

Application of Biochar for Enhancing Cadmium and Zinc Phytostabilization in *Vigna radiata* L. Cultivation

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Received: 11 July 2014 / Accepted: 14 November 2014 / Published online: 26 November 2014
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Abstract Cadmium (Cd) is a toxic and nonessential element. Because of its toxicity, Cd soil contamination is a major environmental risk to living organisms. Several studies have reported on the successful use of biochar to immobilize Cd in soil as it reduces Cd accumulation in plant parts. This research reports on the contrasting effect of biochar on enhancing Cd uptake by plants. A cassava stem biochar produced through low-temperature pyrolysis was applied to natural Cd-contaminated soil that also had a high zinc (Zn) concentration. *Vigna radiata* L. (a green bean) was grown in treatments receiving three biochar rates, i.e., 5, 10, and 15 %, respectively. The results showed that the 10 %

biochar-amended soil had a positive effect on promoting plant growth and seed yield. Unfortunately, 15 % biochar-amended soil caused an adverse effect to plant growth. Cadmium uptake by plants increased with increasing biochar application rate. Zinc uptake by plants tended to decrease with biochar application. Cadmium and Zn bioavailability in soil was significantly reduced with an increasing biochar application rate. The results also showed that the biochar-amended soil could be an alternative and cost-effective method to promote plant growth and decrease Cd mobility in soil. The ratio of Cd concentration in plant root to soil was higher than 1, while the translocation factor from root to shoot was less than 1. These results indicate that the cultivation of *V. radiata* L. coupled with biochar application is an appropriate method to enhance Cd phytostabilization efficiency of *V. radiata* L. in Cd-polluted sites.

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Keywords Cassava stem biochar · Cadmium · Zinc ·
Bioavailability · *Vigna radiata* L. · Phytostabilization

1 Introduction

Cadmium (Cd) is a nonessential, toxic element for living organisms. It is naturally present in the atmosphere, water, and soil (Gallego et al. 2012). As Cd is beneficial in various industrial processes such as electroplating, alloy manufacturing, pigments, plastics, cadmium-nickel batteries, pesticides, mining, pigments and dyes, textile operations, and refining, its use in the industry has been increasing. However, it causes an adverse

effect on people's health (Rao et al. 2010). Cadmium soil contamination occurs from natural and industrial activities. Plants can easily uptake and accumulate Cd even at low concentrations in agricultural soil (Sterckeman et al. 2011). Several plants develop mechanisms to block or eliminate Cd, as it is a nonessential and toxic element to plants (Gallego et al. 2012; White and Brown 2010). The presence of Cd in consumable plants can affect human health (Sterckeman et al. 2011). The consumption of rice containing Cd levels over the long term is a significant health issue (Simmons et al. 2005). Zinc (Zn) at lower concentration is an essential micronutrient for plants (Yadav et al. 2009). However, excess Zn becomes toxic to plants as well (Kamal et al. 2004).

Biochar is a carbon-rich porous material produced by pyrolysis and burning of biomass under conditions of limited oxygen and relatively low temperature. Biochar amendment in soil helps to increase soil fertility and promote plant growth by retaining nutrients in the soil (Houben et al. 2013a; Park et al. 2011). The effect of biochar on plant growth varied with the type of biochar and the amount of application rates (Park et al. 2011). In general, the rate of biochar application ranged between 0.2 and 20 % (Mukherjee and Lal 2014). In addition, biochar can adsorb metal ions and thus is used for heavy metal immobilization (Li et al. 2013; Park et al. 2013; Uchimiya et al. 2010). Heavy metals in soil can be adsorbed on the biochar surface, leading to a decrease in heavy metal bioavailability in soil (Trakal et al. 2011). Biochar persists in soil, while soil organic amendments used to immobilize heavy metals are subject to biodegradation (Lehmann and Joseph 2009; Bolan et al. 2014). Therefore, biochar has received more attention to achieve heavy metal immobilization. The use of biochar as a soil amendment for any specific purposes must be studied case by case, depending on the biochar characteristics and soil properties (Bolan et al. 2014; Uchimiya et al. 2011).

Phytoremediation is a relatively low-cost technology that has the potential to decontaminate soil polluted by heavy metals (Prasad 2003). However, the efficiency of plants on metal remediation depends on plant-generated biomass and several growth limiting factors such as the phytotoxicity of the heavy metals (Prasad 2003; Upadhyay et al. 2007). Many researchers have focused on the application of biochar for heavy metal stabilization in soil (Houben et al. 2013b; Trakal et al. 2011; Yadav et al. 2009). Meanwhile, a number of studies

have revealed that biochar can increase the bioavailability of some toxic metals to plants (Bolan et al. 2014). The application of biochar in heavy metal-contaminated soil for improving plant growth can also lead to an increase in heavy metal uptake by some plants. Some researches have also studied the effect of biochar on plant growth and phytoremediation efficiency (Paz-Ferreiro et al. 2014). Therefore, the study of the effect of biochar on the mobility or stabilization of heavy metals in contaminated soils should be performed (Bolan et al. 2014).

The cassava stem is an agricultural residue, and it is easy to carbonize to biochar in mass production. The aim of this study was to investigate the effect of cassava stem biochar on the efficiency of Cd and Zn phytoremediation by *Vigna radiata* L., a green bean. High levels of Cd- and Zn-contaminated soil were collected from Mae Tao subcatchment, Mae Sot District, Tak Province, Thailand. In addition, the capacity of biochar on Cd adsorption from an aqueous solution was investigated.

2 Materials and Methods

2.1 Preparation of Contaminated Soil

The contaminated soil was collected from an agricultural area at Mae Tao subcatchment. The sampling site location was N 16.673861, E 98.626007. Soil was collected at a depth of 0–20 cm, homogenized, air-dried, ground, and passed through a 2-mm sieve.

2.2 Analysis of Soil Characteristics

The physical and chemical properties of soil, including soil texture, soil pH, cation exchange capacity (CEC), % organic matter (OM), % total nitrogen (N), C/N ratio, available phosphorous (P), extractable potassium (K), extractable calcium (Ca), and extractable magnesium (Mg) were analyzed. To determine the total concentrations of Cd and Zn (acid-digested forms), soil was acid-digested using an open tube digestion method, according to standard US EPA method 3050B (1996). The digested sample was analyzed with a flame atomic adsorption spectrophotometer (FAAS, Perkin Elmer Analyst 800) at wavelengths 326.1 and 213.9 nm for Cd and Zn, respectively. To determine the concentrations of the

bioavailable forms of Cd and Zn, soil was extracted by diethylene triamine pentaacetic acid (DTPA) at the soil extractant ratio of 1:2 and analyzed with FAAS (Quevauviller et al. 1998).

2.3 Biochar Preparation and Its Adsorption Efficiency

Cassava stem was collected and air-dried for 1 month before chopping to the approximate size of 10–15 cm. Mass carbonization was carried out in a 200-L char stove under anoxic condition. The pyrolysis stove temperature was maintained at 350 °C. Carbonization was conducted for 120 min followed by cooling overnight in the stove. The biochar product was then kept in an open container to absorb moisture for further use in the pot experiment. The cassava stem biochar had the following physical and chemical properties: surface area of 6.88 m² g⁻¹, CEC of 93.57 cmol kg⁻¹, 66.7±0.12 % carbon, 2.78±0.16 % hydrogen, 0.22±0.08 % nitrogen, and 30.29±0.34 % oxygen (by difference). Cadmium was not detected in the biochar. The biochar was ground and sieved to particle sizes between 250 and 500 µm for batch static adsorption study.

The Langmuir isotherm is useful for grading the adsorption efficiency of adsorbents (Okeola and Odebunmi 2010), and the Langmuir adsorption isotherm was used to calculate Cd adsorption capacity of biochar in an aqueous solution (Kołodynska et al. 2012; Tanguank et al. 2009; Trakal et al. 2014). A batch adsorption experiment was performed in triplicate, and Cd adsorption capacity of biochar was calculated by using an isotherm model. The batch static experiment was conducted in an aqueous solution of cadmium nitrate [Cd(NO₃)₂] serving as a Cd (II) ion source. The initial concentrations of Cd(NO₃)₂ were varied from 10 to 50 mg L⁻¹. A 0.10-g biochar was accurately weighed, filled in a polyethylene (PE) filter bag, and then placed in 250-mL PE bottles containing 100 mL of Cd(NO₃)₂. The PE bottles then were shaken at 120 rpm for 60 min at room temperature. After that, the biochar bag was removed. The solution was acidified with concentrated HNO₃ to produce a pH less than 2.0. The amounts of Cd (II) ion in acidified solutions were analyzed using FAAS. Cd (II) ion adsorbed on PE filter bags was studied as a control batch to deduct from the total adsorption on biochars.

2.4 Greenhouse Pot Experiment

To study the effect of biochar on plant growth and metal uptake, a pot experiment was conducted in a greenhouse. The local green bean (*V. radiata* L.) plant strain, Kamphaeng Saen 1 (KS1), was obtained from the Department of Agriculture, Thailand. The cassava stem biochar was ground and sieved to a particle size less than 2 mm which conforms to the present soil particle size. The contaminated soil was mixed with biochar at three rates: 5, 10, and 15 % (w/w). The control treatment without biochar application was also performed. The greenhouse study was performed in triplicate, using a completely randomized design. Biochar-amended soils were 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. Each pot was randomly placed in the greenhouse for 2 weeks with daily watering at field capacity before planting. Four green bean seeds were planted in the soil at 1 cm depth. During the process, NPK fertilizer (15-15-15) was added to the soil at week 4 (the blossom stage) and week 6 (the seeding stage) (Garcia and John 1976).

2.5 Plant and Soil Analysis

Plant samples in each treatment were collected at week 2 (the beginning of growth), week 4 (the blossom stage), week 6 (the seed-filling stage), and week 8 (the harvest stage). The harvested plants were thoroughly washed with tap water before rinsing with deionized water. For plant growth determination, root length and stem height were measured. Each plant was separated into shoot (leaf and stem) and root for weighting. Plants were oven-dried to measure dry weight. Plants were acid-digested with an open tube digestion method according to the method of US EPA 3050B (1996). Cadmium and zinc concentrations in plants were analyzed using FAAS. At 8 weeks after planting, the number of bean pods per pot was recorded to determine crop yield. Soil samples were collected every 2 weeks. The soil pH and concentrations of acid-digested (total metals) and bioavailable forms of Cd and Zn were analyzed. In addition, the soil's chemical properties after 8 weeks of planting, including CEC, % OM, % N, available P, extractable K, extractable Ca, extractable Mg, and C/N ratio, were analyzed.

To evaluate plants' metal accumulation, the biological concentration factor (BCF) and translocation factor (TF) were calculated. BCF is the metal concentration ratio of metals in plant root to metal concentration in soil as given in Eq. (1), and TF is described as the ratio of metal concentration in plant shoot to metal concentration in plant root as given in Eq. (2) (Bech et al. 2012; Malik et al. 2010; Yoon et al. 2006).

$$\text{BCF} = \frac{\text{Metals in root}}{\text{Metals in soil}} \quad (1)$$

$$\text{TF} = \frac{\text{Metals in shoot}}{\text{Metals in root}} \quad (2)$$

2.6 Statistical Analysis

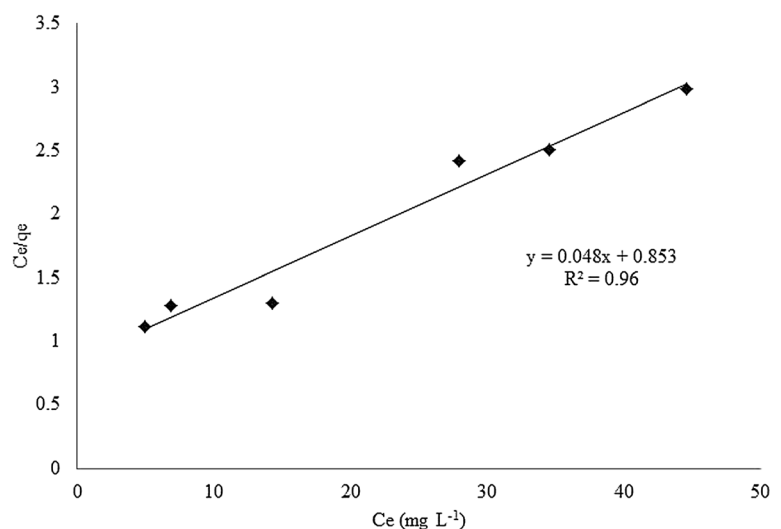
Data of all treatments were analyzed using one-way analysis of variance at 95 % confidence interval. Duncan's multiple range test was used to detect significant differences at $p < 0.05$ using the SPSS 17 program.

3 Results and Discussion

3.1 Adsorption Efficiency of Cd Ion onto Biochar

Figure 1 shows the linear adsorption isotherm of Cd (II) ion on biochar. It shows that the Cd (II) ion adsorption characteristic on the biochar surface fits well with the Langmuir isotherm. The maximum adsorption capacity

Fig. 1 Langmuir isotherm of cassava stem biochar



of biochar from the Langmuir isotherm was 20.53 mg g⁻¹. In this study, cassava stem biochar had a good Cd adsorption efficiency. A comparison of Cd adsorption capacities by various types of biochar produced from different biomaterials, pyrolysis conditions, and activation methods is presented in Table 1.

3.2 Promoting Plant Growth in Contaminated Soil by Biochar Application

The physical and chemical characteristics of soil collected from a contaminated site before planting are presented in Table 2. After directed seeding of *V. radiata* L. in contaminated soil, the percentages of seed germination of all treatments were 100 %. Table 3 shows plant growth in contaminated soil under different biochar application rates. During 2 to 6 weeks after planting, plant growth increased by increasing biochar rates. At week 8, 10 % biochar promoted plant height and fresh weights of shoot and root. In contrast, 15 % biochar caused retardation of plant growth compared to the control treatment without biochar application. In addition, plants showed early mortality symptoms with yellow leaves. It might be postulated that a high biochar rate causes an imbalance of nutrients in the soil. The numbers of total bean pods and ripe bean pods 8 weeks after planting are presented in Fig. 2. The treatment of 10 % biochar application rate had the highest number of bean pods. Meanwhile, the amounts of bean pods in the treatment of 0, 5, and 15 % biochar application showed no significant difference at $p < 0.05$. These findings indicate that the 10 % application rate of biochar in

Table 1 Adsorption capacities of biochars produced from different sources of biomass

Biochar sources	Activation processes	Carbonization temperatures (°C)	Q_{\max} (mg g ⁻¹)	Sources
Swichgrass	KOH	300	34	Regmi et al. (2012)
Pig manure	Nonactivated	600	16.6	Kołodynska et al. (2012)
Manure	–	350	51.4	Xu et al. (2013)
Nut shells	Phosphoric acid Phosphoric acid+SO ₂	475	104.17 142.86	Tajar et al. (2009)
Cashew nut shell	KOH+CO ₂	850	14.29	Tanguank et al. (2009)
Cassava stem	Nonactivated	350	20.53	This study

contaminated soil significantly promoted plant growth and bean pod production.

A high rate of biochar could thus improve soil pH in acidic soil, but over application of biochar could cause high soil alkalinity, resulting in deficiency of plant micronutrients. Zheng et al. (2013) reported that 5 % biochar was the optimum concentration for promoting the growth of maize. A 15 % biochar derived from green waste promoted the highest growth of Indian mustard (Park et al. 2011). On the other hand, Upadhyay et al. (2014) reported that the optimum rate of green waste biochar to lettuce growth was 30 ton ha⁻¹; however, the application of 100 ton ha⁻¹ green waste biochar decreased plant growth.

3.3 Effect of Biochar on Cd and Zn Uptake and Accumulation in *V. radiata* L.

Table 4 shows Cd and Zn contents accumulated in shoot and root biomass of *V. radiata* L. over a growing period.

Table 2 Physical and chemical properties of contaminated soil before planting

Property	Value
Soil texture class	Silty clay loam
pH (1:2 w/v H ₂ O)	7.9
CEC (cmol kg ⁻¹)	13
Organic matter (%)	2.20
Total nitrogen (%)	0.12
Available P (mg kg ⁻¹)	24
Extractable K (mg kg ⁻¹)	180
Extractable Ca	6256
Extractable Mg	353
C/N ratio	10.05

The results showed that Cd and Zn concentrations in roots were higher than those in shoots throughout the cultivation period. Cadmium and Zn accumulation in shoot and root biomass continued to increase with time. At week 4, Cd concentrations in shoot and root biomass were nearly 3- and 2-folds higher than those at week 2, respectively. At week 4, Cd concentration in roots with 5, 10, and 15 % biochar application increased significantly compared to the control treatment without biochar application. The highest Cd content in roots was found at week 4 with all rates of biochar application. At week 6, shoots in the treatment of 5 % biochar application had a maximum Cd accumulation. For all treatments at week 8, Cd accumulation in shoots tended to decrease by about 35 %, due to the completion of the production stage. Cadmium concentrations in bean seed and bean shell in treatments of 10 and 15 % biochar application were higher than those in 5 % biochar and without biochar applications. Cadmium concentrations in bean seed and shell of all treatments ranged between 5.6 and 8.5 mg kg⁻¹, which was much higher than the Codex standard for polished rice (0.4 mg kg⁻¹). Interestingly, Zn concentrations in the shoot and root decreased significantly with biochar application at weeks 8 and 4, respectively. However, no significant change for Zn accumulation in bean seed and bean shell in the presence of biochar was observed 8 weeks after planting.

Xian (1989) reported that Cd has greater uptake affinity 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). Our findings indicate that an increase of plant growth by biochar application promoted Cd uptake by plants. In contrast, several studies that reported on the application of biochar for heavy metal immobilization found that

Table 3 Shoot and root growth of *V. radiata* L. at different biochar rates

Plant growth	Treatment (%)	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 weights are presented in terms of total weight per pot. The means and the S.E. ($n=3$) followed by different lowercase letters within a column are significantly different ($p<0.05$) according to Duncan's multiple range test

ns not significant

biochar stimulated Cd plant uptake, but it tended to reduce Zn uptake in shoots. Regarding the effect of biochar, it can be seen that the increase of metal uptake by plants depends on heterogeneity in response to soil and plant types (Paz-Ferreiro et al. 2014). Fellet et al. (2014) used three feedstock biochars, including orchards' pruning residues, fir tree pellets, and manure

pellets for soil amendment. They found that soil amended with fir tree pellet biochar increases Cd accumulation in three plants species.

To understand the role of biochar in soil, Cd and Zn adsorbed on biochar were analyzed. After harvesting plants, biochar was separated from the soil (only in the treatment of 15 % biochar application rate) and analyzed

Fig. 2 Number of bean pods in each treatment. The error bars represent S.E. ($n=3$), and the different letters above the bar graph denote significant difference ($p<0.05$)

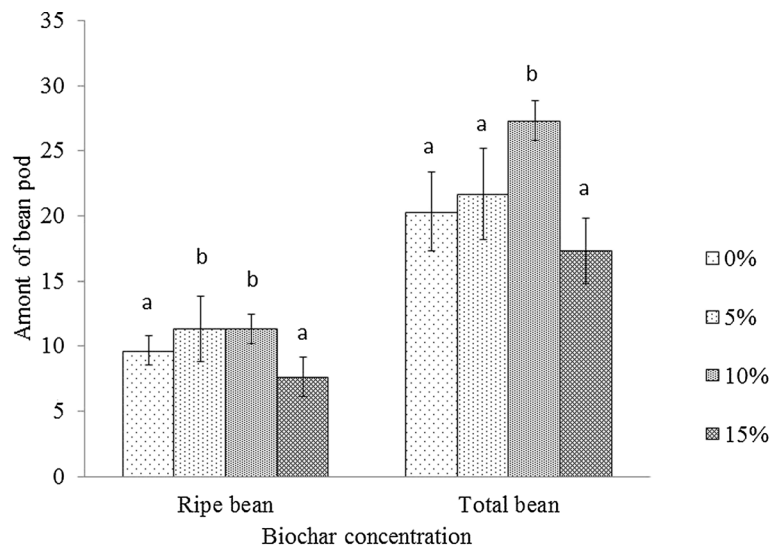


Table 4 Cadmium (Cd) and Zn concentrations in *V. radiata* L. at different growth periods

Metals	Plant parts	Treatment (%)	Metal concentration (mg kg ⁻¹)			
			Week 2 ^a	Week 4	Week 6	Week 8
Cd	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
Zn	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

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

ns not significant

^a Three replicates of plants at week 2 were combined due to less plant dry weight.

for Cd and Zn concentrations. The results showed that the concentrations of Cd and Zn adsorbed on biochar were 15.43 ± 0.06 and 173.33 ± 23.60 mg kg⁻¹, respectively. The amount of Zn adsorption on biochar was 11-fold higher than that of Cd adsorption, indicating that biochar plays an important role in Zn adsorption in high

Zn-contaminated soil. However, Zn concentration in soil (1220 mg kg⁻¹) was 21-fold higher than Cd concentration in soil (58 mg kg⁻¹), indicating that Cd has a higher adsorption affinity than Zn. The findings are similar to those of Rees et al. (2014), who reported that effective metal sorption by biochar affinity increases in

the order of lead (Pb), copper (Cu), Cd, Zn, and nickel (Ni).

3.4 Efficiency of Biochar in Reducing Cd and Zn Bioavailability in Soil

The results of the bioavailability of Cd and Zn extracted by DTPA are presented in Table 5. The results indicate that Cd and Zn bioavailability in soil was efficiently reduced by biochar application. Cd and Zn bioavailability decreased with the increase of biochar rates. Zinc bioavailability was approximately 2–3-folds higher than Cd bioavailability in all treatments. At week 8 of cultivation, Cd bioavailability in soil at all biochar rates was not significantly different, but it significantly decreased when compared to the control treatment without biochar application. Zinc bioavailability in soil with 15 % biochar application decreased significantly when compared to 5 and 10 % biochar applications. These results suggest that 5 % biochar application is a cost-effective biochar application rate to reduce Cd bioavailability.

The mechanism of heavy metal immobilization in contaminated soil depends on soil and amendment properties, the presence of organic matter, and other metals in soil that affect the bioavailability of metals (Romkens et al. 2009). Furthermore, soil OM increases the competitive adsorption mechanism of organic matter and metal ions onto a biochar surface (Chrom et al. 2013). The role of biochar in reducing the bioavailable forms of metals in soil mostly occurs from adsorption of metal ions onto biochar surfaces. Specific biochar

properties perform specific adsorption efficiency, which is induced by different metal immobilization (Park et al. 2011; Uchimiya et al. 2010). Table 6 shows the increase of available P in soil, which is important for plant growth during the blossom and seed-filling stages. The increase of available P in soil stimulated the increase of Cd accumulation in *V. radiata* L., which is similar to the findings of Panwar et al. (1999), who reported that the increase of P concentration led to an increase of Cd concentration in plants.

3.5 Changes of Chemical Properties in Biochar-Amended Soil During Cultivation

The changes of soil pH during plant growth are shown in Fig. 3a. The results indicate that soil pH increased after biochar application but then tended to decrease and stabilize after 2 weeks. At week 8, there was no significant difference of soil pH in all treatments. However, decreasing of pH in biochar-amended soil without plants was less than that in the presence of green beans (Fig. 3b). Hence, it can be indicated that plants do have an effect on soil pH. Fresh biochar application in soil usually affects soil pH. Thomson et al. (1993) reported that soil pH during cultivation of bean crops decreased due to acidification of the rhizosphere for N₂ fixation. Nitrogen for plant uptake is found in three main forms: (i) NO₃⁻ anion, (ii) NH₄⁺ cation, and (iii) nitrogen fixation from N₂ in the air. Uptake of NH₄⁺ cation by plants and N₂ fixation cause the release of H⁺ and lead to acidic soil surrounding the rhizosphere (Bolan et al.

Table 5 Concentrations of Cd and Zn bioavailable forms in soil during planting

Metals	Treatment (%)	Metal concentration (mg kg ⁻¹)			
		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 different lowercase letters within a column are significantly different ($p<0.05$) according to Duncan's multiple range test

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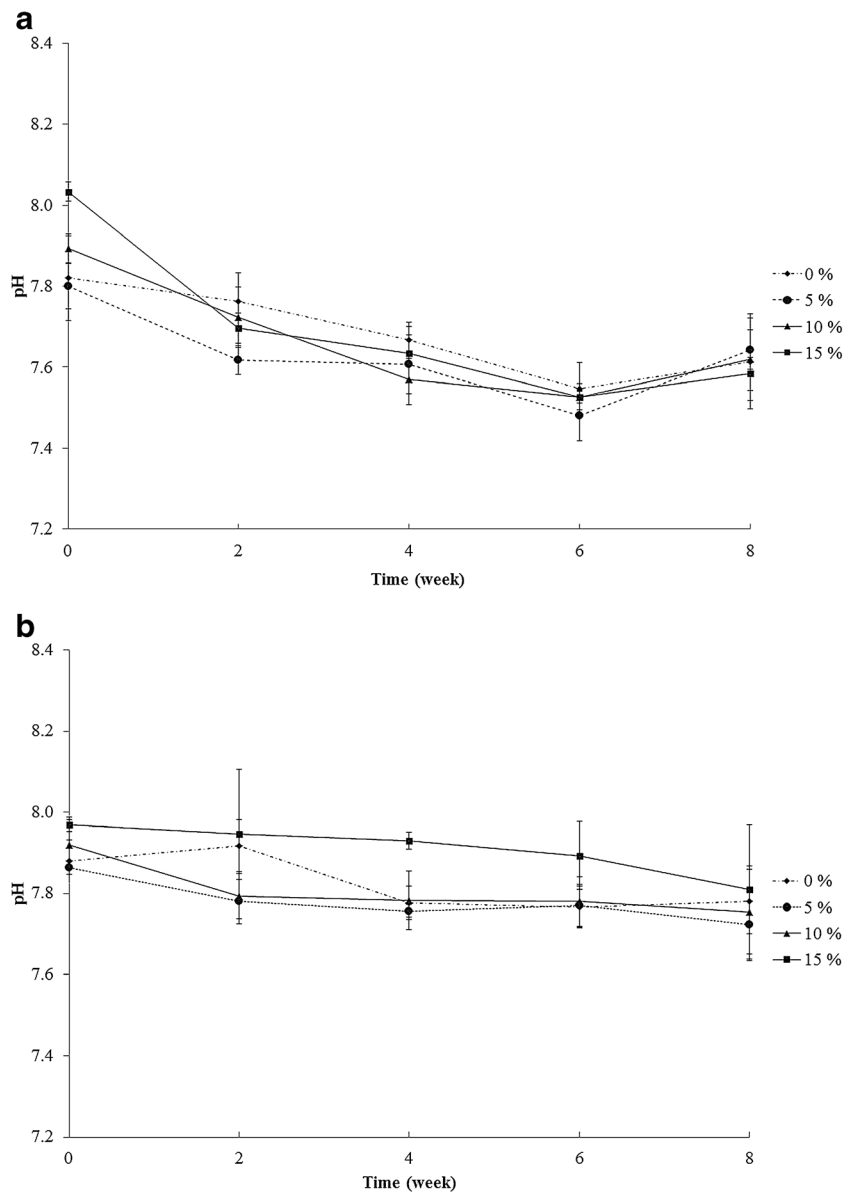
Table 6 Soil chemical properties at each biochar concentration 8 weeks after planting

Biochar rate (%)	CEC (cmol kg ⁻¹)	OM (%)	Total N (%)	Available P (mg kg ⁻¹)	Extractable K (mg kg ⁻¹)	Extractable Ca (mg kg ⁻¹)	Extractable Mg (mg kg ⁻¹)	C/N ratio
0	14.00	2.55	0.13	24	171	6185	334	11.38
5	16.00	4.44	0.17	50	459	5729	344	15.15
10	19.00	6.16	0.18	99	807	5760	415	19.85
15	23.20	8.99	0.26	151	1367	7035	674	20.06

1991). Biochar has oxidation and adsorption properties of organic acids as a result of soil pH shift (Houben et al.

2013b). In general, biochar can increase soil pH and maintain a stable soil pH level in the presence of biochar

Fig. 3 Soil pH change during *V. radiata* L. cultivation: **a** pot with plant and **b** pot without plant



carbonate contents due to its chemical buffering capacity (Cao et al. 2009; Yuan et al. 2011). In this study, the presence of rich concentrations of Zn and Cd also presented an adsorption mechanism for metals to be adsorbed onto the carbonate, which correlates with the presence of hydroxyl, carboxylate, and carbonyl groups (Lee et al. 2010). Therefore, metal adsorption on these functional groups results in less alkaline biochar-amended soil and alkaline soil property.

Soil properties are important factors that affect metal uptake by plants (Tangahu et al. 2011). The production of biochar at a low pyrolysis temperature results in high biochar yields and low ash content, which also leads to lower alkaline pH (Singh et al. 2010; Song and Guo 2012; Hassan and Aarts 2011). Table 6 presents the soil characteristics of each treatment after 8 weeks of cultivation. The soil, a silty clay loam, had a moderately fine texture soil. The soil pH of biochar amendments of 5, 10, and 15 % biochar was slightly alkaline. It was observed that soil OM increased by increasing the biochar rate. In the soil environment, biochar mobilized and released OM, N, P, and soluble K (Ameloot et al. 2013; Mukherjee and Zimmerman 2013). Organic matter served as the nutrients for microbial growth and degradation (Mclatchey and Reddy 1998). This study found

that the increase of other soil nutrients such as K, Ca, and Mg in the treatment with biochar application resulted from an increase of OM. Since CEC correlated with the increase of O and C contents, CEC in biochar-amended soil increased by increasing the biochar rate (Lee et al. 2010). Therefore, soil with biochar application had an increase in soil fertilities. These results are similar to the previous findings of Liang et al. (2006) and Houben et al. (2013a). The increase of microbial population resulted in the decomposition of organic matter by biological action; nevertheless, the increase of microbial did not result in a biotransformation process of valence change. Since Cd exists naturally only in a valence state of +2, microbial oxidation or reduction of Cd is improbable (Riser-Roberts 1998).

3.6 Performances of Biochar on Cd and Zn Accumulation and Translocation in *V. radiata* L.

Owing to the positive effects of biochar on promoting plant growth, stimulating Cd accumulation in plant root and limiting Cd accumulation in plant shoot, biochar can be used for Cd phytostabilization. Table 7 shows BCF values of Cd and Zn during plant cultivation in contaminated soil. The results showed that BCF_{Cd}

Table 7 Bioconcentration factor (BCF) and translocation factor (TF) of Cd and Zn in green beans planted in biochar-amended soil

Metals	Factor	Treatment (%)	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±0.02 ns	0.20±0.04 ns	0.24±0.06 ns

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

ns not significant

increased with plant growth, except at week 8 when plant growth declined due to a declining growth phase. BCF_{Cd} values greater than 1 were observed in the treatments with biochar application at weeks 4 and 6. BCF_{Cd} values increased with an increase in the biochar rate. These findings suggest that biochar application increases BCF_{Cd} and Cd hyperaccumulation in *V. radiata* L. during the blossom and seed-filling stages. BCF_{Zn} was lower than BCF_{Cd} at all cultivation periods. However, biochar had no effect on BCF_{Zn} , except at week 4. BCF_{Zn} significantly decreased in the treatments with biochar application compared to the control treatment at week 4 after planting.

The TF value reflects plant's ability to translocate metals from root to shoot. The results of TF_{Cd} and TF_{Zn} in *V. radiata* L. are presented in Table 6. The results show that TF_{Cd} increased with time of growth period. However, TF_{Cd} was less than 1 at all cultivation periods. At week 4, TF_{Cd} was reduced in the treatment with biochar application. In addition, TF_{Cd} was greater than TF_{Zn} at all cultivation periods. These results indicate that *V. radiata* L. has limited Cd and Zn translocation from root to shoot. However, the plant with high BCF and low TF is considered as a good phytostabilizing plant (Cui et al. 2007; Malik et al. 2010; Yoon et al. 2006). These findings suggest that *V. radiata* L. has the ability to retain Cd in its root and limit Cd translocation from root to shoot. Thus, *V. radiata* L. is suitable for Cd phytostabilization in Cd and Zn co-contaminated soil.

4 Conclusions

Cassava stem biochar improved soil fertilities by increasing organic matter and soil nutrients. The optimum rate of biochar application in soil to increase plant growth and bean pod yield was 10 %. However, 15 % biochar had an adverse effect on plant growth. Cd accumulated in *V. radiata* L. root was higher than that in its shoot. Any rate of biochar application can promote Cd accumulation in plant roots. In addition, *V. radiata* L. had less Cd translocation from root to shoot, and biochar application reduced Cd translocation from root to shoot. These findings recommend the application of biochar for soil amendment during planting to enhance Cd phytostabilization. However, bean seed contained high levels of Cd. Therefore, *V. radiata* L. seed is not suitable for consumption. Zinc was accumulated more in the root

than in the shoot. In addition, the accumulation of Zn on biochar was 11 times higher than that of Cd. These findings suggest that the application of biochar to immobilize Cd in contaminated soil with high Zn levels should be considered case by case.

Acknowledgments This research was supported by grants awarded through the 90th Year Chulalongkorn Scholarship, Graduate School, Chulalongkorn University.

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