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Effect of Biochar Amendment on Bioavailability and Accumulation of Cadmium and Trace Elements in *Brassica chinensis* L. (Chinese Cabbage)

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Abstract

This study aims to investigate the potential use of maize stalk (MS), bamboo (BB) and cow manure (CM) derived biochar as a soil amendment on the cadmium (Cd) and trace metals (Mn, Zn, Fe) accumulation by two Cd accumulator cultivars; low (Aijiaoheiye 333) (AJ) and high (Zhouyeheiyoudonger) (ZH) of *Brassica chinensis* L. The effect of different biochar (4% w/w) on both cultivars grown on artificially Cd contaminated Alfisol soil was studied. All selected biochar decreased the bioavailability of Cd in soil and its phytoavailability for both cultivars of *B. chinensis* L. followed the order: MS > CM > BB. In particular, with increase soil pH MS biochar significantly reduced Cd bioavailability in soil by 54% and accumulation in shoots of AJ and ZH cultivars by 35% and 41%, as soil amendment. These results indicated the effectiveness of biochar by reducing the availability and phytotoxicity of Cd, while enhanced shoot dry biomass by promoting microbial activity and availability of essential trace metals.

Keyword: bamboo, cow manure, maize stalk, immobilization, soil contamination, trace metal

1. Introduction

Cadmium (Cd) contamination in soil, sediment and water has become a major environmental issue due to its higher toxicity and mobility to plants and further in the food chain (Tang et al., 2009), and has become a major human health problem when those soils are used in agriculture purpose to produce food for human consumption (Lee et al., 2005; Liu et al., 2005). Due to higher mobility of Cd in soil-plant system, it is easily taken up in edible parts of plants. Leafy vegetables are commonly consumed by human as a source of food and accumulate higher concentrations of Cd as compared to other crops (Yang et al., 2010). *Brassica chinensis* L. known as Chinese cabbage is an important leafy vegetable grown and consumed throughout the world; especially in China. Chinese cabbage grown in Cd contaminated soil has higher capacity to accumulate Cd in its edible parts, hence can pose potential health risks (Yan et al., 2009). Cd minimization is set priority issue in China to avoid serious health risks and to ensure food safety. To reduce the Cd contamination through the food chain, therefore it is very important to minimize Cd pollution and its availability in soils.

Minimum Cd accumulation in edible parts of crop plants through soil amendments is a better approach that can directly affects its mobilization to avoid the human health risks attributed to Cd. Growing low Cd accumulating cultivars of crops with soil amendments may be more efficient for reducing Cd entrance in food chain as compared to growing low-Cd cultivars alone (Liu et al., 2010). There has been increasing interest in the reduction of heavy metals in soils with the application of different types of biochar and biosolids amendment (Namgay et al., 2010). Biochar are stable carbonaceous by-products synthesized through pyrolysis of organic materials (Ahmad et al., 2014). They are used as soil amendment to reduce heavy metal mobility and bioavailability (Me´ndez et al., 2012), because of having various immobilization characteristics of contaminants including active functional groups, microporous structure, high pH and cation exchange capacity (Chen & Lin, 2001; Jiang et al., 2012). The sorption process affected by functional groups of biochar depends on the nature of their surface charge so that both transition metals and non-transition metals can be occluded onto the surface of their particles (Amonette & Joseph, 2009). Biochar derived from different source materials have different characteristics that affect its performance regarding to remediation capacity of Cd. Therefore, the choice of biochar for immobilizing heavy metals, characteristics of biochar should also be taken into consideration. Several kinds of source materials can be used to make biochar; like woodchips, animal manure and crop residues (Tang et al., 2013).

Trace metals such as Zn, Mn and Fe are essential elements to promote plant growth and required to accomplish metabolic functions, such as energy metabolism, primary and secondary metabolism, cell protection, gene regulation, hormone perception, signal transduction and reproduction (Hänsch & Mendel, 2009; Hebbern et al., 2009). Fang et al. (2012) reported that Chinese cabbage grown in heavy metals contaminated soil is prone to decrease the nutrients uptake. However, biochar application significantly increases the amounts of essential trace metals and plant growth (Lehmann et al., 2003).

Due to the rapid industrialization and wide application of agrochemicals, China is now facing issues of heavy metal contamination in farmland soil. According to national survey of soil pollution from 2005-2013, about 6.3×106 km^2 of land throughout China and 19.40% of the farmland soil was polluted mainly with heavy metals (Bulletin of National Soil Pollution Survey, 2014). Alfisol is an important soil type and occupied about 1.25 million square km, about 13% of the land area in China (about the same percentage occurred as Alfisols in U.S.) (Xiao, 1992). Plants grown in Cd contaminated Alfisol soil accumulated higher Cd contents because of relatively higher percentage of sand in this soil which enhance Cd accumulation and ultimately decreased eco-physiological components such as plant growth, by interfering metabolic processes, mineral nutrition and photosynthetic activity (Sebastian & Prasad, 2014; Rafiq et al., 2014).

The effects of various biochar on immobilization of heavy metals have been studied in different soils but less information is available on plant and animal manure derived biochar effect on Cd immobilization to affirm the root uptake and shoot translocation in low and high Cd accumulation cultivars of Chinese cabbage grown in a Cd contaminated Alfisol soil. This study investigated the effects of different biochar on: 1) immobilization of Cd in a Chinese Alfisol soil, 2) their impact to availability of Zn, Mn and Fe; 3) plant growth and metals mobility in Cd accumulation contrasting cultivars of Chinese cabbage.

2. Materials and Methods

2.1 Biochar Production and Characterization

Maize stalks were collected from a local grower in Hangzhou, China; bamboo saw dust was obtained from Dalian songsen products Co. Ltd and cow manure was collected from a cattle operation in Shaoxing, Zhejiang, China. The maize stalk (MS), bamboo (BB), and air dried cow manure (CM) was oven dried at 65 °C for 48 h, prior to pyrolyzed. All biochar were produced at 500 °C pyrolysis temperature, combusted in an automated biomass pyrolyzing furnace (TH-01, China) for 2 hr at heating rate of 10^o/min. After the pyrolysis process, the biochar were ground to pass through a 0.5 mm sieve. All the biochar were analyzed for pH by adding biochar to deionized water at the ratio of 1:20 (Inyang et al., 2012). Elemental (N, C, H) analysis was performed by using an elemental analyzer (Flash-EA112, Thermo Finnigan). For the identification of surface functional groups, FTIR analysis (Nicolet 6700) was performed in the 400 and 4000 cm^{-1} region with 50 scans taken at 2 cm^{-1} resolutions. Scanning Electron Microscope (SEM) imaging analysis by using scanning microscope (FEI QUANTA FEG 650) was performed to compare the biochar structure. Surface element analysis was done at the same surface location with SEM using energy dispersive X -ray spectroscopy (EDS, EDAX Inc. Genesis XM). The specific surface areas of the biochar were measured by N_2 adsorption isotherms at 77 K with the Brunauer-Emmett-Teller (BET) method and by CO_2 isotherms at 273 K using a Quadrasorb Si-MP surface area analyzer.

2.2 Soil and Amendments

The soil (Alfisol) used in this study was collected from the upper horizon at a depth of up to 20 cm from an uncontaminated site of Shaoxing, Zhejiang, China. The soil was air-dried, grounded, screened through 2 mm sieve and analyzed for pH with a pH meter in 1:5 soil water suspensions after 30 min (Tang et al., 2015) and organic matter contents (Rashid et al., 2001). The physiochemical properties of soil are listed in Table 1.

Table 1. Physiochemical properties of soil

Soil Type	Sand %	Silt %	Clay %	OM %	Total Cd (mg kg^{-1})	Total Mn (mg kg^{-1})	Total Zn (mg kg^{-1})	Total Fe (mg kg^{-1})
Alfisol	45	25	24	3.00	0.05	60	79	70

Soil was spiked with Cd as $Cd(NO_3)_2$ in an aqueous solution at the rate of 2 mg kg^{-1}. Uncontaminated tap water was used in soil to maintain 70% water holding capacity for the period of two months. Each soil treatment had been sieved (2 mm sieve) again and total Cd concentration was determined. CM, MS and BB biochar were applied at the rate of 4% w/w to each Cd contaminated potted soil and incubated for two weeks to reach equilibrium at 60% water holding capacity (Park et al., 2011).

2.3 Mehlich-3 Extraction

Bioavailability of Cd and trace metals was determined in soils with and without biochar amendment through Mehlich-3-extraction by following the extraction method as described by Mehlich (1984). Soil samples without biochar were used as blank sample. Sample concentration in filtrate was analyzed for metals by ICP-MS (Agilent, 7500a, USA). All samples were conducted in triplicates.

2.4 Plant and Greenhouse Experiment

A pot experiment was performed under natural light in greenhouse at Zhejiang University (China). Two Cd accumulation contrasting cultivars of *B. chinensis* L. low; *Aijiaoheiye 333* (AJ) and high; *Zhouyeheiyoudonger* (ZH) Cd accumulator cultivar (Chen et al., 2012), were used for the experiment. Sieved soil (2 kg) was placed in each (20 cm × 20 cm) plastic pots set on plastic saucers. After two weeks of incubation period, 15 seeds were sown in each pot. The treatments were arranged in completely randomized design in triplicates (pots). Ten days later, uniform seedlings were thinned to three per pot. Plants were grown for two months at average temperature of 25-28 °C with regular watering. After harvesting, plants were separated into roots and shoots (including leaves and stem) and were washed with tap water and then with Milli-Q water to remove attached soil particles. The samples were blotted dry and then dried at 65 °C for 72 h. Dry weight (DW) of shoots was measured in triplicates.

2.5 DHA and BSR of Soil

At the end of the experiment, each fresh moist soil samples from pots were used to analyze soil basal respiration (SBR) and dehydrogenase activity (DHA). SBR was measured by CO_2 evolution according to procedure described by Islam and Weil (2000). Briefly, about 20 g (dry weight basis) of each fresh soil was weighted in a plastic tube (perforate d at the top for gas exchange) adjusted to 60% water holding capacity and inserted in Schott bottle containing 20 ml 0.5 M NaOH for 10 days at 25 °C in the dark. After incubation period, the CO_2 absorbed in the NaOH was immediately measured by using a TOC analyzer (Analytikjena, multi N/C 3100, China). The SBR was calculated as follows:

$$SBR = (CO_2\text{-}C_{soil} - CO_2\text{-}C_{air})/10 \text{ days} \quad (1)$$

Where, CO_2-C_{soil} is the amount of CO_2 evolved from soil and CO_2-C_{air} is the absorbed atmospheric CO_2 by 0.5 M NaOH in a blank flask.

For dehydrogenase activity (DHA) analysis, 3 g (dry weight basis) of each fresh soil sample was weighed into 50 mL sterile centrifuge tubes with 3 mL of 0.5% triphenyltetrazolium chloride (TTC) solution in 0.1 M tris buffer (pH 7.6-7.8) (Casida et al., 1964; J. Singh & D. K. Singh, 2005). After incubation for 24 h at 37 °C, 10 mL of methanol was added to each sample, shaked and centrifuged to extract triphenyl formazan (TPF) which was measured at 485 nm against blank (sterile Milli-Q water) using spectrophotometer (Shimadzu C-R3A).

2.6 Cadmium and Trace Metal Analysis

For the determination of total Cd and trace metals (Fe, Mn, Zn) concentration in soil, 0.2 g of each soil sample was digested with HNO_3-HF-$HClO_4$ (5:1:1) (Shentu et al., 2008). Whereas, for plant samples 0.2 g of dried tissue of each treatment was digested in 15 mL HCl/HNO3 /$HClO_4$ (3:1:2 v/v) at 150 °C until the solution became clear. After cooling the digest was transferred to a volumetric flask, diluted to 30 ml volume (Tang et al., 2015). Solutions were filtered and stored at 4 °C prior to analysis. Metal concentrations were analyzed in the filtrate by using ICP-MS (Agilent, 7500a). All results were conducted in triplicates.

2.7 Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test was used to compare the means of the treatments using the statistical software package SPSS (version 16.0). The mean values with standard error were presented and a $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Characteristics of Soil and Biochar

The pH of MS and CM biochar were alkaline while BB biochar had neutral pH and contains mainly C and a small proportion of N. The C content of biochar followed the order CM < MS < BB (Table 2). These results were used to calculate the atomic H:C and C:N ratios to evaluate the aromaticity of biochar.

Table 2. Physiochemical properties of biochar

Sample	pH	Component %			Atomic Ratio		SA (m^2/g)
		C	H	N	H/C	C/N	
MS	8.36	65.89	1.93	1.94	0.35	42.23	1.624
BB	6.97	80.44	2.46	1.06	0.36	95.71	6.376
CM	8.35	41.71	1.2	1.89	0.34	25.70	8.559

Note. MB, BB and CMB represents biochars derived from Maize stalk, bamboo and cow manure respectively.

The nature of functional groups was identified in MS, BB and CM by FTIR (Fourier transform infrared spectroscopy) analysis (Figure 1). According to Coates (2000), the band at 3405 cm^{-1} of MS biochar represents the hydroxyl group, OH stretch. The band at 1418 and 1593 cm^{-1} represents the C=C-C aromatic ring stretch and carboxylate bond and the band at 1418 cm^{-1} represents the vinyl C-H in plane bends of olefin, respectively. The band at 1074 cm^{-1} represents the C-I, C-N, C-F, C-O and aromatic C-H in plane bend and 821 cm^{-1} assigned the aromatic C-H out of plane bend. In BB, the band at 3415 cm^{-1} represents the H-bonded -OH stretch hydroxyl group. The bands at 1588 and 1598 cm^{-1} assigned to aromatic ring stretch and at 1163 cm^{-1} aromatic C-H in plane bend. In CM, the band at 3396 cm^{-1} represents the H-bonded -OH stretch hydroxyl group. The band at 1417 cm^{-1} assigned to vinyl C-H in plane bend, carboxylate and carbonate ion and 1077 cm^{-1} assigned to C-O-C cyclic ethers and C-O stretch, respectively. The bands at 1588 and 798 cm^{-1} represents the aromatic ring stretch and aromatic C-H out of plane bend, respectively. Surface area of the biochar ranged from 1.622 to 8.559 m^2 g^{-1} (Table 2).

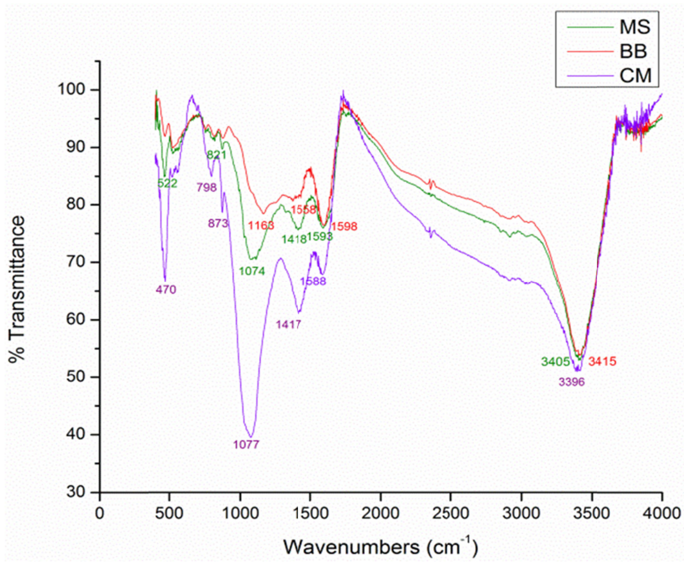
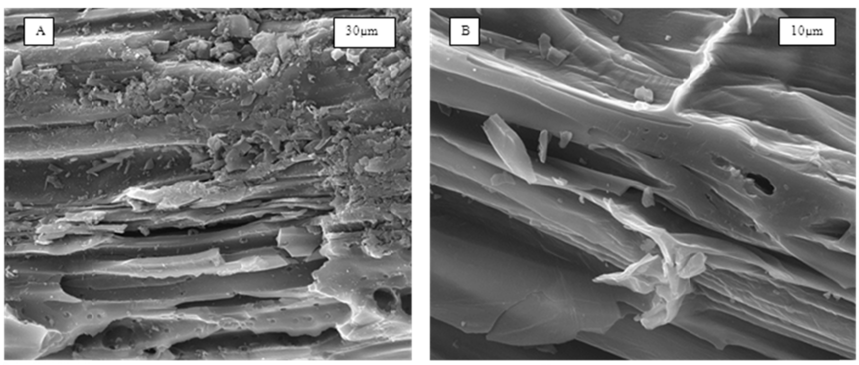
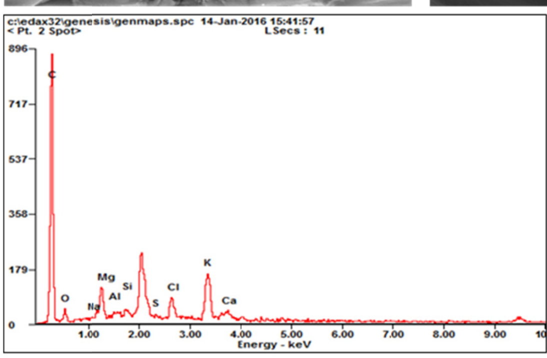


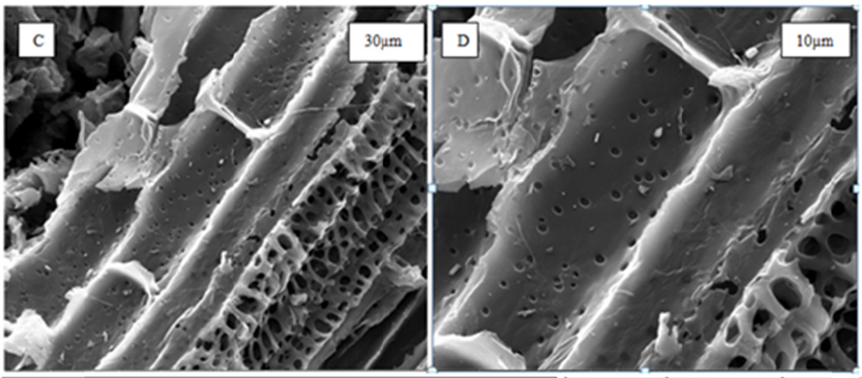
Figure 1. FTIR spectra of maize stalk (MS), bamboo (BB) and cow manure (CM) derived biochars

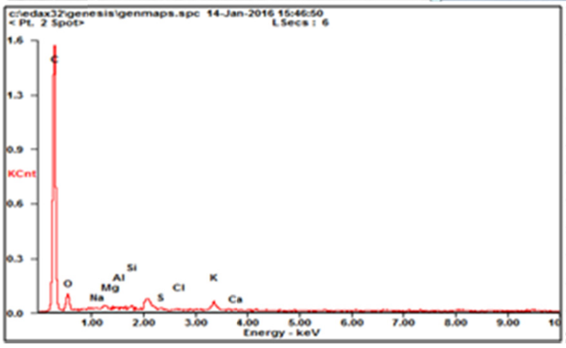
SEM images of biochar and their surface elemental analysis results are shown in Figure 2. The SEM images showed the irregular and porous surfaces of all biochar, although the MS and BB biochar showed relatively higher porous feature (Figure 2). The EDS spectrum of biochars showed that they have C and O dominated the surface of biochar with Na, Mg, Al, S, Cl and Ca present at different proportions (Figure 2).



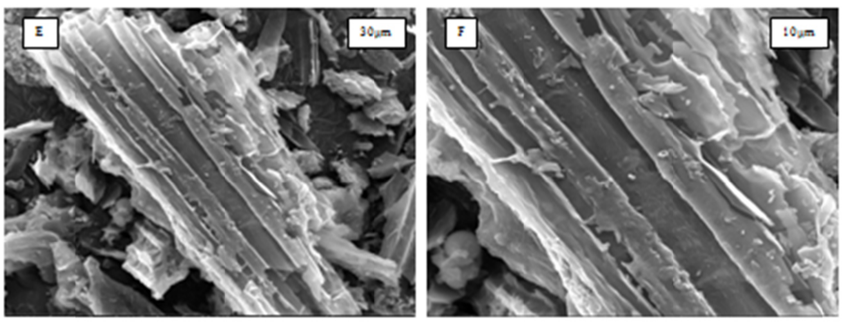


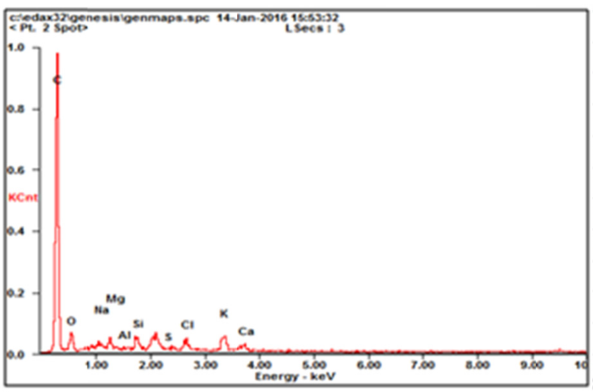
Element	*Wt%*	*At%*
C	78.20	86.95
O	09.74	08.13
Na	00.40	00.24
Mg	02.67	01.46
Al	00.23	00.11
Si	00.47	00.22
S	00.65	00.27
Cl	00.40	00.15
K	05.81	01.98
Ca	01.43	00.48
Matrix	Correction	ZAF





Element	Wt%	At%
C	82.50	88.38
O	12.01	09.66
Na	00.39	00.22
Mg	00.00	00.00
Al	00.19	00.09
Si	00.11	00.05
S	00.30	00.12
Cl	00.00	00.00
K	04.50	01.48
Ca	00.00	00.00
Matrix	Correction	ZAF





Element	Wt%	At%
C	65.20	81.35
O	05.50	05.15
Na	00.97	00.63
Mg	01.03	00.64
Al	00.23	00.13
Si	09.96	05.31
S	00.98	00.46
Cl	04.29	01.81
K	10.04	03.85
Ca	01.81	00.68
Matrix	Correction	ZAF

Figure 2. SEM images and Surface element analysis (EDS) of biochars; (A and B) maize stalk, (C and D) bamboo and (E and F) cow manure derived biochars

3.2 Change in Soil pH

Application of CM and MS biochar increased soil pH as they were alkaline in nature (Table 3). The highest mean value of pH occurred in soil treated with MS and CM biochar but BB had no effect on soil pH, while the lowest values of pH were found in the control soil untreated with biochar.

Table 3. pH, Mehlich-3 Extractable Cd and trace elements (mg kg^{-1}) with and without biochar amendments

Treatments	pH	Cd	Mn	Zn	Fe
Soil	7.33±0.03c	0.76±0.11a	35.31±1.16b	41.61±1.33c	52.24±2.18b
CM+SOIL	7.70±0.03b	0.46±0.06b	55.82±1.62a	61.09±2.04b	48.06±1.75b
BB+SOIL	7.38±0.02c	0.42±0.03b	33.16±1.68b	64.03±1.47b	46.73±1.31b
MS+SOIL	7.78±0.02a	0.35±0.04b	57.8±1.28a	77.39±1.20a	66.43±1.27a

Note. MB, BB and CM represents biochars derived from Maize stalk, bamboo and cow manure amended soil, respectively. Each value represents the mean of three replicates ± standard error, and the different letters within the same column are significantly different at $p < 0.05$.

3.3 Bioavailability of Cd and Trace Metals in Soil

Cadmium availability was significantly ($P < 0.05$) influenced by the application of biochar in soil and was very effective in reducing Cd extractability (Mehlic-3 extraction) (Table 3). Particularly, MS biochar showed higher decline in extractable Cd content by 54%, as compared to the un-amended soil. While BB and CM biochar addition reduced Cd availability by 45% and 39%, respectively.

The addition of biochar significantly ($P < 0.05$) effects the trace metal availability of Cd contaminated Alfisol soil (Table 3). The highest increase of trace metals occurred with addition of MS biochar as compared to the untreated soil by 26, 94 and 86% for Fe, Mn and Zn, respectively.

3.4 Effect of Biochar on DHA and BSR of Soil

Application of biochar also considerably ($P < 0.05$) enhanced the DHA and SBR (Table 4). Addition of MS and CM biochar had the highest increased in soil respiration. DHA also increased more significantly ($P < 0.05$) with addition of BB and CM than MS biochar.

Table 4. Microbial activity (DHA and BSR) and Dry biomass (g $plant^{-1}$) of shoots of *B. chinensis* L. cultivars Aijiaoheiye 333 (AJ) and Zhouyeheiyoudonger (ZH) grown on Alfisol soil with different biochar amendment

Soil treatments	Dry Biomass		DHA		BSR	
	AJ	ZH	AJ	ZH	AJ	ZH
SOIL	1.75± 0.07b	1.36± 0.15b	1.98±0.05c	1.98±0.04c	0.55±0.01c	0.6±0.01d
MS	2.67 ±0.21a	2.26±0.18a	2.68±0.02b	2.67±0.01b	2.77±0.02a	2.74±0.02a
BB	2.66 ±0.09a	2.26 ±0.09a	2.79±0.02a	2.8±0.01a	1.76±0.01b	1.89±0.01c
CM	2.66 ±0.15a	2.43± 0.18a	2.87±0.02a	2.85±0.02a	2.81±0.01a	2.58±0.01b

Note. MB, BB and CM represents biochars derived from Maize stalk, bamboo and cow manure amended soil, respectively.

3.5 Effect of Biochar on Plant Dry Biomass and Metals Accumulation

As expected from the observed decrease Cd availability in soil, all the biochar were effective in decreasing Cd accumulation in the shoots of both cultivars of Chinese cabbage (Figure 3). MS and BB biochar were reduced Cd accumulation in shoots of AJ cultivar by 36 and 35%, as compared to the control which did not receive amendment. In ZH cultivar, MS biochar had the highest reduction in Cd accumulation in shoots as compared to the control by 41%. However, application of BB and CM biochar also reduced the concentration of Cd in shoots by 32 and 10%, respectively. Cd concentration in roots was also affected by the addition of biochar in both cultivars of Chinese cabbage (Figure 3). The highest decrease in Cd content was recorded in roots of AJ grown in BB biochar amended soil as compared to the control and other biochar amendments, respectively.

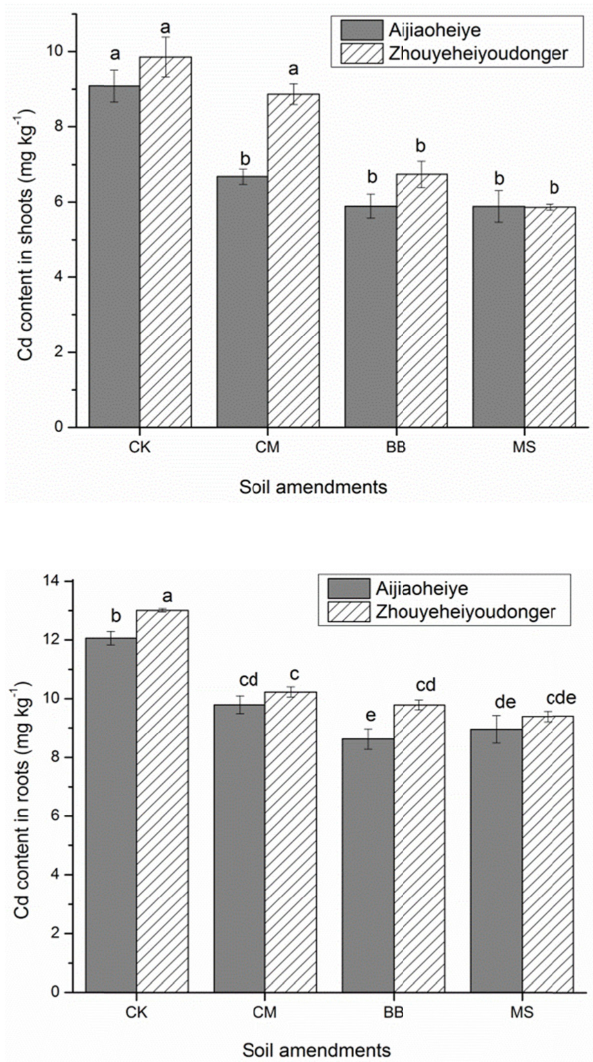


Figure 3. Effects of biochar amendments on Cd concentration in shoots and roots of *B.chinensis* L. cultivars: *Aijiaoheiye 333* (AJ) and *Zhouyeheiyoudonger* (ZH). BB and CM represent biochars derived from maize stalk, bamboo and cow manure amended soil. Each value represents the mean of three replicates ± standard error, and the different letters are significantly different at $p < 0.05$

All biochar addition had also impacted on trace metal availability and accumulation in shoots of both cultivars of Chinese cabbage (Figure 4). Specifically, MS biochar significantly ($P < 0.05$) increased Mn and Zn content in shoots of AJ cultivar by 32% and 5% and ZH cultivars by 18% and 23%. While, Fe concentration decreased in AJ by 45% and 26% in ZH cultivar with MS biochar addition with respect to their control. However, BB biochar addition had decreased the accumulation of Zn, Fe and Mn in shoots of ZH cultivar while in shoots of AJ cultivar Zn and Fe increased by 17.8% and 32.4% but there was no effect on Mn as compared to the control. CM biochar increased Mn content in the shoots of AJ and ZH cultivars by 16% and 20% while decreased Fe accumulation by 43% and 31%.

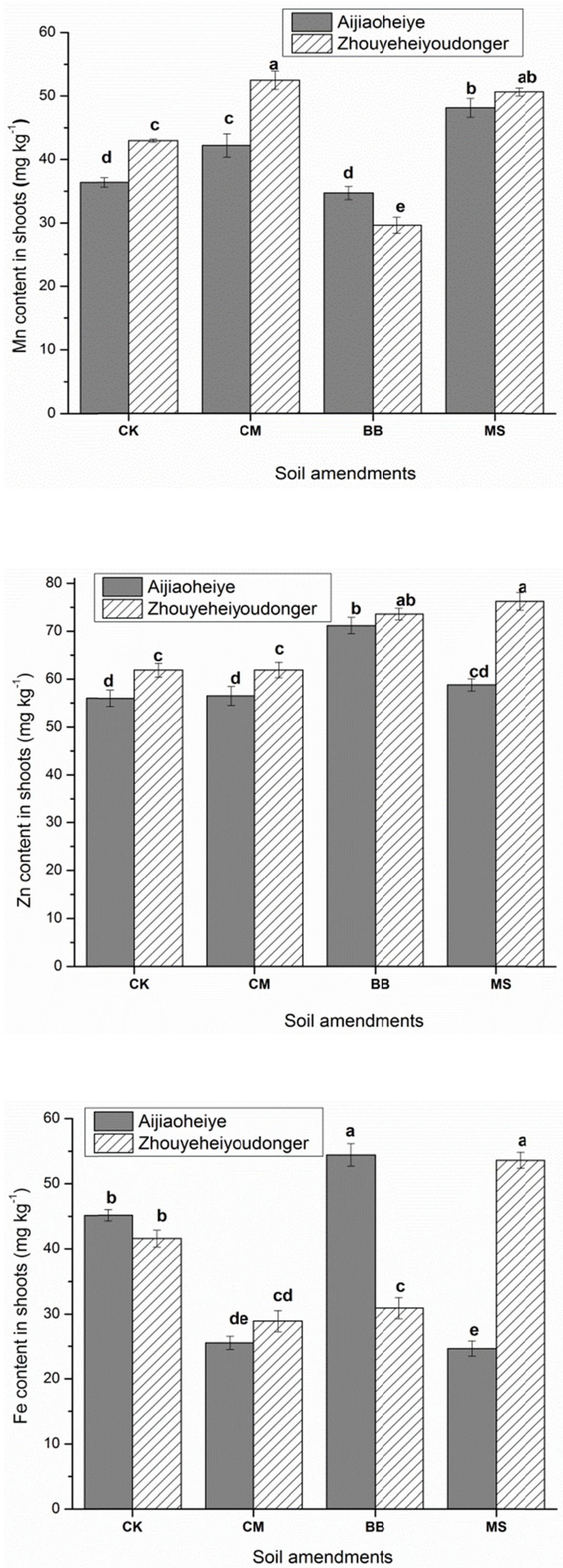


Figure 4. Mn, Zn and Fe concentration in shoots of *B.chinensis* L. cultivars *Aijiaoheiye 333* (AJ) and *Zhouyeheiyoudonger* (ZH) with soil amendments. BB and CM represent biochars derived from maize stalk, bamboo and cow manure amended soil. Each value represents the mean of three replicates ± standard error, and the different letters are significantly different at $p < 0.05$

The application of biochar significantly ($P < 0.05$) increased the dry biomass of plant shoots of both cultivars as compared to the control (Table 4). The addition of CM biochar increased the DW of ZH and AJ cultivars by 79 and 52%. Both MS and BB biochar increased shoot DW of AJ and ZH by 52% and 66%, respectively.

4. Discussion

Various studies have revealed that biochar used as a soil amendment are able to increase soil pH and induced liming effect (Lucchini et al., 2014; Ameloot et al., 2013; Nguyen & Lehmann, 2009). Our results showed that biochar were primarily in the alkaline range and increased the soil pH except bamboo biochar which demonstrated that pH of biochar was dependent on the type of feedstock (Table 3). With increased biochar pH results increase in soil pH which directly enhanced the negative charge groups and this might be the reason that BB has no effect on soil pH. The alkalinity of biochar may be due to the separation of alkali salts during pyrolysis (Mohan et al., 2014) and the release of basic cations into the soil that could be responsible for increasing soil pH (Nguyen & Lehmann, 2009). Application of MS and CM biochar increased the soil pH from 7.33 to 7.78 and 7.70 and subsequently immobilized Cd in an Alfisol soil; however, this increase could results in the precipitation of Cd as $Cd(CO)_3$ (Mousavi et al., 2010).

Numerous studies have also been reported that application of biochar is effective in Cd immobilization, thereby reducing the bioavailability and phytotoxicity (Zhou et al., 2008; Namgay et al., 2010; Jiang et al., 2012). Mehlich-3 extractable Cd was found to be more improved indicator and used as soil Cd thresholds in Chinese cabbage for potential dietary toxicity (Rafiq et al., 2014). The effects of biochar on metal adsorption varied with the source material used to produce it and the type of metal. MS, BB and CM biochar amendment significantly declined the extractability of Cd in an Alfisol soil. BB biochar reduced the Cd availability even at neutral pH. Similarly, it was reported that BB biochar can absorb Cu, Hg, Ni, and Cr from both soils and water while Cd in polluted soils (Cheng et al., 2006; Skjemstad et al., 2002). The possible mechanism may be that the BB biochar has a high amount of amorphous C, thereby supporting the formation of micropores and increasing the sorption capacity (Haghseresht et al., 1999).

Biochar could indirectly enhance plant growth through supplying nutrients and trace elements and improving soil physical and biological properties (Lehmann et al., 2006). Application of biochar (4% w/w) significantly reduced Cd accumulation in both cultivars of Chinese cabbage and increased essential trace metals that may subsequently attribute to the increased plant biomass. Matovic (2010) also reported that in agricultural soil, optimum biochar addition ranged between 1 to 5% w/w of soil. The overall addition of MS, CM and BB biochar had a positive effect on plant growth i.e increased the dry weight of shoot as compared to the control (untreated with biochar). Similarly, the application of Eucalyptus spp. wood chips derived biochar produced higher biomass of spring onion as compared to cultivated in control soils (Yu et al., 2009).

With the MS, CM and BB biochar amendment, Cd concentration significantly reduced in shoots and roots of Cd accumulating contrasting cultivars of Chinese cabbage. MS and BB had more decline in Cd accumulation and facilitate more essential trace metal concentration in shoots of both cultivars than CM. Similarly, Lehmann et al. (2003) also reported that application of biochar increased plant yield and this increase attributed to the increase in soil available nutrients. Park et al. (2011) also reported that biochar application resulting in increased plant biomass and reduced metal uptake in plants because of dilution and immobilization of metals.

In this study, MS biochar had the highest N content while BB biochar had lowest. However, in previous studies it has been observed that N contents in biochar depend on feedstock types, as plant material derived biochar had more C and N than animal manure derived biochar (Ahmad et al., 2014; Gaskin et al., 2008). MS and BB biochar were showed higher C/N ratio than CM biochar, such events has been attributed to aromaticity of biochar and will cause slow decomposition and higher stability in soil (Lehman, 2007). Hence, it seems that BB biochar would have the longest stability, whereas CM biochar has the least stability in soil. This was attributed to the high lignin content of bamboo which contributes to the high content of aromatic C of biochar (Cesarino et al., 2012).

However, the total H and C were notably higher in BB and MS biochar as compared to the CM biochar. Whereas, in this study all biochar had same H/C ratio and indicating lower aromatic structure while increase occurred in the preserved organic C because higher H/C ratios of biochar specified lower carbonization and aromaticity (Mohan et al., 2014). Lower aromatic structure as a result may have more sorption sites for inorganic contaminants (Ahmad et al., 2014; Chen et al., 2001). In reducing availability and uptake of Cd, the MS and BB biochar were more effective than CM biochar which had a larger surface area. According to Cui et al. (2016) *Zizania caduciflora* and *Vetiveria zizanioides* derived biochar had a larger surface area but lower sorption capacity of ammonium. In our results, the EDS spectrum of biochar showed that C and O dominated the surfaces of biochar, suggesting that they have more C and O bonds which adsorbed Cd from soil. SEM images also showed a highly porous surface of plant derived biochar which indicated the more sorption capacity of heavy metals. The functional groups of biochar also have a significant role in Cd sorption. A large number of functional groups mentioned in FTIR analysis were responsible for the adsorption of Cd. It was reported that biochar could adsorb heavy metal ions due to the presence of many functional groups including phenolic, carboxyl, and hydroxyl groups in its carbon-based structure (Bogusz et al., 2015; Jeong et al., 2012). The FTIR spectra of MS, BB and CM (Figure 1) indicated that adsorption attributed may be due to coordination of -OH, C=C-C, C-H, C-O, C-O-C groups exhibited on the surface of biochar because surface complex of Cd with oxygen-containing groups of biochar considerably contributes to Cd^{2+} sorption (Cui et al., 2016). Similarly, Ahmad et al. (2014) and Xu et al. (2013) reported that O containing groups could form strong surface complexes with Cd. However, contradictory explanations have been reported on the mobility of metals within biochar (Beesley et al., 2010). Therefore, specifically detailed studies on metal binding and their transformation are still required to understand the mechanism.

Soil basal respiration (SBR) and Dehydrogenase activity (DHA) are interrelated and key parameters for the rapid measurement of changes in soil fertility and determination of the intensity of microbial metabolism in soil (Trasar-Cepeda et al., 2012). Application of MS, BB and CM biochar improved the soil basal respiration and dehydrogenase activity which may also contribute to decrease the Cd bioavailability, phytotoxicity and enhanced phytobioavailability of essential trace metals. The application of biochar enhanced soil microbial activity and microbial population have also been previously observed (Verheijen et al., 2010; Lehmann et al., 2011), even though the mechanism is not fully understood for biochar induced stimulation of microbial activity (Warnock et al., 2007). Similarly, green waste and chicken manure derived biochar amendment also considerably decreased Cd and Pb, stimulated microbial activity, nutrients uptake and plant growth which may be due to enhanced soil respiration (Park et al., 2011).

5. Conclusion

Application of MS, BB and CM derived biochar as soil amendment for immobilization of Cd were promising materials for agricultural soils by altering chemical properties of soil. The results have clearly confirmed that biochar amendment to Cd contaminated Alfisol soil has the potential to suppress the bioavailability and phytotoxicity. In addition, biochar amendment enhanced plant growth and dry biomass by improving essential trace metal availability. Plant derived biochar were more effective than cow manure derived biochar in Cd immobilization and increasing phytoavailability of trace metals and may be due to lower C/N ratio and higher H and C contents of MS and BB biochar. Therefore, biochar has the potential to reduce the Cd uptake in both cultivars of *B. chinensis* L. which could be used to lower the human health risk caused by the Cd contamination. In this study biochar were effective to reduce Cd and increase the Zn, Mn and Fe availability into the plant soil system even though it varies depending on the type of biochar. However, further additional field study required to explore the applications of findings and sustainability of biochar in an Alfisol soil.

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