	50.729	3	16.910	7.958	.009
Error	17.000	8	2.125		
Total	5380.750	12			
Corrected Total	67.729	11			

a. R Squared = .749 (Adjusted R Squared = .655)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	18.0000	
5	3	20.3333	20.3333
15	3		22.6667
10	3		23.1667
Sig.		.086	.052

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.125.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I4: Plant growth analysis (root fresh weight)

SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\plant shoot weight.sav' /COMPRESSED. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\plant root weight.sav' /COMPRESSED. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\plant root weight.sav' /COMPRESSED. UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 15 นาฬิกา 29 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant root weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.031
	Elapsed Time	0:00:00.045

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant root weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.010[a]	3	.003	.620	.622
Intercept	4.284	1	4.284	827.841	.000
Treatment	.010	3	.003	.620	.622
Error	.041	8	.005		
Total	4.335	12			
Corrected Total	.051	11			

a. R Squared = .189 (Adjusted R Squared = -.116)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset 1
0	3	.5633
15	3	.5833
5	3	.6033
10	3	.6400
Sig.		.254

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .005.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 15 นาฬิกา 29 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant root weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.063
	Elapsed Time	0:00:00.194

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant root weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.813[a]	3	.271	9.841	.005
Intercept	33.134	1	33.134	1202.673	.000
Treatment	.813	3	.271	9.841	.005
Error	.220	8	.028		
Total	34.167	12			
Corrected Total	1.034	11			

a. R Squared = .787 (Adjusted R Squared = .707)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	1.2500	
5	3		1.6367
10	3		1.8267
15	3		1.9333
Sig.		1.000	.069

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .028.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 15 นาฬิกา 30 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant root weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.248

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant root weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.587[a]	3	4.529	24.041	.000
Intercept	175.415	1	175.415	931.157	.000
Treatment	13.587	3	4.529	24.041	.000
Error	1.507	8	.188		
Total	190.509	12			
Corrected Total	15.094	11			

a. R Squared = .900 (Adjusted R Squared = .863)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
0	3	2.4000		
5	3		3.2200	
10	3			4.6300
15	3			5.0433
Sig.		1.000	1.000	.277

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .188.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 15 นาฬิกา 30 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\plant root weight.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.232

[DataSet1] F:\Paper\Paper 3 plant\Stat\plant root weight.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.532[a]	3	1.177	41.158	.000
Intercept	181.274	1	181.274	6336.410	.000
Treatment	3.532	3	1.177	41.158	.000
Error	.229	8	.029		
Total	185.035	12			
Corrected Total	3.761	11			

a. R Squared = .939 (Adjusted R Squared = .916)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
0	3	3.0900		
5	3		3.6900	
15	3			4.3100
10	3			4.4567
Sig.		1.000	1.000	.319

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .029.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I5: Production yield (amount of total bean)

SAVE OUTFILE='H:\Paper 3 plant\amount bean pod.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\amount bean pod.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\amount bean pod.sav' /COMPRESSED. UNIANOVA number BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 12 นาฬิกา 15 นาที
Comments		
Input	Data	H:\Paper 3 plant\amount bean pod.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA number BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.701

[DataSet5] H:\Paper 3 plant\amount bean pod.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:number

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	158.000[a]	3	52.667	6.945	.013
Intercept	5633.333	1	5633.333	742.857	.000
Treatment	158.000	3	52.667	6.945	.013
Error	60.667	8	7.583		
Total	5852.000	12			
Corrected Total	218.667	11			

a. R Squared = .723 (Adjusted R Squared = .619)

Post Hoc Tests

Treatment

Homogeneous Subsets

number

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	17.33	
0	3	20.33	
5	3	21.67	
10	3		27.33
Sig.		.102	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.583.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I6: Production yield (amount of ripe bean)

SAVE OUTFILE='H:\Paper 3 plant\number bean pod ripe.sav' /COMPRESSED. UNIANOVA number BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 12 นาฬิกา 20 นาที
Comments		
Input	Data	H:\Paper 3 plant umber bean pod ripe.sav
	Active Dataset	DataSet5
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA number BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.062

[DataSet5] H:\Paper 3 plant\number bean pod ripe.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:number

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.333[a]	3	9.111	3.216	.083
Intercept	1200.000	1	1200.000	423.529	.000
Treatment	27.333	3	9.111	3.216	.083
Error	22.667	8	2.833		
Total	1250.000	12			
Corrected Total	50.000	11			

a. R Squared = .547 (Adjusted R Squared = .377)

Post Hoc Tests

Treatment

Homogeneous Subsets

number

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	7.67	
0	3	9.67	9.67
5	3		11.33
10	3		11.33
Sig.		.184	.278

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2.833.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I7: Cadmium concentration in plant (shoot)

SAVE OUTFILE='H:\Paper 3 plant\Cd shoot.sav' /COMPRESSED. UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 6 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd shoot.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.020

[DataSet1] H:\Paper 3 plant\Cd shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.573[a]	3	1.858	1.089	.408
Intercept	2593.080	1	2593.080	1520.125	.000
Treatment	5.573	3	1.858	1.089	.408
Error	13.647	8	1.706		
Total	2612.300	12			
Corrected Total	19.220	11			

a. R Squared = .290 (Adjusted R Squared = .024)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1
5	3	13.8333
0	3	14.2333
10	3	15.3667
15	3	15.3667
Sig.		.213

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.706.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

```
UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE
/POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
```

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 7 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd shoot.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.073

[DataSet1] H:\Paper 3 plant\Cd shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.489[a]	3	1.496	2.285	.156
Intercept	3934.941	1	3934.941	6007.543	.000
Treatment	4.489	3	1.496	2.285	.156
Error	5.240	8	.655		
Total	3944.670	12			
Corrected Total	9.729	11			

a. R Squared = .461 (Adjusted R Squared = .259)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	17.1333	
10	3	18.1333	18.1333
15	3	18.3667	18.3667
5	3		18.8000
Sig.		.111	.361

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .655.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

SAVE OUTFILE='H:\Paper 3 plant\Cd shoot.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Cd root.sav' /COMPRESSED.

I8: Cadmium concentration in plant (root)

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd root.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.063

[DataSet2] H:\Paper 3 plant\Cd root.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3233.456[a]	3	1077.819	34.194	.000
Intercept	48392.080	1	48392.080	1535.257	.000
Treatment	3233.456	3	1077.819	34.194	.000
Error	252.164	8	31.521		
Total	51877.700	12			
Corrected Total	3485.620	11			

a. R Squared = .928 (Adjusted R Squared = .901)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	35.7733	
15	3		66.8567
5	3		75.1867
10	3		76.1967
Sig.		1.000	.086

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 31.521.

a. Uses Harmonic Mean Sample Size = 3.000.

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	35.7733	
15	3		66.8567
5	3		75.1867
10	3		76.1967
Sig.		1.000	.086

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 31.521.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd root.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.048

[DataSet2] H:\Paper 3 plant\Cd root.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	662.862[a]	3	220.954	3.745	.060
Intercept	50930.361	1	50930.361	863.254	.000
Treatment	662.862	3	220.954	3.745	.060
Error	471.985	8	58.998		
Total	52065.208	12			
Corrected Total	1134.847	11			

a. R Squared = .584 (Adjusted R Squared = .428)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	56.5633	
5	3	59.4433	
15	3	69.7767	69.7767
10	3		74.8067
Sig.		.078	.446

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 58.998.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd root.sav
	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.031

[DataSet2] H:\Paper 3 plant\Cd root.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	103.527[a]	3	34.509	1.497	.288
Intercept	9252.964	1	9252.964	401.383	.000
Treatment	103.527	3	34.509	1.497	.288
Error	184.422	8	23.053		
Total	9540.912	12			
Corrected Total	287.948	11			

a. R Squared = .360 (Adjusted R Squared = .119)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1
5	3	25.1333
0	3	25.4400
15	3	28.0333
10	3	32.4667
Sig.		.117

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 23.053.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I9: Cadmium concentration in plant (bean seed)

SAVE OUTFILE='H:\Paper 3 plant\Cd seed.sav' /COMPRESSED. UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 51 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd seed.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.046

[DataSet4] H:\Paper 3 plant\Cd seed.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.206[a]	3	4.735	20.804	.000
Intercept	611.327	1	611.327	2685.774	.000
Treatment	14.206	3	4.735	20.804	.000
Error	1.821	8	.228		
Total	627.353	12			
Corrected Total	16.027	11			

a. R Squared = .886 (Adjusted R Squared = .844)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	5.8333	
5	3	6.3500	
15	3		7.8467
10	3		8.5200
Sig.		.221	.122

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .228.

a. Uses Harmonic Mean Sample Size = 3.000.

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	5.8333	
5	3	6.3500	
15	3		7.8467
10	3		8.5200
Sig.		.221	.122

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .228.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I10: Cadmium concentration in plant (bean shell)

SAVE OUTFILE='H:\Paper 3 plant\Cd seed shell.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Cd seed shell.sav' /COMPRESSED. UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 53 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd seed shell.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.046
	Elapsed Time	0:00:00.046

[DataSet4] H:\Paper 3 plant\Cd seed shell.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.550[a]	3	5.183	14.458	.001
Intercept	621.648	1	621.648	1733.945	.000
Treatment	15.550	3	5.183	14.458	.001
Error	2.868	8	.359		
Total	640.067	12			
Corrected Total	18.418	11			

a. R Squared = .844 (Adjusted R Squared = .786)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	5.6067	
5	3	6.6300	
15	3		8.2500
10	3		8.3033
Sig.		.070	.916

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .359.

a. Uses Harmonic Mean Sample Size = 3.000.

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	5.6067	
5	3	6.6300	
15	3		8.2500
10	3		8.3033
Sig.		.070	.916

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .359.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I11: Zinc concentration in plant (shoot)

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 47 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.017

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	54.783[a]	3	18.261	.557	.658
Intercept	49910.101	1	49910.101	1521.843	.000
Treatment	54.782	3	18.261	.557	.658
Error	262.367	8	32.796		
Total	50227.250	12			
Corrected Total	317.149	11			

a. R Squared = .173 (Adjusted R Squared = -.137)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

		Subset
Treatment	N	1
5	3	61.0000
0	3	64.5000
10	3	66.2000
15	3	66.2667
Sig.		.319

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 32.796.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 47 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.063

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	770.249[a]	3	256.750	3.801	.058
Intercept	53133.521	1	53133.521	786.581	.000
Treatment	770.249	3	256.750	3.801	.058
Error	540.400	8	67.550		
Total	54444.170	12			
Corrected Total	1310.649	11			

a. R Squared = .588 (Adjusted R Squared = .433)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

		Subset	
Treatment	N	1	2
15	3	53.3667	
5	3	67.0333	67.0333
0	3		71.7667
10	3		74.0000
Sig.		.076	.348

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 67.550.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of VarianceNotes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 47 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.063
	Elapsed Time	0:00:00.062

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1124.110[a]	3	374.703	5.374	.026
Intercept	76193.203	1	76193.203	1092.676	.000
Treatment	1124.110	3	374.703	5.374	.026
Error	557.847	8	69.731		
Total	77875.160	12			
Corrected Total	1681.957	11			

a. R Squared = .668 (Adjusted R Squared = .544)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

		Subset	
Treatment	N	1	2
15	3	69.4333	
5	3	76.5000	
10	3	77.2000	
0	3		95.6000
Sig.		.306	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 69.731.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I12: Zinc concentration in plant (root)

GET FILE='H:\Paper 3 plant\Zn DTPA.sav'. DATASET CLOSE DataSet2. GET FILE='H:\Paper 3 plant\Zn shoot.sav'. SAVE OUTFILE='H:\Paper 3 plant\Zn shoot.sav' /COMPRESSED. UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 43 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.046
	Elapsed Time	0:00:00.046

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31314.236[a]	3	10438.079	4.097	.049
Intercept	862477.701	1	862477.701	338.496	.000
Treatment	31314.236	3	10438.079	4.097	.049
Error	20383.753	8	2547.969		
Total	914175.690	12			
Corrected Total	51697.989	11			

a. R Squared = .606 (Adjusted R Squared = .458)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

		Subset	
Treatment	N	1	2
5	3	234.6000	
15	3	236.3333	
10	3	245.1333	
0	3		356.3000
Sig.		.812	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2547.969.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

DATASET CLOSE DataSet3. UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 43 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.031
	Elapsed Time	0:00:00.031

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5839.310[a]	3	1946.437	2.181	.168
Intercept	973446.403	1	973446.403	1090.833	.000
Treatment	5839.310	3	1946.437	2.181	.168
Error	7139.107	8	892.388		
Total	986424.820	12			
Corrected Total	12978.417	11			

a. R Squared = .450 (Adjusted R Squared = .244)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

		Subset
Treatment	N	1
15	3	268.0000
5	3	274.0000
10	3	274.5000
0	3	322.7667
Sig.		.068

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 892.388.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 43 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn shoot.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.032

[DataSet4] H:\Paper 3 plant\Zn shoot.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5944.753[a]	3	1981.584	1.401	.312
Intercept	1283671.253	1	1283671.253	907.275	.000
Treatment	5944.753	3	1981.584	1.401	.312
Error	11318.913	8	1414.864		
Total	1300934.920	12			
Corrected Total	17263.667	11			

a. R Squared = .344 (Adjusted R Squared = .098)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

		Subset
Treatment	N	1
15	3	295.4333
5	3	323.6000
10	3	331.3333
0	3	357.9000
Sig.		.093

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1414.864.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

SAVE OUTFILE='H:\Paper 3 plant\Zn root.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Zn shoot.sav' /COMPRESSED.

I13: Zinc concentration in plant (bean seed)

SAVE OUTFILE='H:\Paper 3 plant\Zn seed.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Zn seed.sav' /COMPRESSED. UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 54 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn seed.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.016

[DataSet4] H:\Paper 3 plant\Zn seed.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.907[a]	3	12.636	1.634	.257
Intercept	20441.857	1	20441.857	2642.899	.000
Treatment	37.907	3	12.636	1.634	.257
Error	61.877	8	7.735		
Total	20541.640	12			
Corrected Total	99.784	11			

a. R Squared = .380 (Adjusted R Squared = .147)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	39.2667
10	3	40.6167
5	3	41.0833
0	3	44.1267
Sig.		.079

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.735.

a. Uses Harmonic Mean Sample Size = 3.000.

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	39.2667
10	3	40.6167
5	3	41.0833
0	3	44.1267
Sig.		.079

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.735.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I14: Zinc concentration in plant (bean shell)

SAVE OUTFILE='H:\Paper 3 plant\Zn seed shell.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Zn seed shell.sav' /COMPRESSED. UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 10 นาฬิกา 55 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn seed shell.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.015

[DataSet4] H:\Paper 3 plant\Zn seed shell.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45.555[a]	3	15.185	2.358	.148
Intercept	10929.385	1	10929.385	1697.437	.000
Treatment	45.555	3	15.185	2.358	.148
Error	51.510	8	6.439		
Total	11026.450	12			
Corrected Total	97.065	11			

a. R Squared = .469 (Adjusted R Squared = .270)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1
5	3	28.5833
15	3	28.9633
10	3	29.6867
0	3	33.4833
Sig.		.057

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6.439.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I14: Concentration of Cd in soil (DTPA) available form

GET FILE='H:\Paper 3 plant\Cd DTPA 0 day.sav'. >Warning. Command name: GET FILE >SPSS Statistics system file "H:\Paper 3 plant\Cd DTPA 0 day.sav" is written in a character encoding (windows-1252) >incompatible with the current LOCALE setting. It may not be readable. >Consider changing LOCALE or setting UNICODE on. (DATA 1721) UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 08 นาฬิกา 57 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd DTPA 0 day.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.125

[DataSet1] H:\Paper 3 plant\Cd DTPA 0 day.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc0day

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.065[a]	3	1.022	3.020	.094
Intercept	1284.518	1	1284.518	3797.074	.000
Treatment	3.065	3	1.022	3.020	.094
Error	2.706	8	.338		
Total	1290.290	12			
Corrected Total	5.772	11			

a. R Squared = .531 (Adjusted R Squared = .355)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc0day

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	9.7867	
5	3	10.2050	10.2050
10	3	10.2257	10.2257
0	3		11.1673
Sig.		.401	.088

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .338.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 08 นาฬิกา 58 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd DTPA 0 day.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.046

[DataSet1] H:\Paper 3 plant\Cd DTPA 0 day.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.828[a]	3	2.943	13.622	.002
Intercept	1062.220	1	1062.220	4916.980	.000
Treatment	8.828	3	2.943	13.622	.002
Error	1.728	8	.216		
Total	1072.776	12			
Corrected Total	10.557	11			

a. R Squared = .836 (Adjusted R Squared = .775)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset		
		1	2	3
15	3	8.3860		
10	3	9.0083	9.0083	
5	3		9.5173	
0	3			10.7220
Sig.		.140	.217	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .216.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

SAVE OUTFILE='H:\Paper 3 plant\Cd DTPA 0 day.sav' /COMPRESSED. UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 08 นาฬิกา 59 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd DTPA 0 day.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.048

[DataSet1] H:\Paper 3 plant\Cd DTPA 0 day.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.389[a]	3	3.796	8.220	.008
Intercept	1055.700	1	1055.700	2285.677	.000
Treatment	11.389	3	3.796	8.220	.008
Error	3.695	8	.462		
Total	1070.785	12			
Corrected Total	15.084	11			

a. R Squared = .755 (Adjusted R Squared = .663)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,b]

Treatment	N	Subset		
		1	2	3
15	3	8.1060		
10	3	9.1000	9.1000	
5	3		9.4887	
0	3			10.8233
Sig.		.111	.504	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .462.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 9 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd DTPA 0 day.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.031
	Elapsed Time	0:00:00.063

[DataSet1] H:\Paper 3 plant\Cd DTPA 0 day.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.272[a]	3	5.091	37.049	.000
Intercept	946.004	1	946.004	6884.929	.000
Treatment	15.272	3	5.091	37.049	.000
Error	1.099	8	.137		
Total	962.375	12			
Corrected Total	16.371	11			

a. R Squared = .933 (Adjusted R Squared = .908)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset		
		1	2	3
15	3	7.5310		
5	3		8.5617	
10	3		8.7660	
0	3			10.6567
Sig.		1.000	.519	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .137.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

```
UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE
/POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
```

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 10 นาที
Comments		
Input	Data	H:\Paper 3 plant\Cd DTPA 0 day.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.062

[DataSet1] H:\Paper 3 plant\Cd DTPA 0 day.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.163[a]	3	6.388	7.463	.010
Intercept	897.282	1	897.282	1048.429	.000
Treatment	19.163	3	6.388	7.463	.010
Error	6.847	8	.856		
Total	923.291	12			
Corrected Total	26.009	11			

a. R Squared = .737 (Adjusted R Squared = .638)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset	
		1	2
15	3	7.5000	
10	3	7.7200	
5	3	8.6693	
0	3		10.6993
Sig.		.176	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .856.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

SAVE OUTFILE='H:\Paper 3 plant\Zn DTPA.sav' /COMPRESSED. SAVE OUTFILE='H:\Paper 3 plant\Zn DTPA.sav' /COMPRESSED.

```
UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3)
/INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN)
/CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
```

I14: Concentration of Zn in soil (DTPA) available form

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 44 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn DTPA.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.046
	Elapsed Time	0:00:00.204

[DataSet1] H:\Paper 3 plant\Zn DTPA.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc0day

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.237[a]	3	1.746	3.742	.060
Intercept	9315.047	1	9315.047	19966.879	.000
Treatment	5.237	3	1.746	3.742	.060
Error	3.732	8	.467		
Total	9324.016	12			
Corrected Total	8.969	11			

a. R Squared = .584 (Adjusted R Squared = .428)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc0day

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	26.8957	
10	3	27.6290	27.6290
5	3		28.3160
0	3		28.6047
Sig.		.225	.132

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .467.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 46 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn DTPA.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.032

[DataSet1] H:\Paper 3 plant\Zn DTPA.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.093[a]	3	.698	1.918	.205
Intercept	8961.944	1	8961.944	24640.628	.000
Treatment	2.093	3	.698	1.918	.205
Error	2.910	8	.364		
Total	8966.947	12			
Corrected Total	5.002	11			

a. R Squared = .418 (Adjusted R Squared = .200)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	26.6173
0	3	27.4390
10	3	27.6277
5	3	27.6287
Sig.		.090

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .364.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 53 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn DTPA.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.056

[DataSet1] H:\Paper 3 plant\Zn DTPA.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.672[a]	3	12.224	9.789	.005
Intercept	7058.302	1	7058.302	5652.600	.000
Treatment	36.672	3	12.224	9.789	.005
Error	9.989	8	1.249		
Total	7104.963	12			
Corrected Total	46.661	11			

a. R Squared = .786 (Adjusted R Squared = .706)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	22.3537	
10	3	23.4130	
5	3	24.1793	
0	3		27.0647
Sig.		.091	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.249.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 54 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn DTPA.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.047

[DataSet1] H:\Paper 3 plant\Zn DTPA.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.318[a]	3	8.773	8.714	.007
Intercept	6666.256	1	6666.256	6621.811	.000
Treatment	26.318	3	8.773	8.714	.007
Error	8.054	8	1.007		
Total	6700.628	12			
Corrected Total	34.372	11			

a. R Squared = .766 (Adjusted R Squared = .678)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

		Subset	
Treatment	N	1	2
15	3	22.1900	
5	3	22.9867	
10	3	23.0333	
0	3		26.0680
Sig.		.352	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.007.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		09-พ.ค.-2557, 09 นาฬิกา 55 นาที
Comments		
Input	Data	H:\Paper 3 plant\Zn DTPA.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.071

[DataSet1] H:\Paper 3 plant\Zn DTPA.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	82.246[a]	3	27.415	38.715	.000
Intercept	5836.062	1	5836.062	8241.424	.000
Treatment	82.246	3	27.415	38.715	.000
Error	5.665	8	.708		
Total	5923.973	12			
Corrected Total	87.911	11			

a. R Squared = .936 (Adjusted R Squared = .911)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
15	3	19.1110		
10	3		21.1823	
5	3		21.6430	
0	3			26.2760
Sig.		1.000	.521	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .708.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I15: Soil pH change

GET FILE='F:\Paper\Paper 3 plant\Stat\Plant shoot growth.sav'. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Soil pH.sav' /COMPRESSED. UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 16 นาฬิกา 8 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Soil pH.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc0day BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.023

[DataSet1] F:\Paper\Paper 3 plant\Stat\Soil pH.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc0day

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.096[a]	3	.032	4.792	.034
Intercept	747.341	1	747.341	112101.125	.000
Treatment	.096	3	.032	4.792	.034
Error	.053	8	.007		
Total	747.490	12			
Corrected Total	.149	11			

a. R Squared = .642 (Adjusted R Squared = .508)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc0day

Duncan[a,b]

Treatment	N	Subset 1	Subset 2
5	3	7.8000	
0	3	7.8333	
10	3	7.9000	7.9000
15	3		8.0333
Sig.		.188	.081

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .007.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 16 นาฬิกา 8 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Soil pH.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.036

[DataSet1] F:\Paper\Paper 3 plant\Stat\Soil pH.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.035[a]	3	.012	3.479	.070
Intercept	711.480	1	711.480	215056.927	.000
Treatment	.035	3	.012	3.479	.070
Error	.026	8	.003		
Total	711.541	12			
Corrected Total	.061	11			

a. R Squared = .566 (Adjusted R Squared = .403)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc15days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
5	3	7.6167	
15	3	7.6967	7.6967
10	3	7.7233	7.7233
0	3		7.7633
Sig.		.061	.210

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .003.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 16 นาฬิกา 8 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Soil pH.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.024

[DataSet1] F:\Paper\Paper 3 plant\Stat\Soil pH.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.161[a]	3	.054	8.483	.007
Intercept	695.097	1	695.097	110041.806	.000
Treatment	.161	3	.054	8.483	.007
Error	.051	8	.006		
Total	695.309	12			
Corrected Total	.211	11			

a. R Squared = .761 (Adjusted R Squared = .671)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
15	3	7.5000	
10	3	7.5367	
5	3	7.6067	
0	3		7.8000
Sig.		.153	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .006.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 16 นาฬิกา 8 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Soil pH.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.049

[DataSet1] F:\Paper\Paper 3 plant\Stat\Soil pH.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.038[a]	3	.013	2.781	.110
Intercept	673.051	1	673.051	148467.222	.000
Treatment	.038	3	.013	2.781	.110
Error	.036	8	.005		
Total	673.126	12			
Corrected Total	.074	11			

a. R Squared = .511 (Adjusted R Squared = .327)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
10	3	7.4267	
15	3	7.4700	7.4700
5	3	7.4800	7.4800
0	3		7.5800
Sig.		.379	.091

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .005.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		15-พ.ค.-2557, 16 นาฬิกา 9 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Soil pH.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Conc67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.000
	Elapsed Time	0:00:00.023

[DataSet1] F:\Paper\Paper 3 plant\Stat\Soil pH.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Conc67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.038[a]	3	.013	1.435	.303
Intercept	688.265	1	688.265	78658.804	.000
Treatment	.038	3	.013	1.435	.303
Error	.070	8	.009		
Total	688.372	12			
Corrected Total	.108	11			

a. R Squared = .350 (Adjusted R Squared = .106)

Post Hoc Tests

Treatment

Homogeneous Subsets

Conc67days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	7.5167
10	3	7.5200
0	3	7.6133
5	3	7.6433
Sig.		.158

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .009.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I16: Cd_{BCF} factor

GET FILE='F:\Paper\Paper 3 plant\Stat\Cd root.sav'. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Cd BCF.sav' /COMPRESSED. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Cd BCF.sav' /COMPRESSED. UNIANOVA Factor15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 09 นาฬิกา 53 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor15days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.021

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor15days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.345[a]	3	.115	153.511	.000
Intercept	5.227	1	5.227	6969.600	.000
Treatment	.345	3	.115	153.511	.000
Error	.006	8	.001		
Total	5.579	12			
Corrected Total	.351	11			

a. R Squared = .983 (Adjusted R Squared = .977)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor15days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2	Subset 3
0	3	.4133		
5	3		.6433	
10	3		.6933	
15	3			.8900
Sig.		1.000	.056	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 09 นาฬิกา 53 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.047
	Elapsed Time	0:00:00.035

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.663[a]	3	.221	13.054	.002
Intercept	21.870	1	21.870	1291.535	.000
Treatment	.663	3	.221	13.054	.002
Error	.135	8	.017		
Total	22.669	12			
Corrected Total	.799	11			

a. R Squared = .830 (Adjusted R Squared = .767)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	.9567	
5	3		1.4033
15	3		1.4667
10	3		1.5733
Sig.		1.000	.163

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .017.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 09 นาฬิกา 53 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.032
	Elapsed Time	0:00:00.028

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.632[a]	3	.211	9.640	.005
Intercept	19.102	1	19.102	874.550	.000
Treatment	.632	3	.211	9.640	.005
Error	.175	8	.022		
Total	19.908	12			
Corrected Total	.806	11			

a. R Squared = .783 (Adjusted R Squared = .702)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor45days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	.9733	
5	3	1.1000	
10	3		1.4833
15	3		1.4900
Sig.		.325	.957

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .022.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 09 นาฬิกา 53 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.030

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.087[a]	3	.029	3.289	.079
Intercept	3.663	1	3.663	416.259	.000
Treatment	.087	3	.029	3.289	.079
Error	.070	8	.009		
Total	3.820	12			
Corrected Total	.157	11			

a. R Squared = .552 (Adjusted R Squared = .384)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor67days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
0	3	.4633	
5	3	.4733	.4733
15	3	.6200	.6200
10	3		.6533
Sig.		.085	.054

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .009.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I17: Zn_{BCF} factor

SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Zn BCF.sav' /COMPRESSED. UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.031
	Elapsed Time	0:00:00.019

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.023[a]	3	.008	3.449	.072
Intercept	.740	1	.740	336.379	.000
Treatment	.023	3	.008	3.449	.072
Error	.018	8	.002		
Total	.780	12			
Corrected Total	.040	11			

a. R Squared = .564 (Adjusted R Squared = .400)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
5	3	.2167	
15	3	.2233	
10	3	.2300	
0	3		.3233
Sig.		.747	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.018

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.004[a]	3	.001	1.813	.223
Intercept	.864	1	.864	1164.989	.000
Treatment	.004	3	.001	1.813	.223
Error	.006	8	.001		
Total	.874	12			
Corrected Total	.010	11			

a. R Squared = .405 (Adjusted R Squared = .181)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor45days

Duncan[a,,b]

Treatment	N	Subset 1
5	3	.2567
15	3	.2567
10	3	.2600
0	3	.3000
Sig.		.105

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 38 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn BCF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.014

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn BCF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.006[a]	3	.002	1.146	.388
Intercept	1.463	1	1.463	904.954	.000
Treatment	.006	3	.002	1.146	.388
Error	.013	8	.002		
Total	1.482	12			
Corrected Total	.018	11			

a. R Squared = .301 (Adjusted R Squared = .038)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor67days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	.3200
5	3	.3433
10	3	.3533
0	3	.3800
Sig.		.124

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Zn BCF.sav' /COMPRESSED. SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Zn TF.sav' /COMPRESSED.

I18: Cd_{TF} factor

SAVE OUTFILE='F:\Paper\Paper 3 plant\Stat\Cd TF.sav' /COMPRESSED. UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 34 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.019

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.011[a]	3	.004	3.782	.059
Intercept	.594	1	.594	604.144	.000
Treatment	.011	3	.004	3.782	.059
Error	.008	8	.001		
Total	.613	12			
Corrected Total	.019	11			

a. R Squared = .587 (Adjusted R Squared = .431)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor30days

Duncan[a,,b]

Treatment	N	Subset 1	Subset 2
5	3	.1867	
10	3	.2033	
15	3	.2333	.2333
0	3		.2667
Sig.		.119	.229

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 35 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.021

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.010[a]	3	.003	1.933	.203
Intercept	.969	1	.969	545.920	.000
Treatment	.010	3	.003	1.933	.203
Error	.014	8	.002		
Total	.994	12			
Corrected Total	.024	11			

a. R Squared = .420 (Adjusted R Squared = .203)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor45days

Duncan[a,,b]

Treatment	N	Subset 1
10	3	.2433
15	3	.2700
0	3	.3067
5	3	.3167
Sig.		.080

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 35 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Cd TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.015
	Elapsed Time	0:00:00.021

[DataSet1] F:\Paper\Paper 3 plant\Stat\Cd TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.022[a]	3	.007	1.078	.412
Intercept	2.493	1	2.493	359.626	.000
Treatment	.022	3	.007	1.078	.412
Error	.055	8	.007		
Total	2.571	12			
Corrected Total	.078	11			

a. R Squared = .288 (Adjusted R Squared = .021)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor67days

Duncan[a,,b]

Treatment	N	Subset 1
10	3	.4033
15	3	.4433
0	3	.4533
5	3	.5233
Sig.		.136

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .007.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

I19: Zn_{TF} factor

UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 41 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor30days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.000
	Elapsed Time	0:00:00.007

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor30days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.017[a]	3	.006	2.215	.164
Intercept	.770	1	.770	301.029	.000
Treatment	.017	3	.006	2.215	.164
Error	.020	8	.003		
Total	.808	12			
Corrected Total	.037	11			

a. R Squared = .454 (Adjusted R Squared = .249)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor30days

Duncan[a,,b]

Treatment	N	Subset 1
0	3	.1900
5	3	.2600
10	3	.2800
15	3	.2833
Sig.		.067

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .003.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 42 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor45days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.020

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor45days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.007[a]	3	.002	1.238	.358
Intercept	.677	1	.677	335.640	.000
Treatment	.007	3	.002	1.238	.358
Error	.016	8	.002		
Total	.701	12			
Corrected Total	.024	11			

a. R Squared = .317 (Adjusted R Squared = .061)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor45days

Duncan[a,,b]

Treatment	N	Subset 1
15	3	.2033
0	3	.2267
5	3	.2500
10	3	.2700
Sig.		.126

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.

Univariate Analysis of Variance

Notes

Output Created		16-พ.ค.-2557, 10 นาฬิกา 42 นาที
Comments		
Input	Data	F:\Paper\Paper 3 plant\Stat\Zn TF.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		UNIANOVA Factor67days BY Treatment /METHOD=SSTYPE(3) /INTERCEPT=INCLUDE /POSTHOC=Treatment(DUNCAN) /CRITERIA=ALPHA(0.05) /DESIGN=Treatment.
Resources	Processor Time	0:00:00.016
	Elapsed Time	0:00:00.018

[DataSet1] F:\Paper\Paper 3 plant\Stat\Zn TF.sav

Between-Subjects Factors

		N
Treatment	0	3
	5	3
	10	3
	15	3

Tests of Between-Subjects Effects

Dependent Variable:Factor67days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.002[a]	3	.001	.299	.826
Intercept	.720	1	.720	430.030	.000
Treatment	.002	3	.001	.299	.826
Error	.013	8	.002		
Total	.735	12			
Corrected Total	.015	11			

a. R Squared = .101 (Adjusted R Squared = -.237)

Post Hoc Tests

Treatment

Homogeneous Subsets

Factor67days

Duncan[a,,b]

Treatment	N	Subset 1
10	3	.2333
5	3	.2400
15	3	.2433
0	3	.2633
Sig.		.422

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Appendix J: Mass-balance calculation

Assumption for calculation

Biochar adsorption capacity 20.53 mg/g

			% adsorption
Cd adsorb in biochar	15.43	mg/kg	8.174401356
Zn adsorb in biochar	173.33	mg/kg	91.82559864
Total adsorb	188.76		100

Cadmium	soil weight (g)	Biochar weight (g)	Treatments	Total Cd (mg/kg)	amount Cd (mg)
	7000	0	0%	55.36	387.54
	6650	350	5%	53.21	353.85
	6300	700	10%	49.65	312.82
	5950	1050	15%	45.16	268.67

non-bioavailable mg	Soil bioavailable metals (mg/kg)	Soil bioavailable (mg)
312.64	10.70	74.90
296.20	8.67	57.65
264.18	7.72	48.64
224.05	7.50	44.63

Cd in shoot (mg/kg)	Cd in shoot (mg)	root weight (g)	Cd in root (mg/kg)	Cd in root (mg)	seed weight (g)	Cd in seed (mg/kg)
11.5	0.007	0.16	25.4	0.004	3.66	5.8
12.7	0.010	0.19	25.1	0.005	4.75	6.4
12.7	0.004	0.22	32.5	0.007	5.06	8.5
12.4	0.007	0.07	28.0	0.002	2.66	7.8

Cd in seed (mg)	shell weight (g)	Cd in shell (mg/kg)	Cd in shell (mg)	Cd in biochar (mg)	Total (mg)
0.021	0.86	5.6	0.005	0	387.58
0.030	1.15	6.6	0.008	5.4005	359.30
0.043	1.24	8.3	0.010	10.801	323.69
0.021	0.52	8.3	0.004	16.2015	284.91

Percentage of Cd (%)						
non available	available	shoot	root	bean	biochar	Total
80.67	19.32	0.00	0.00	0.01	0.00	100.00
82.44	16.05	0.00	0.00	0.01	1.50	100.00
81.62	15.03	0.00	0.00	0.02	3.34	100.00
78.64	15.66	0.00	0.00	0.01	5.69	100.00

Zinc	soil weight (g)	Biochar weight (g)	Treatments	Total Zn (mg/kg)	amount Zn (mg)
	7000	0	0%	943.63	6,605.43
	6650	350	5%	944.17	6,278.71
	6300	700	10%	945.37	5,955.81
	5950	1050	15%	933.93	5,556.90

non-bioavailable mg	Soil bioavailable metals (mg/kg)	Soil bioavailable (mg)	shoot weight (g)
6,421.50	26.28	183.93	0.61
6,134.78	21.64	143.93	0.78
5,822.36	21.18	133.45	0.28
5,443.19	19.11	113.71	0.54

Zn in shoot (mg/kg)	Zn in shoot (mg)	root weight (g)	Zn in root (mg/kg)	Zn in root (mg)	seed weight (g)	Zn in seed (mg/kg)
95.6	0.058	0.16	357.9	0.058	3.66	44.1
76.5	0.060	0.19	323.6	0.062	4.75	41.1
77.2	0.022	0.22	331.3	0.072	5.06	40.6
69.4	0.038	0.07	295.4	0.021	2.66	39.3

Zn in seed (mg)	shell weight (g)	Zn in shell (mg/kg)	Zn in shell (mg)	Zn in biochar (mg)	Total (mg)
0.161	0.86	33.5	0.029	0	6,605.74
0.195	1.15	28.6	0.033	60.6655	6,339.72
0.205	1.24	29.7	0.037	121.331	6,077.48
0.105	0.52	29.0	0.015	181.9965	5,739.08

Percentage of Zn(%)						
non available	available	shoot	root	bean	biochar	Total
97.21	2.78	0.00	0.00	0.00	0.00	100.00
96.77	2.27	0.00	0.00	0.00	0.96	100.00
95.80	2.20	0.00	0.00	0.00	2.00	100.00
94.84	1.98	0.00	0.00	0.00	3.17	100.00

VITA

Name: Mr.Songkrit Prapagdee

Date of Birth: 26 June, 1970

Place of Birth: Prachinburi, Thailand

Educational Record

- Bachelor of Science (Aquatic Science),
  Burapha University, Cholburi, Thailand, 1992
- Master of Science (Environmental science)
  Chulalongkorn University, Bangkok, Thailand, 1995

Office Address: Environmental Research Institute, Chulalongkorn University,
Pathumwan, Bangkok 10330, Thailand

Home Address: 41 Boromratchonni Soi 18, Talingchan, Bangkok 10170, Thailand

E-mail: songkrit.p@gmail.com

Scholarship:The 90th Year Chulalongkorn Scholarship, Graduate School, Chulalongkorn University.

Publications related to the thesis:

1) Prapagdee, S., Piyatiratitivorakul, S., & Petsom, A., (2014). Activation of cassava stem biochar by physico-chemical method for stimulating cadmium removal efficiency from aqueous solution. Environment Asia, 7(2), 60-69.

2) Prapagdee, S., Piyatiratitivorakul, S., & Petsom, A., (2014). Physico-chemical activation on rice husk biochar for enhancing of cadmium removal from aqueous solution. Asian Journal of Water, Environment and Pollution, (Submitted)

3) Prapagdee, S., Piyatiratitivorakul, S., Petsom, A., & Tawinteung, N., (2014). Application of biochar for enhancing cadmium and zinc phytostabilization in Vigna radiata L. cultivation. Water, Air, and Soil Pollution, 225:2233 DOI 10.1007/s11270-014-2233