Environ Sci Pollut Res (2016) 23:12323–12331
DOI 10.1007/s11356-016-6264-6

RESEARCH ARTICLE

Effects of biochar amendment on relieving cadmium stress and reducing cadmium accumulation in pepper

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Received: 10 November 2015 / Accepted: 7 February 2016 / Published online: 15 March 2016

Abstract Biochar is widely used in agricultural soils or heavy metal-polluted soils to improve the quality of the soils, which would affect the growth of the plant. However, the information of biochars' effect on the plant growth was still lacking, especially for the physiological response of the plant. Pot experiments were used to examine the effect of willow-derived biochars at two temperatures (450 and 600 °C) on cadmium (Cd) accumulation in pepper and to reveal the response of physiological parameters to exogenous Cd stress (1 and 5 mg/kg). The results showed that the accumulation of Cd in pepper roots was higher than that in pepper shoots. For low level of Cd treatments, high additional rates of the biochars could obviously reduce the accumulation of Cd in the pepper roots. Moreover, there was a negative correlation between the C content of the biochar-amended soils and the Cd content of the pepper root, suggesting that the application of biochar to the soil decreased the Cd accumulation in the root. A positive relationship between the H/C ratios of biochar-amended soils and their corresponding Cd concentrations in pepper root indicated that low thermal temperature-derived biochar could play an important role in immobilizing Cd in the soil. Furthermore, on the condition of low Cd level of treatments, the malondialdehyde content decreased in biochar-amended soils, especially at high biochar application rate. The chlorophyll content increased with increasing the rates of the biochar application. The physiological parameters indirectly proved that the application of biochar did not always alleviate the toxic effects of Cd on pepper leaves at high Cd concentration.

Keywords Cadmium accumulation · Biochar properties · Biochar-amended soil · Physiological parameters · Pepper

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Introduction

Cd is a major heavy metal in the biosphere that results from mining, industrial, agricultural and geochemical processes (David et al. 2011; Radotic et al. 2000; Liu et al. 2008). The toxicity of Cd is of growing concern because Cd in soil can be transferred to plants, resulting in the disturbances of cellular redox control and inductions of reactive oxygen species (ROS) production (David et al. 2011). Thus, it is imperative to remediate Cd-contaminated soils or to relieve the toxicity of Cd for plant growth.

Plant metabolism is influenced by Cd, which is translocated to different parts of plant. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production. The increase levels of MDA may result from the overproduction of ROS (Choudhary et al. 2007). In general, metal stress in plants can promote the production of ROS that are naturally formed in plants cells (Monteiro et al. 2009; Noctor et al. 2007). If ROS remain at acceptable levels under the defence of antioxidant systems, oxidative damage does not occur. However, oxidative damage can result from the overproduction of ROS, which could lead to protein oxidation, enzyme inhibition, DNA and RNA damage, and membrane lipid peroxidation generating MDA (Cho and Seo 2005; Lin et al. 2007; Mittler 2002). Moreover, previous study has demonstrated that a reduction in chlorophyll can be attributed to Cd toxicity (Liu et al. 2011). Because Cd reduces plant growth by inhibiting cell elongation and/or division, it leads to a decrease in chlorophyll biosynthesis, photosynthesis and respiration (Lefèvre et al. 2009). Therefore, the MDA and chlorophyll contents are the primary indicators of the plant under the heavy metals' stress.

Similar to activated carbons, biochars are produced by combustion processes under conditions of low temperatures and minimal oxygen (Beesley and Marmiroli 2011), and they have been applied to soils to adsorb inorganic contaminants (Dong et al. 2011; Fellet et al. 2011; Uchimiya et al. 2011b). Recently, attention has been given to biochar as a soil amendment because of its ability to sequester heavy metal contaminants and its beneficial effects on the physico-chemical characteristics of the soil. The carbon content and cation exchange capacity (CEC) of biochar has been reported to increase with increasing pyrolysis temperature (Keiluweit et al. 2010; Uchimiya et al. 2011c). Oxygen-containing carboxyl, hydroxyl and phenolic surface functional groups of soil organic and mineral components play central roles in binding metal ions, and biochar amendment could increase the amount of ligands, which could complex and reduce the bioavailability of metals (Uchimiya et al. 2011a). Karami et al. (2011) employed biochars to amend soils to decrease lead (Pb) and copper (Cu) mobility. They discovered that hardwood-derived biochar was more effective in reducing pore water Cu concentrations, and ryegrass shoot Cu and Pb levels were reduced after biochar amendment. Uchimiya et al. (2010) found that the cation exchange of biochar-amended soils played a more important role than other factors, such as the coordination of π electrons (C=C) of carbon and precipitation. In addition, different dosages of biochar added to soil might impact plant growth or heavy metal bioavailability (Fellet et al. 2011). They found that as the biochar content increased in the substrates, the pH and nutrient retention increased, and the bioavailability of Cd, Pb, Tl and Zn of the mine tailings decreased. Therefore, different dosages of biochar would likely increase the fertility of soils and cause changes in plant growth, such as variations in plant biomass (Hartley et al. 2009; Hossain et al. 2010; Rillig et al. 2010), yield (Asai et al. 2009; Graber et al. 2010) and germination (Jones et al. 2010).

Biochar has been proposed as a soil amendment to ameliorate heavy metal pollution of soils, and its potential mechanisms are presented above. Because higher pH should enhance the electrostatic attraction between soil surfaces and cations (Sposito, 1989), previous studies on biochar amendment have focused on acidic soils (Beesley et al. 2010; Novak et al. 2009; Uchimiya et al. 2011a, 2011b) in order to promote the soil pH. However, few studies used the alkaline soils in previous research. This might be attributed to the research of the sorption mechanism, which should eliminate the precipitation in the soils with high pH level. The soils in the north of China contain a lot of organic matters, and the pH is slightly high. In recent year, more and more regions in the north of China applied the biochars to amend the soils (Zhang et al. 2014). However, as the biochar's application become widely in northern China, the effect of biochars on alkaline soils was not deeply investigated. Furthermore, it remains unclear whether biochar can cause stress to plants when immobilizing Cd. Liao et al. (2014) suggested the ubiquitous presence of free radicals in biochars, which may be harmful for plant. Therefore, the objectives of this study were (1) to investigate the effect of biochar on cadmium accumulation in pepper and (2) to study the response of pepper leaf physiological parameters to cadmium stress.

Materials and methods

Soil and biochar preparation

The experimental soil (0–0.15 m) was collected from a farmland in the Tongzhou district of Beijing, China. Beijing is a warm temperate zone with semi-humid continental monsoon climate. The average temperature of this region is 10–12 °C. This soil was classified as a loamy soil according to the China Soil Classification. The collected soil was air-dried at room temperature. Large debris and biological remnants were removed from the soil samples. Then, the soil was passed through a 0.25-mm nylon sieve.

Willow chips collected from the Tongzhou district of Beijing was used as the feedstock for biochar production. The willow chips were pyrolyzed at different temperatures (i.e. 450 and 600 °C). The willow-derived biochars are hereafter referred to as BLX, where X indicates the final heating treatment temperature (HTT). The process used to prepare the biochars is described elsewhere (Chen et al. 2008). Briefly, the dried feedstocks were rinsed with deionized water and cut to a length of 1 cm. The willow chips were charred at different temperatures in a closed ceramic pot for 4 h under oxygen-limited conditions in a muffle furnace. Then, the biochars were gently ground and homogenized to pass through a 0.25-mm sieve.

Pot experiment

The biochar-amended soil used in this study was prepared by mixing the soil and two different biochars (BL450 and BL600) in different ratios (0.2, 1 and 5 %, *w*/*w* = biochar/soil). Each pot contained 500 g of the soil mixed with the different biochars similar to Hartley et al. (2009). The amended soil was thoroughly homogenized and allowed to equilibrate in plastic bags for 2 weeks at room temperature at 70 % soil water capacity. The biochar-amended soil containing BLX was denoted as SLX. The elemental composition and physicochemical characteristics of all SLXs are listed in Table 2. A Cd $(NO_3)_2$ solutions at two concentrations (1 and 10 mg/kg) was added to the biochar-amended soil. After sufficient mixing, the samples were transferred to plastic pots and placed on plastic saucers to prevent leachate draining from the soils. In accordance with the report by Lin et al. (2007), the pot samples were incubated for 2 weeks. A randomized block design was used, which was repeated three times. The control treatment (CK) did not receive the biochar or the Cd solutions.

Pepper seeds were surface-sterilized with 30 % sodium hypochlorite for 10 min, and thoroughly washed with distilled water and then placed on moist filter paper for germination. After germination, ten seeds were sown in each pot to a depth of 0.5 cm. After 30 days, the seedlings were culled and three seedlings were retained in each pot. The experiment was conducted in a greenhouse. Conventional fertilization and irrigation was used throughout the growing season. The plants were grown for 70 days. Before the peppers were harvested, the physiological parameters (i.e. MDA and chlorophyll) of the leaves were determined. Fresh samples were collected (the third leaf from the top), and then the leaves were cleaned and extracted by the extraction solution. Thereafter, the liquid supernatant was analysed.

Analytical methods

The C, H, and N content of the soil, the two biochars samples (BL450 and BL600) and the biochar-amended soils (SL450 and SL600) were determined using an Elemental Vario EIII elemental analyzer (Elementar Company, Germany) by complete combustion. Therefore, bulk elemental composition obtained from elemental analyzer represented the C, H, N and O content per mass of biochar and biochar-amended soils, which were measured in duplicate to obtain the average data. The surface elemental composition of the top surface layer for the biochars (depth, 3–5 nm) (Sun et al. 2012) was determined using X-ray photoelectron spectroscopy (XPS) (including an elemental survey scan and a high-resolution scan at the C_{1s} edge) with a Kratos Axis Ultra electron spectrometer using monochromated Al Kα source operated at 225 W, and more detailed information of characterization is described elsewhere (Sun et al. 2013; Yang et al. 2011). The binding energies for the high-resolution spectra were calibrated by setting C to 1s at 284.6 eV. Deconvolution processing of C_{1s} spectra was done with CasaXPS software package and the C_{1s} binding energy levels were assigned as following: 284.6 eV to C-C, 286.2 eV to C-H, 287.6 eV to C=O and 289.1 eV to COO (Yang et al. 2011). Additionally, the spectra of the Fourier transform infrared (FTIR) of the biochars were characterized by a Nexus 670 FTIR spectrophotometer (Thermo Nicolet Corporation, USA) with transmission sample accessory and a deuterated triglyceride sulphate (DTGS) detector. The sample concentration for FTIR determination was about 2 % in KBr and then ground with an agate mortar and pestle. The milled sample was immediately transferred to a sample holder, and its surface was smoothed with a glass microscope slide. Before analysis, the diffuse-reflectance cell containing the samples was flushed with dry N_2 gas to eliminate interference from CO_2 and moisture. To obtain FTIR spectra, 32 scans were collected at a resolution of 4 cm^{-1}, and the spectra with numerical value for major peak wave numbers and intensities were recorded. A strong acid digestion method ($HNO_3 + H_2O_2 + HF$) was used to dissolve heavy metals in solution by heating (Gao et al. 2008). The digested solution of the cations of the soil, biochars and biochar-amended soils were measured using an inductively coupled plasma optical emission spectrometer (ICP-AES; SPECTRO Company, Germany) (Silber et al. 2010).

The peppers were harvested and separated into roots and shoots. The shoots were carefully rinsed with tap water. The root and shoot samples were cleaned ultrasonically, re-washed with deionized water, and dried in an oven at 80 °C for 2 days (Hartley et al. 2009). The dried samples were ground to pass through a 40-mesh sieve for further use. The samples (200 mg) were digested with HNO_3 and H_2O_2 at 160 °C for 4 h to determine the concentration of cations in the roots and shoots (Huang and Wang 2010) using an ICP-AES.

The MDA content was measured using the method described by Heath and Packer (1968). Briefly, approximately 200 mg of fresh leaves was ground in 0.25 % thiobarbituric acid (TBA) in a 10 % trichloroacetic acid (TCA) solution using a mortar and pestle. The tubes were centrifuged at 10, 000×*g* for 20 min at 4 °C. The supernatant with 5 % TBA was heated at 95 °C for 30 min in a water bath and then rapidly cooled in an ice bath. The heated supernatant was centrifuged at 10,000×*g* for 10 min. The absorbance of the supernatant was read at 532 and 600 nm using an UV–visible spectrophotometer (Varian, USA) (Tang et al. 2010). TBA (0.25 %) in 10 % TCA was used for the blank.

Approximately 100 mg of fresh mature leaves of the pepper was cut into 1–2-cm^2 pieces. Each leaf sample was extracted for 24 h in the dark at 22 °C with 10 mL 80 % acetone. The supernatant of the extraction was used to measure the contents of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). The UV–visible spectrophotometer was used to determine the absorbance of the supernatant at 663 and 646 nm, respectively. The total chlorophyll content was calculated as the sum of Chl *a* and Chl *b* and was expressed as Chl *a* + Chl *b* (Wang 2008).

Table 1 Chemical properties of the soil and biochar used in the pot experiment

Properties	Soil	BL450	BL600
pH	8.11	7.81	9.64
C (%)	14.6	70.38	80.19
H (%)	6.87	2.75	1.94
N (%)	1.14	1.20	1.34
O (%)	–	20.4	8.96
H/C	5.65	0.47	0.29
O/C	–	0.22	0.08
Cd (mg/kg)	0.37	1.97	0.71

Data analysis

The statistical analyses were conducted using SPSS 16.0 for Windows. One-way ANOVA followed by a Duncan's test was performed to analyse significant differences among the treatment means ($p < 0.05$).

Results and discussion

The properties of the biochars

The elemental compositions of the biochars are listed in Table 1. The C content of BL600 was higher than that of BL450, indicating that the degree of carbonization was accelerated with increasing HTT (Kim et al. 2012). The pH values of the biochars increased with the thermal temperature, which were in agreement with previous study (Cao and Harris 2010). They suggested that the C started to become ashed and alkali salts began to separate from the organic matrix, increasing the BC pH to above 10. With increasing the additional rates of biochars (BL450 and BL600), the C content of biochar-amended soils increased, and the H/C ratios of the biochar-amended soils decreased (Table 2), suggesting that the addition of biochars into the soil make the degree of carbonization of the soil increase, and reduce the aromaticity of the soil.

XPS was used to characterize surfaces of the biochars particles in comparison to the properties of the interior of the biochars particles. The maximum depth that XPS can probe is about 10 nm, allowing it to distinguish between surface and bulk properties using the element analysis (Binh et al. 2009; Sun et al. 2012; Sun et al. 2013). In terms of the surface elemental composition of the biochars in Table 1, the surface polarity (O/C) of BL450 was higher than that of BL600, implying that a higher number of polarity groups (e.g. -COOH, -CO or –OH) is on the surface of BL450. According to a previous report (Yao et al. 2010), the C_{1s} envelope contains four signals that are attributed to aliphatic/aromatic carbon groups (CHx, C-C/C=C) (284.6 eV), hydroxyl and ether groups (-C-OR) (285.8 eV) and carbonyl groups (-COOR) (288.8–288.9 eV) (Table 3). The XPS result showed that the low temperature biochar (BL450) contained higher proportions of carbonyl and carboxylic groups than the high temperature biochar (BL600). This result was demonstrated in the analysis of the surface polarity in this study (Table 3) and in agreement with the report by Sun et al. (2013). They indicated an increasing degree of carbonization of chars following dehydration, decarboxylation and decarbonylation during pyrolysis (Sun et al. 2013; Keiluweit et al. 2010).

The FTIR spectra of the biochars are presented in Fig. 1. The band at 3369–3412 cm^{-1} was attributed to hydrogen-bonded hydroxyl groups (Uchimiya et al. 2011b). The bands at 1740 and 1597 cm^{-1} were assigned to the carbonyl/carboxyl C=O and the aromatic C=C stretching modes, respectively (Sun et al. 2011). These peaks were detected on the BL450 and BL600, respectively. The intensity of the aromatic carbonyl/carboxyl C=O peak of BL450 was stronger than that of BL600. In addition, Uchimiya et al. (2011b) used the molar ratios of elements to estimate the aromaticity (H/C) and polarity (O/C) of the biochars. This information was obtained by the element analysis. As shown in Table 1, the total polarity of the biochars decreased with increasing HTT; however, the aromaticity of the biochars exhibited the opposite trend. This result was similar to the previous study by Sun et al (2013).

Table 2 The elemental composition and the physico-chemical characteristics of the biochar-amended soils

Property	0.2 %SL450	1 %SL450	5 %SL450	0.2 %SL600	1 %SL600	5 %SL600
pH	8.39	8.45	8.47	8.41	8.62	8.87
C (%)	17.34	22.75	35.18	17.09	18.53	31.40
H (%)	6.70	6.57	5.46	6.13	4.98	4.32
N (%)	1.01	1.04	0.94	0.91	0.72	0.73
O (%)	–	–	–	–	–	–
H/C	4.64	3.47	1.86	4.30	3.23	1.65
O/C	–	–	–	–	–	–
Cd (mg/kg)	0.65	0.39	0.62	0.39	0.41	0.49

Table 3 Surface elemental composition, surface polarity and surface functional groups of the biochars measured by XPS

Samples	C %	O %	N %	Na %	Si %	Ca %	O/C	(O + N)/C
BL450	76.59	19.09	1.46	0.33	UL	2.53	0.19	0.20
BL600	85.03	13.05	1.11	UL	0.81	UL	0.12	0.13
	H-C-C %	C-O %	C=O %	O-C=O %	Oxidized C %			
BL450	76.24	10.34	7.59	5.84	23.77			
BL600	82.40	9.04	2.18	6.38	17.60			

The C_{s1} binding energy levels were assigned as follows: 284.6 eV to C-C, 286.2 eV to C-H, 287.6 eV to C=O and 289.1 eV to COO (Yang et al. 2011)

UL under the limit of detection

The Cd accumulation in different pepper components

The accumulation of Cd in the pepper root and shoot is shown in Fig. 2. Compared with the control (0 mg/kg Cd), the Cd concentrations in the pepper root and shoot increased with the increment of Cd concentration. An increase in the BL450 rate caused a reduction of Cd concentrations in the pepper root in the SL450 treatments. Compared with the soil treatment, the pepper root Cd concentrations in the 5 %SL450 treatment was reduced by 22 and 38 % in response to 1 and 10 mg/kg Cd addition ($p < 0.05$), respectively. In addition, at a higher rate of BL600 in the 1 mg/kg Cd treatment, the Cd accumulation in the pepper root declined and was slightly different compared with that measured in the soil treatment. However, when high Cd (10 mg/kg) was added into SL600, the Cd concentration of the pepper root was reduced by 15, 5 and 15 % at the three biochar addition rates (0.2, 1 and 5 %, respectively) comparing with the soil treatment. Therefore, high rates of BL450 and BL600 addition decreased the Cd accumulation in the pepper root, especially the BL450. This result is in agreement with the conclusion of Cui et al. (2011), who indicated that rice grain Cd concentration decreased with increasing biochar rates in the soil, attributing to the elevation in pH and soil organic carbon. Park et al. (2011) suggested that the higher the biochar addition rate, the lower the Cd accumulation in Indian

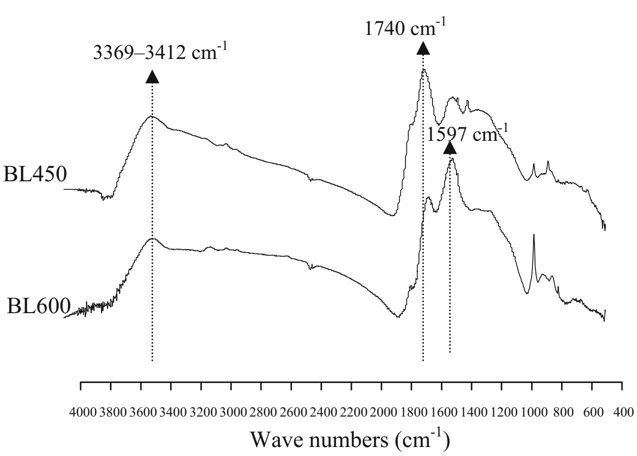


Fig. 1 FTIR spectra of the two biochars

mustard. A similar result was observed in the current research (Fig. 2a). A negative correlation was observed between the C contents of biochar-amended soils (Table 2) and respective Cd concentrations in pepper root (Fig. 3a) ($p < 0.05$) in 1 mg/kg Cd treatments. This manifested that Cd content in the soil had been affected by the organic carbon in the soil following the addition of the biochar. The results of the elemental composition, XPS and FTIR (Tables 1, 2, 3 and Fig. 1) also indicated that the soil's organic carbon was increased in this study. Thus, the Cd accumulation in the pepper root might be reduced due to the addition of biochars.

In addition, in the 1 mg/kg Cd treatment, the Cd concentration of the root decreased by 22 and 11 % in the 5 %SL450 and 5 %SL600 treatments, respectively, compared to the soil treatment (Fig. 2). In the 10 mg/kg treatments, the 5 %SL450 and 5 %SL600 treatments decreased by 38 and 15 %, respectively, compared to the soil treatment (Fig. 2). The results indicated that high biochar application rates (5 %) could reduce the accumulation of Cd in the root, and a low thermal temperature-derived biochar (BL450) could immobilize more Cd in the soil than a high thermal temperature-derived biochar (BL600). Furthermore, there was a positive relationship between the H/C ratios of biochar-amended soils and their corresponding Cd concentrations in pepper root (Fig. 3c) in 1 mg/kg Cd treatments ($p < 0.05$), suggesting that low thermal temperature-derived biochar (BL450) should play an important role in immobilize Cd in the soil. Park et al. (2011) reported that the immobilization of heavy metals varied depending on the type of biochar present (e.g. -COOH, -OH). The FTIR spectra and surface functionalities of BL450 and BL600 manifested that BL450 would complex with Cd^{2+} in the soil (Uchimiya et al. 2010, 2011c). The greater the amount of Cd immobilized in the soil, the lower the Cd accumulation in the pepper root.

The accumulation of Cd in the pepper shoot is shown in Fig. 2b. Compared with the control, the Cd concentrations in the pepper shoot increased with the increment of Cd concentration. Moreover, the Cd accumulation of the pepper shoot decreased with increasing the additional rate of the biochars

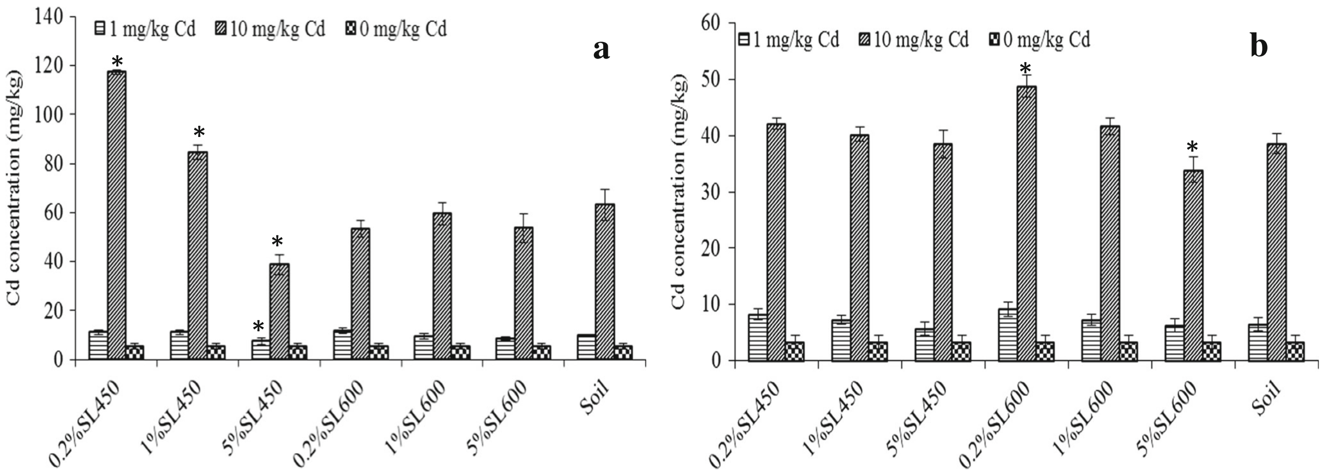


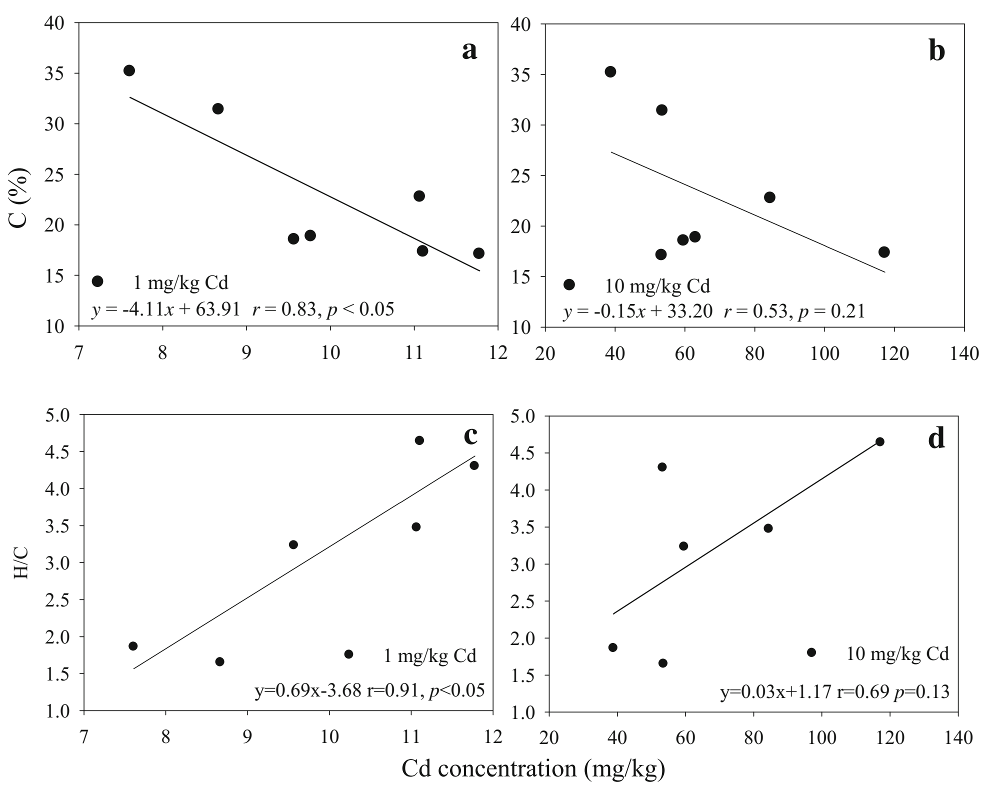
Fig. 2 The accumulation of Cd in **a** roots and **b** shoots of pepper grown in soil amended with 0.2, 1 and 5 % biochar produced at 450 °C (SL450) or 600 °C(SL600) (*asterisk* indicates significant differences at $p < 0.05$)

(BL450 and BL600). In the 1 mg/kg Cd treatment, comparing to the soil treatment, the Cd concentrations of the 5 %SL450 and 5 %SL600 in the pepper shoot reduced by 12 and 4 %, respectively. While in the 10 mg/kg Cd treatment, the Cd concentration of the 5 %SL450 and 5 %SL600 in the pepper shoot decreased by 0 and 12 %, respectively. In terms of the previous report by Xu et al (2013), they indicated that Cd could complex with the organic matter of cell wall in order to reduce the damage of the leaf tissues by Cd. Furthermore, the Cd concentrations in the root were higher than that in the shoot. Thus, the lower Cd accumulated in the pepper shoot, the lighter might destroy to the pepper leaves.

Physiological parameters of the pepper leaves

Cd can induce the intracellular production of ROS (Luna et al. 1994), which would lead to oxidative stress in cellular components (Lopez et al. 2006) and the induction of detectable biochemical responses in organisms. MDA is an oxidation product of the ROS interaction with polyunsaturated fatty

Fig. 3 Correlation between the Cd concentration in the pepper roots and the carbon content and H/C ratios of the biochar-amended soils



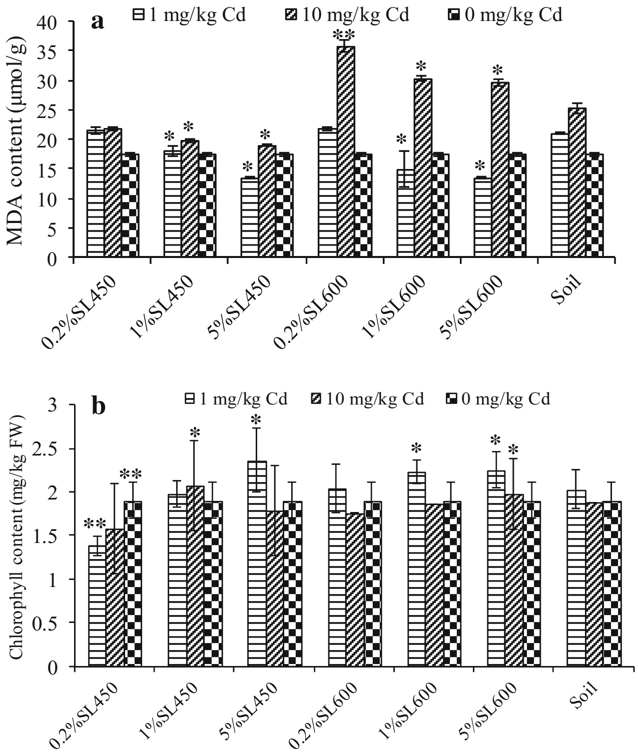
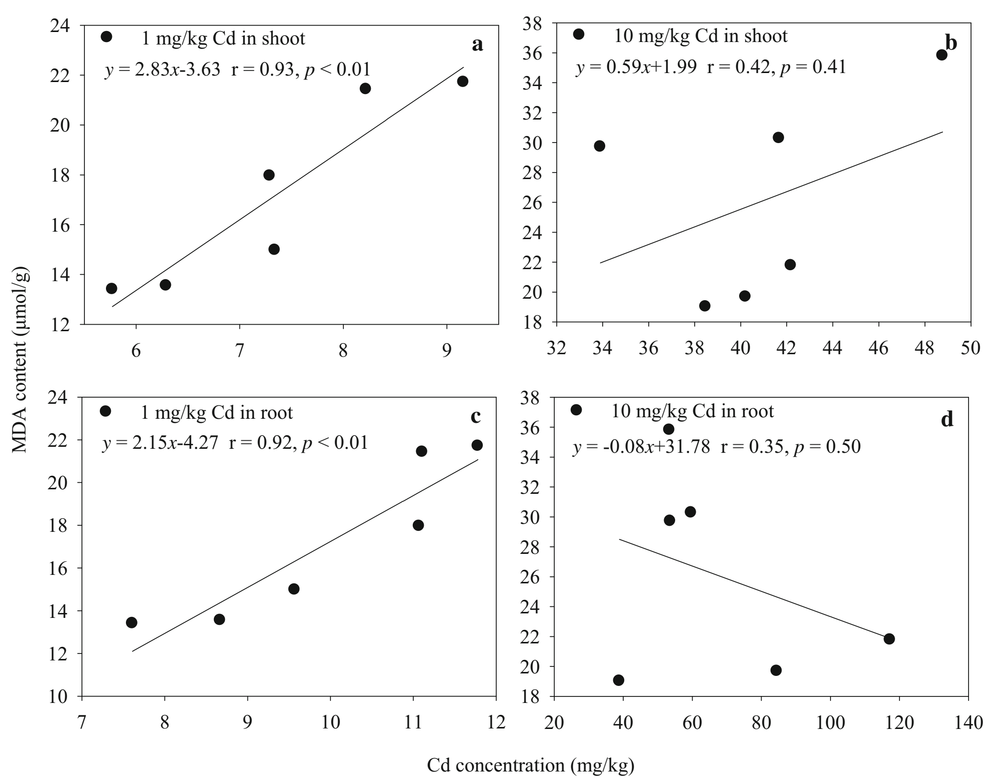


Fig. 4 MDA (**a**) and chlorophyll (**b**) contents of pepper leaves (*single asterisk* indicates significant differences at $p < 0.05$; *double asterisks* indicates significant differences at $p < 0.01$)

acids and an indicator of free radical production and consequent tissue damage (Song et al. 2009). The Cd toxicity in the pepper was evaluated based on the MDA and chlorophyll contents (Fig. 4). In the soil treatment, the MDA content of the pepper leaves increased with increasing Cd concentration. This result is similar to a previous report (Dong et al. 2011). Moreover, for 1 mg/kg Cd treatments, the MDA contents of the biochar-amended soil treatments (SL450 and SL600) decreased with increasing additional rate of the biochar (Fig. 4a). The correlation between the MDA contents of the pepper leaves and the Cd concentrations in the plant (Fig. 5a, c) exhibited a positive correlation ($p < 0.05$), for the 1 mg/kg Cd treatment, implying that the higher additional rate of the biochar to the soil, the lower MDA content was detected in the pepper leaves. Especially, the MDA content in the 5 %SL450 and 5 %SL600 treatments were significant lower than that in the soil ($p < 0.05$). This suggested that Cd would be immobilized by the biochar, which would relieve the harmful effects of the Cd on pepper at low Cd level in the soil. In 10 mg/kg Cd treatments, the addition of different biochar to the soil could result in a change in the MDA content. The MDA content in the SL450 treatments was lower than that in the soil, while the MDA content in the SL600 treatments was significant higher than that in the soil. This result indicated that the BL600 could not relieve the Cd damage to the pepper in high Cd treatment. This phenomenon might be attributed to the saturation of Cd binding sites in the BL600 at 10 mg/kg Cd (Xu et al. 2014). They illustrated that the biochar and biochar-amended soils all have the maximum adsorptive capacity. Therefore, biochar is like a buffer, which would

Fig. 5 Correlation between the MDA contents in the pepper leaves and the Cd accumulation in the pepper



combine with the Cd in order to alleviate the absorption of Cd by plant's roots in low Cd polluted soil.

Among the variety of targets reported for heavy metals in plants, the photosynthetic apparatus seems the most sensitive. Accordingly, the level of photosynthetic pigments is often used to assess the impact of environmental stresses in plants because the change of photosynthetic pigments is linked to visual symptoms of plant illness and photosynthetic productivity (Huang and Wang 2010; Ben Ghnaya et al. 2007). The chlorophyll content (Chl a + Chl b) of the pepper leaves is shown in Fig. 4b. The chlorophyll content of the leaves increased with the increment of the biochar (BL450 and BL600) application in the low Cd treatment. For 1 mg/kg Cd treatment, the chlorophyll content of the 0.2 %SL450 treatment significantly decreased by 32 % comparing to the soil ($p < 0.01$), while the chlorophyll contents of the 5 %SL450, 1 %SL600 and 5 %SL600 increased by 16, 9 and 10 %, respectively ($p < 0.05$). In 10 mg/kg Cd treatment, the chlorophyll content of the 0.2 %SL450 treatment significantly decreased by 19 % ($p < 0.01$). Interestingly, the chlorophyll contents measured in the 5 %SL450, 1 %SL600 and 5 %SL600 were higher than those measured in the soil treatment and the control, suggesting that the chlorophyll content rose after the addition of the biochars to the soil in the low Cd treatment. However, except for 1 %SL450 and 5 %SL600, the chlorophyll contents of the biochar treatment were lower than those of the soil and the control in the high Cd treatment. Therefore, reduction in chlorophyll indicates that with high Cd concentrations, the application of biochar did not alleviate the toxic effects of Cd on pepper leaves.

Conclusion

This research showed that the accumulation of Cd in pepper roots was higher than that in pepper shoots. High additional rates of the biochars could obviously reduce the accumulation of Cd in the pepper roots in low Cd treatment. Moreover, there was a negative and positive correlation between the C content and the H/C ratios of the biochar-amended soils and the Cd content of the pepper root, respectively. The correlation suggested that the application of biochars to the soil decreased the Cd accumulation in the root, and the low thermal temperature-derived biochar (BL450) should play an important role in immobilizing Cd in the soil. Furthermore, for low level of Cd treatments, the MDA content decreased in biochar-amended soils, especially at high biochar application rate. The chlorophyll content increased with increasing biochar application rate. The physiological parameters indirectly proved that with high Cd concentrations, the application of biochar did not always alleviate the toxic effects of Cd on pepper leaves.

Acknowledgments This research was supported by the Postdoctoral Science Foundation of China (2015M571072) and the Public Interest Scientific Research Fund of the Ministry of Water Resource (No. 201501019).

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