Mitigating cadmium accumulation in greenhouse lettuce production using biochar

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Abstract Greenhouse experiments were conducted to investigate the influence of rice straw biochar (RSB) on soil cadmium (Cd) availability and accumulation in lettuce. The RSB was applied either in bands or broadcast in the test site of four greenhouses with soil Cd concentrations ranging from 1.70–3.14 $\mu\text{g g}^{-1}$. Biochar doses applied in bands were half of those broadcast. The Cd levels in the shoots of lettuce were observed to be reduced by up to 57% with increasing RSB application rate (0, 6, 12, 18 t ha⁻¹). Following RSB application, shoot Cd concentrations of lettuce were reduced to below the Chinese threshold value set for food, and hazard quotients for Cd associated with vegetable consumption were reduced from 0.70–1.11 to 0.42–0.65. A decrease in soil bulk density (11%) and increases in water holding capacity (16%), available

phosphorus (30%), available potassium (197%), and lettuce yield (15%) were observed after RSB application. Multiple linear regression analysis suggested that the soil extractable Cd level (but not biomass dilution) and soil bulk density, as influenced by RSB addition, were the dominant contributors to the shoot Cd levels in lettuce and lettuce yield, respectively. These results highlight the potential for RSB to mitigate the phytoaccumulation of Cd and thereby to reduce human exposure from vegetable consumption. Application of biochar in band, rather than broadcasting over the entire area, represents an opportunity to halve the biochar cost while retaining a good remediation effect.

Keywords Biochar · Cadmium · Leaf lettuce · Greenhouse · Hazard quotient

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Introduction

Cadmium (Cd) is a nonessential and toxic metal element. Exposure to Cd has been associated with many diseases in humans, such as nephrotoxicity, osteoporosis, neurotoxicity, and adverse endocrine and reproductive effects (EFSA 2009). Among the toxic metals and metalloids, Cd is notable for its ease of uptake by crops and thus its ability to enter the food chain. Many studies have shown the contamination of farmland soils by Cd in China, and consequently, Cd contamination of food crops and vegetables have been frequently reported (Wang et al. 2005; Li et al. 2006; Khan et al. 2008; Zeng et al. 2008; Zheng et al. 2015a). In peri-urban areas of China, the main agricultural activities are livestock production and vegetable cultivation. Organic fertilizers from livestock slurry and wastewater sludge are easily available and widely used in peri-urban arable lands. Thus, soils can be contaminated with Cd through the application of livestock manures, fertilizers and agrochemicals,

sewage irrigation, and atmospheric deposition (Luo et al. 2009). Urban and peri-urban agriculture such as vegetable greenhouses are more vulnerable to metal contamination because of the frequent and heavy input of fertilizers and irrigation. Indeed, vegetables produced in peri-urban greenhouses have been reported to be contaminated with Cd because of the excessive use of Cd-containing manures (Ju et al. 2007; Luo et al. 2009; Yang et al. 2009; Yang et al. 2013).

Biochar is receiving increasing attention because of its potential benefits in agricultural and environmental contexts such as mitigation of global warming (Sui et al. 2016), improvement of soil quality (Olmo et al. 2016), and the increase of soil nutrient availability and water retention capacity (Liu et al. 2016; Sorrenti and Toselli 2016), and improvement of crop yield (Khan et al. 2013; Olmo et al. 2016). In recent years, there has been growing evidence that the application of biochar in soil could immobilize Cd and thereby reduce its accumulation within plants (Park et al. 2011; Zheng et al. 2012; Khan et al. 2014; Zheng et al. 2015a). Incorporation of biochars into soil has been shown to result in considerable immobilization of Cd in soils and reduced Cd accumulation in Indian mustard, wheat and rice (Park et al. 2011; Zheng et al. 2013, 2015a). It follows that biochar could decrease the transfer of Cd from soils to other vegetables and reduce Cd exposure associated with vegetable consumption.

Many studies have reported the effect of broadcast biochar on the immobilization of Cd in soils. However, biochar cost remains too high for such an approach to be popularized at a large scale. The biochar cost can be halved by using band application instead of broadcast application; however, the effectiveness of band placement versus broadcast application on Cd immobilization remains unclear. In addition, there remains insufficient information regarding the effect of biochar amendment on Cd accumulation in greenhouse vegetables that are subject to excessive irrigation and inputs of fertilizers and manures. In the present study, both broadcast and band applications of biochar were employed for the purpose of Cd sequestration in a suburban greenhouse soil. Specifically, the research investigated the following: (i) the influence of biochar additions on Cd immobilization and accumulation in leaf lettuce grown in greenhouses; (ii) the effectiveness of broadcast and band biochar application for the mitigation of Cd accumulation in vegetables; and (iii) the reduction in Cd intake by humans and hazard quotient (HQ) associated with vegetable consumption.

Materials and methods

Study area and biochar description

The experiment was carried out in greenhouses in a vegetable garden in Hebei province, less than 2 km from Beijing, China,

and was initiated in 2015. Vegetables produced in this garden are primarily supplied to suburban residents of Beijing. The vegetable garden soil was historically contaminated with Cd due to excessive inputs of livestock manure in the 1990s.

Biochar was produced from rice straw in a pyrolysis system with a rotatory kiln (12 m long and 0.9 m diameter) by Nanjing Qinfeng Crop Straw Technology Company, Jiangsu Province, China. Rice straw was placed into the rotatory kiln and charred in the absence of air for 1 h after the kiln temperature was raised to 500 °C by heating up for about 3 h. The obtained biochar was ground and passed through 2-mm mesh sieves. Selected physicochemical properties of the biochar are presented in Table 1.

Experimental design

Soil Cd concentrations in soil samples from 21 greenhouses of the vegetable garden were tested before experiments commenced (Table 2). Soil Cd concentrations were determined from three soil samples per greenhouse. Each sample was bulked from three subsamples that were randomly taken from each of the greenhouses using the “S” sampling method (soil was collected from a depth between 0 and 20 cm; about 200 g per subsample). Greenhouse 18 (G18) was identified as having a soil Cd concentration ($2.28 \mu\text{g g}^{-1}$) in the middle of the range of concentrations observed (1.34 to $3.14 \mu\text{g g}^{-1}$). G18 was therefore selected for a preliminary study (Experiment 1). Thereafter, Greenhouses 5, 6, 20, and 21 (G5, G6, G20, and G21) were selected for a further study (Experiment 2). G5 and G6 provided soils with relatively lower Cd concentrations (1.70 – $1.76 \mu\text{g g}^{-1}$), while G20 and G21 had higher Cd concentrations (2.62 – $3.14 \mu\text{g g}^{-1}$).

Experiment 1 Rice straw biochar (RSB) was applied in G18 using the broadcasting method at different rates (0, 6, 12, 18 t ha⁻¹). Four plots (6.5 m × 3.8 m) were used for each RSB addition rate. Biochar (<2 mm) was added to the soil and mixed thoroughly with the topsoil (0–20 cm depth) using rotary cultivator plowing. In keeping with common practice of local farmers, cattle manure and chemical fertilizers were applied as basal fertilizers at 20 t manure ha⁻¹, 0.1 t P₂O₅ ha⁻¹, and 0.2 t nitrogen (N) ha⁻¹ (no potassium (K) was added). The Cd concentrations of the applied manure and phosphorus (P) fertilizer were $1.24 \mu\text{g g}^{-1}$ (dry weight manure) and $0.95 \mu\text{g g}^{-1}$ P₂O₅, respectively. Following RSB and fertilizer application, leaf lettuce (*Lactuca sativa* L. var. *ramosa* Hort.) seedlings, which had been raised for 4 weeks, were transplanted into experimental plots. Water management was performed following conventional practices by the local farmers.

Experiment 2 RSB was applied in G5, G6, G20, and G21 to allow lower (1.70 – $1.76 \mu\text{g g}^{-1}$) and higher levels (2.62 –

Table 1 Physicochemical properties and elemental concentrations in biochar

pH	TC (%)	TN (%)	Total heavy metals ($\mu\text{g g}^{-1}$)				Water-soluble heavy metals ($\mu\text{g g}^{-1}$)				Water-soluble nutrients (mg g^{-1})	
			Cd	Zn	Pb	As	Cd	Zn	Pb	As	P	K
10.7	45.1	1.59	0.145	266.3	14.4	0.77	0.004	0.34	0.081	0.25	0.66	47.6

3.14 $\mu\text{g g}^{-1}$) of Cd (Table 1) to be considered for both broadcast and band RSB applications.

Broadcast application of RSB was at a rate of 10 t ha⁻¹ in the lower Cd treatments (G5) and at an increased rate of 16 t ha⁻¹ in the higher Cd treatments (G21). The decision to use two different rates of application was informed by Experiment 1 (see “Results” section) wherein an application of 10 t ha⁻¹ was deemed to be a probable minimum application to reduce leaf Cd concentrations to below regulatory limits (see “Results;” Fig. 1) in the lower Cd concentration soil (G5). Given that soil Cd concentration in G5 was approximately two thirds (Table 1) of that in the high concentration soil (G21), the biochar application was scaled accordingly (16 t ha⁻¹) in the treatment of the soil with the higher Cd concentration.

To contrast with the broadcast application of RSB, G6 (lower Cd) and G20 (higher Cd) were amended with RSB using band application. Band application focused the RSB onto only half of the plot area (the zone around the growing lettuce plants). Thus, applications of only 5 and 8 t ha⁻¹ were made in G6 and G20, respectively.

Table 2 Cd concentrations in soils ($\mu\text{g g}^{-1}$) in 21 greenhouses. Values are given as mean $\pm$ standard deviation ($n = 3$)

Greenhouses	Total soil Cd	Greenhouses	Total soil Cd
G1	1.34 $\pm$ 0.14 h ^a	G12	2.01 $\pm$ 0.20 cdef
G2	1.34 $\pm$ 0.14 h	G13	2.04 $\pm$ 0.22 cdef
G3	1.40 $\pm$ 0.21 h	G14	2.10 $\pm$ 0.34 cdef
G4	1.49 $\pm$ 0.26 gh	G15	2.11 $\pm$ 0.31 cdef
<i>G5^d</i>	1.70 $\pm$ 0.32 fgh	G16	2.16 $\pm$ 0.29 cde
<i>G6</i>	1.76 $\pm$ 0.15 efgh	G17	2.19 $\pm$ 0.26 bcde
G7	1.78 $\pm$ 0.28 efgh	<i>G18</i>	2.28 $\pm$ 0.30 bcd
G8	1.88 $\pm$ 0.24 defg	G19	2.32 $\pm$ 0.37 bc
G9	1.96 $\pm$ 0.17 cdef	<i>G20</i>	2.62 $\pm$ 0.24 ab
G10	1.98 $\pm$ 0.27 cdef	<i>G21</i>	3.14 $\pm$ 0.36 a
G11	2.00 $\pm$ 0.23 cdef		
Background soil	0.094 ^b	Regulatory limit	0.6 ^c

Different letters indicate significant difference at $p < 0.05$ between greenhouses ($n = 3$)

^a Concentration in background soil of Hebei, China (CNEMC 1990)

^b Environmental quality standard for agricultural soils of China, GB15618-1995

^c Greenhouses indicated in *italic font* were selected for the present study

In all instances, four plots (6.5 m $\times$ 7.5 m) were produced for each treatment type in every greenhouse (four control plots, without RSB addition, were also established in each green house). Regarding treatment in band RSB application, lettuce was planted within the biochar addition band, and soil and plant samples were also taken in these bands. The water and fertilizer management were same as in Experiment 1.

Sampling and chemical analysis

Topsoil samples were taken at five random points, away from the edge of the experimental plots, 2 weeks after RSB application. These five samples were combined to give a composite sample for the plot. Soil samples were air-dried, crushed, and sieved with 2-mm mesh prior to analysis (see below). Two soil samples were taken using cutting rings from the surface layer (0–10 cm) of each plot for analysis of soil bulk density (SBD) and water holding capacity (WHC).

Plant samples were taken after 2 months of growth. At harvest, shoots of lettuce were cut above the soil surface, and their fresh weight was recorded immediately. Samples were then washed with tap water to remove any attached particles, thoroughly rinsed with deionized water three times, then homogenized in an electric blender and kept in clean polyethylene bags and refrigerated at 2 °C until analysis. The corresponding root samples (0–20 cm depth) with attached soil were taken and washed with deionized water to dislodge adherent soil particles then dried at 70 °C for 48 h. Aliquots (2.0 g) of the fresh shoot samples were weighed into 50 mL clean triangular flasks. Mixed acid of concentrated HNO₃ and HClO₄ (4:1 v/v) (10 mL) was added and allowed to stand overnight. The following day, after placing a small funnel on the mouth of the flasks, samples were digested on an electric hot plate (4 h at 180 °C). The digested solution was cooled and diluted to 25 mL with Millipore ultrapure water then filtered prior to elemental analysis (Yang et al. 2009). After grinding to a fine powder, aliquots (0.15 g) of the dry root samples were weighed and digested in the same way as shoot samples.

Finely ground (<0.149 mm) soil (0.5 g) and biochar samples (0.2 g) were digested in a glass tube using HCl, HNO₃, and HClO₄ (3:1:1 v/v) (10 mL) for determination of total element concentrations (Lu 2000; Zheng et al. 2015a). Available concentrations of Cd, zinc (Zn), iron (Fe), manganese (Mn) in the soil were assessed using diethylenetriaminepentaacetic

Fig. 1 DTPA-extractable Cd concentrations, under biochar additions at different rates (0, 6, 12, 18 t ha⁻¹) in greenhouse G18, in soils (a) and shoot Cd concentrations of leaf lettuce (*Lactuca sativa* L. var. *ramosa* Hort.) (b). Different letters above the bars indicate significant differences between treatments at $p < 0.05$ ($n = 4$)

acid (DTPA) solution as the extractant (1:5 w/v) (Lu 2000; Ettler et al. 2007). The DTPA solution contained 0.005 M DTPA, 0.01 M CaCl₂, and 0.1 M triethanol amine at pH 7.3. The DTPA (50 mL) was added to soil samples (10 g) contained within 100 mL flasks and shaken for 2 h. Water extracts of biochar were carried out using 1:10 suspensions, shaken for 3 h and the supernatants filtered through a 0.45-µm pore-size Millipore filter. Measurements of elemental concentrations were carried out using inductively coupled plasma mass spectroscopy (ICP-MS, 7500a, Agilent Technologies, USA) with indium isotopes (In¹¹⁵) as internal standards (10 µg L⁻¹) for Cd and ICP optical emission spectroscopy (ICP-OES, Optima 2000, PerkinElmer Co., USA) for P, K, calcium (Ca), magnesium (Mg), Fe, Mn, and Zn. Certified reference material GBW07603 (bush twigs and leaves), spikes, and blanks were used for quality assurance. The recovery of these reference materials and spikes ranged from 85% to 112%.

Total carbon (TC) and nitrogen (TN) analyses of biochar were conducted on a solid TC/TN analyzer (Vario EL III, Elementar Analysen systeme GmbH, Germany). Soil available concentrations of N, P, and K were determined using the alkaline hydrolysis diffusion method, sodium bicarbonate leaching-molybdenum antimony colorimetric method, and ammonium acetate extraction-flame photometric method, respectively (Lu 2000).

Daily intake of Cd and HQ

The human estimated daily intake (EDI) of Cd through consumption of vegetables was calculated using the following equation (Eq. 1):

$$EDI = \frac{IR_v \times [Cd]_v}{BW} \quad (1)$$

where IR_v, [Cd]_v, and BW represent the vegetable intake rate per capita for rural resident in Beijing (kg day⁻¹; 279 g day⁻¹) (BSY 2015), Cd concentrations in vegetable and

average body weight (62 kg) (calculated according to CSY 2015 and NCDSR 2015), respectively.

The HQ indices for Cd in vegetable were calculated using the equation described by the US Environmental Protection Agency (USEPA 2014) (Eq. 2):

$$HQ = \frac{EDI}{RfD} \quad (2)$$

where RfD represents the oral reference dose (1 µg kg⁻¹ day⁻¹ for Cd) (USEPA 2014).

Statistical analysis

All data were subjected to one-way analysis of variance. Significant differences were identified using the least significant difference test ($p < 0.05$). Multiple linear regressions using a stepwise method were obtained. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., USA).

Results

Characteristics of the soils and biochar

Soil Cd concentrations were elevated in all greenhouses (1.34–3.14 µg g⁻¹) relative to background soil (0.094 µg g⁻¹) (CNEMC 1990) by factors ranging from 14.3 to 33.4. These Cd concentrations also exceeded the agricultural soil regulation guidance value (0.6 µg g⁻¹) (GB15618-1995) by factors from 2.2 to 5.2 (Table 2).

The high pH (10.7) and high carbon level (45%) of RSB were in agreement with other studies (Atkinson et al. 2010; Yuan et al. 2011; Luo et al. 2014) (Table 1). In addition, RSB used in the current study also contained plant essential nutrients, including N (1.59%) and water-soluble P and K at 0.66 and 47.6 mg g⁻¹, respectively. These nutrient concentrations are in agreement with those reported for other rice straw

biochar (Zheng et al. 2012). The Cd concentration in RSB was much lower than in the greenhouse soils (Table 1). The concentrations of Cd, Zn, Pb, and As were all well below the threshold of sludge for agricultural use in China (GB18918-2002). The Cd concentrations in RSB were an order of magnitude lower than maximum values reported for a range of biochars, while Zn, Pb, and As concentrations were within a factor of 3 (Freddo et al. 2012). Concentrations of water soluble Cd, Zn, Pb, and As in RSB were sufficiently low to be considered negligible.

Influence of biochar on soil properties and lettuce yield

In greenhouse G18, significant ($p < 0.05$) decreases in SBD (of 6% to 11%) and increases in WHC (of 10% to 16%) were observed with increasing RSB addition. Soil pH increased significantly ($p < 0.05$) by 0.2 to 0.8 units under RSB addition compared with the control (no RSB amendment). Concentrations of available P and K in soils increased by factors of up to 1.3 and 2.7 ($p < 0.05$), respectively, with the increase of RSB addition. However, the available N concentration did not change significantly under RSB amendment ($p < 0.05$) (Table 3). The improvement of soil properties, such as SBD and WHC, and increases in available nutrients following biochar addition, are usually conducive to crop production (Park et al. 2011; Uzoma et al. 2011; Albuquerque et al. 2014). In the present study, shoot weights (lettuce yield) increased significantly ($p < 0.05$) with increasing addition of RSB. Lettuce yield increased by factors of 1.08 and 1.15 under RSB addition at rates of 12 and 18 t ha⁻¹, respectively. Among soil properties (available N, P, K concentrations, SBD and WHC), the decrease in SBD following RSB addition was a major contributor to the increase in lettuce yield ($p < 0.001$) (Table 4). The SBD is an important indicator of soil physical properties; its decrease is accompanied by loosening of soil and increasing soil porosity, which is conducive to root elongation and water retention, thus assisting improved uptake of essential nutrients for plant growth (Mukherjee et al. 2014; Ulyett et al. 2014). It is therefore reasonable to speculate that RSB addition increases root biomass and root length in lettuce

Table 4 Multiple linear regressions between Cd concentrations in lettuce shoot ([Cd]_v) vs. DTPA-extractable Cd concentrations in soils ([Cd]_s) and shoot fresh weight of lettuce (SFW), between [Cd]_s vs. soil pH and available P ([P]_s), and between lettuce yield (Y) vs. soil available N, P, K concentrations, bulk density ([BD]) and water holding capacity in G18 ($n = 16$) using a stepwise method. Variables in regression equations were all significant ($p < 0.05$)

Model	<i>r</i> value	Significance
$[Cd]_v = -0.160 + 0.817 \cdot [Cd]_s$	0.719	$p < 0.01$
$[Cd]_s = 1.329 - 0.105 \cdot pH - 0.002 \cdot [P]_s$	0.892	$p < 0.001$
$Y = 46.703 - 17.787 \cdot [BD]$	0.780	$p < 0.001$

although this were not tested. In addition, there were also significant correlations between lettuce yield vs. available concentrations of K ($p = 0.003$) and WHC ($p = 0.018$), respectively (Fig. S1). The high level of available nutrients in all of the greenhouse soils may be attributed to the excessive application of fertilizers, as indicated by a previous report (Ju et al. 2007). Given the already elevated P and K status, the addition of biochar-associated P and K is unlikely to have contributed to the observed increase in lettuce yield.

In G5, G6, G20, G21, concentrations of available K in all soils increased significantly ($p < 0.05$) (by 59%–197%) after RSB addition. In the case of nil K applied in all greenhouses, lettuce yield increased by 8%–11% ($p < 0.05$) regardless of whether RSB was added using the band or broadcast method (Table S1). Available concentrations of soil N and P were not significantly influenced ($p < 0.05$) by RSB addition. Soil pH in G20 and G21 were significantly elevated ($p < 0.05$) by 0.4 and 0.4 units after RSB addition, respectively; however, the soil pH did not change significantly in G5 and G6 ($p < 0.05$).

DTPA-extractable concentrations of Cd

Biochar addition significantly ($p < 0.05$) decreased DTPA-extractable concentrations of soil Cd (0.46 μg g⁻¹) by 8%–25%, especially at the addition rate of 18 t ha⁻¹ in G18 ($p < 0.001$) (Fig. 1a). These decreases in Cd extractability may be attributed to the increases in pH and available P concentrations following RSB amendment. There were

Table 3 Influence of biochar additions on soil pH, soil bulk density, water holding capacity (WHC), available nutrient concentrations, and lettuce yield in G18. Values are given as mean ± standard deviation ($n = 4$)

Biochar addition (t ha ⁻¹)	Soil pH	Soil bulk density (g cm ⁻³)	WHC (%)	Available nutrient (mg g ⁻¹)			Lettuce yield (t ha ⁻¹)
				N	P	K	
0	7.6 ± 0.1 c	1.34 ± 0.05 a	20.1 ± 1.4 c	0.19 ± 0.04 a	0.43 ± 0.06 b	0.47 ± 0.1 c	22.9 ± 0.9 c
6	7.8 ± 0.1 b	1.26 ± 0.04 b	21.0 ± 1.2 bc	0.18 ± 0.02 a	0.46 ± 0.06 ab	0.73 ± 0.2 b	23.8 ± 0.7 bc
12	8.0 ± 0.2 b	1.23 ± 0.03 bc	22.2 ± 1.5 ab	0.19 ± 0.01 a	0.48 ± 0.06 ab	0.86 ± 0.1 b	24.6 ± 0.9 b
18	8.4 ± 0.1 a	1.19 ± 0.02 c	23.4 ± 0.7 a	0.18 ± 0.01 a	0.56 ± 0.11 a	1.27 ± 0.2 a	26.3 ± 0.7 a

Different letters in the same column represent significant difference between treatments ($p < 0.05$) ($n = 4$)

significant correlations between DTPA-extractable concentrations of Cd with soil pH ($p < 0.001$) and with available P ($p = 0.008$) (respectively, Fig. 2a, b). Both variables (soil pH and available P concentration) contributed significantly ($p < 0.05$) to the immobilization of soil Cd (Table 4).

Following RSB amendment in broadcast application in G5 (10 t ha^{-1}) and G21 (16 t ha^{-1}) and in band application in G6 (5 t ha^{-1}) and G20 (8 t ha^{-1}), DTPA-extractable concentrations of Cd decreased significantly ($p < 0.05$) by 20%, 30%, and 32% in G21, G6, and G20, respectively (although no significant reduction was observed in G5) (Fig. 3a). The decrease rates of DTPA-extractable concentrations of Cd caused by RSB amendment were increased with increasing extractable Cd in soils initially in the four greenhouses.

Concentrations of DTPA-extractable Fe, Mn, and Zn in soils were measured as these can influence the fate of Cd in soil by affecting its availability in soils (Table S2). Soil concentrations of DTPA-extractable Fe and Mn in G18 decreased with increasing RSB additions by up to 33% ($p < 0.01$) and 28% ($p < 0.05$), respectively. The DTPA-extractable Zn concentrations did not change significantly ($p < 0.05$) after RSB addition. In G5, G6, G20, and G21, concentrations of DTPA-extractable Fe decreased by 22%–31% ($p < 0.05$) after RSB addition; however, Zn concentrations were not significantly affected ($p < 0.05$). The DTPA-extractable Mn concentrations reduced by 24% in G20 only ($p < 0.05$).

Cd accumulation in lettuce

Shoot Cd concentrations in lettuce were reduced by up to 57% ($p < 0.001$) ($0.22 \mu\text{g g}^{-1}$ fresh weight, FW, initially) with increasing RSB additions in G18 (Fig. 1b). There was a significant ($p = 0.002$) positive correlation between shoot Cd concentrations in lettuce and DTPA-extractable concentrations of Cd in soils (Fig. 2c). In addition, lettuce yield, namely, shoot biomass (SFW), was also linearly correlated with shoot Cd concentrations ($p = 0.003$) (Fig. 2d). The SFW was excluded in multiple linear regression analysis between Cd concentrations in lettuce shoot ($[\text{Cd}]_s$) vs. DTPA-extractable Cd concentrations in soils ($[\text{Cd}]_e$) and SFW using a stepwise method. This analysis indicated that it was the immobilization of Cd in soils rather than the biomass dilution that underpinned the reduction in Cd concentrations in lettuce shoots following RSB addition (Table 4).

In G6 and G20, where RSB was applied in bands, Cd concentrations in lettuce shoots decreased by 29% and 42%, respectively. Where RSB was applied by broadcasting (in G21 and G5), a significant ($p < 0.05$) decrease in shoot Cd concentration of lettuce was only observed in the higher Cd concentration soils (Fig. 3b). Compared with the national standard, shoot Cd concentrations under un-amended treatments in G6, G18, G20 were 0.1%, 10%, and 23% higher, respectively, than the Chinese regulation limit set for food ($0.2 \mu\text{g g}^{-1}$ FW) (MHPRC and SAC 2012). Shoot Cd concentrations of lettuce in these three greenhouses were reduced to below the

Fig. 2 Relationship between DTPA-extractable concentration of Cd, under biochar additions at different rates (0, 6, 12, 18 t ha^{-1}) in greenhouse G18 vs. soil pH (a) vs. soil-available P concentration (b). The relationship between shoot Cd concentrations vs. DTPA-extractable concentrations of Cd (c) vs. shoot biomass of lettuce (lettuce yield) (d)

Fig. 3 DTPA-extractable Cd concentrations in four greenhouses (G5, G6, G20, G21) in soils (a) and shoot Cd concentrations of leaf lettuce (*L. sativa* L. var. *ramosa* Hort.) (b), in the un-amended control treatment (□), broadcast biochar application (▨) and band biochar application (▤)

(▤). Different letters above the bars indicate significant differences at $p < 0.05$ comparing biochar application treatment with the un-amended control treatment ($n = 4$)

regulation limit following RSB amendment. Shoot Cd concentrations in lettuce did not exceed the maximum allowable limit in G5 and G21, although soil Cd concentrations were higher than the guidance value for agricultural soil in China.

Root Cd concentrations in lettuce, based on dry weight, are shown in Table S2. Root Cd concentrations decreased by up to 53% ($p < 0.01$) with increasing RSB additions in G18. Decreases in root Cd concentrations of 34% and 42% ($p < 0.05$) were caused by band RSB additions in G6 and G20, respectively. Broadcast application of RSB significantly reduced root Cd concentrations by 33% ($p < 0.05$) in G21, but not in G5.

Estimated daily intake of Cd and HQs

The EDIs and HQs of Cd are listed in Table 5. The EDIs of Cd in un-amended treatments in the five experimental

greenhouses ranged from 0.70 to $1.11 \mu\text{g kg}^{-1} \text{ day}^{-1}$. After RSB addition, EDIs for Cd decreased by up to 57%, 22%, 23%, 29%, and 42% in G18, G5, G21, G6, and G20, respectively. Among these five greenhouses, Cd EDI was above the oral reference dose ($1.0 \mu\text{g kg}^{-1} \text{ day}^{-1}$) set by the USEPA only in the un-amended treatment of G20 and as a consequence its HQ was >1 (Table 5). However, the Cd HQ in G20 decreased from 1.11 in the control to 0.65 under RSB amendment.

Discussion

To our knowledge, this is the first report to investigate the influence of biochar on the accumulation of Cd in lettuce under greenhouse vegetable production, and the first study to apply biochar under band application. The soils of all experimental greenhouses were tainted by Cd due to historically

Table 5 EDI and HQ for Cd associated with the consumption of vegetable grown in control soils and soils amended with biochar. Where HQ was >1 this has been indicated in *italics*

Greenhouses	Application ways	Biochar dose (t ha^{-1})	EDI ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)	HQ
G18	Control	0	0.99	0.99
	Broadcast	6	0.82	0.82
	Broadcast	12	0.66	0.66
	Broadcast	18	0.42	0.42
G5	Control	0	0.70	0.70
	Broadcast	10	0.54	0.54
G21	Control	0	0.83	0.83
	Broadcast	16	0.64	0.64
G6	Control	0	0.90	0.90
	Band	5	0.64	0.64
G20	Control	0	1.11	<i>1.11</i>
	Band	8	0.65	0.65

Control, broadcast, and band represent un-amended with biochar, biochar application in broadcast placement, and biochar application in band placement, respectively

frequent and large applications of manure containing high Cd levels. Both band and broadcast applications of RSB successfully reduced shoot Cd concentrations in lettuce (Fig. 3b). The band application, of course, halved the mass of biochar required for soil amelioration and therefore represents an opportunity to reduce remediation costs.

Soil pH and P were the key factors decreasing DTPA-extractable concentrations of soil Cd after RSB addition. The biochar-induced pH increase (0.2–0.8 units) was strongly correlated with the immobilization of Cd (Tables 3 and 4; Fig. 2a). Because the number of negatively charged surface sites in soil increases with increasing pH, the sorption capacity of soil to cationic metals correspondingly increases (Bradl 2004). The pH increase after the application of biochar could reduce metal extractability by promoting metal (co)precipitation and metal adsorption on specific biochar mineral phases (Rees et al. 2014; Yang et al. 2016). Moreover, other studies have shown that metal mobility reduced in the presence of biochar because of the metal adsorption on the biochar surface (especially on carboxylic and phenolic groups) (Uchimiya et al. 2011a, b). In addition to an increase in soil pH, P entering the soil with RSB application may also have been a contributing factor in immobilizing Cd through the formation of phosphate precipitates (McGowen et al. 2001; Zheng et al. 2011) (Table 1). Availability of soil P, after RSB addition, was observed to increase, and a significant correlation between concentrations of soil available P and DTPA-extractable concentrations of soil Cd was shown (Table 3, Fig. 2b).

Soils of the five experimental greenhouses exhibited a wide range of Cd concentrations decreasing in the following order: G5 < G6 < G18 < G20 < G21. This was noticeably different to the order observed for extractable Cd: G5 < G18 < G21 < G6 < G20 (Table 2, Fig. 3a). This may be attributed to the difference of soil properties between the greenhouses, which was gradually caused by different fertilizer, water, and vegetable planting management over the years. For instance, soil pH (7.3–7.8), available K (0.25–0.54 mg g⁻¹), and available P (0.31–0.48 mg g⁻¹) were notably different among the five experimental greenhouses. In addition to these factors, Fe and Mn oxides, competitive ions (e.g., Zn), salinity, and chloride levels in the greenhouse soils could also influence the availability of soil Cd (Adams et al. 2004). Contrasting Cd concentration/availability and soil properties may have been caused by the variability in application of animal manure containing high levels of organic matter. These applications were large and frequent (>40 t ha⁻¹ year⁻¹) in all greenhouses. The Cd concentration can be strongly immobilized through specific adsorption reactions with organic matter (Cui et al. 2016; van Herwijnen et al. 2007; Karlsson et al. 2007), although it can also occur when binding with dissolved organic matter (Del Castilho et al. 1993; Kalbitz and Wennrich 1998).

The magnitude of decrease in DTPA-extractable concentrations of Cd was in the order G5 < G21 < G6 < G20. This order was the same as that of initial extractable Cd in soil (Fig. 3a). The effect of RSB amendment on reducing DTPA-extractable concentrations of Cd increased with increasing initial extractable Cd in soil. One possible interpretation is that a lesser amount of extractable Cd was present in the soil and thus a smaller amount of Cd was able to diffuse to the biochar surface or become dissolved in soil solution to be adsorbed or precipitated. This supports the suggestion that diffusion of Cd ions to the root surface is the dominant pathway in soil (Lorenz et al. 1994). In addition, the decreases in DTPA-extractable concentrations of Fe in soils of all greenhouses and that of Mn in G20 ($p < 0.05$) (Table S2) may further immobilize soil Cd through co-precipitation with Fe/Mn oxides, which are abundant in soils and have great capacity to sorb Cd (Suda and Makino 2016).

Shoot Cd concentrations in lettuce decreased significantly after RSB addition, except in G5 (Figs. 1b and 3b). The decreases in shoot Cd concentration were attributed primarily to the decreases in extractable Cd in soil though there was a significant ($p = 0.003$) negative correlation between shoot biomass (lettuce yield) and shoot Cd concentration (Fig. 2c, d, Table 4). The Cd concentrations in lettuce shoot of G6, G18, and G20 among the five experimental greenhouses exceeded the Chinese regulation limit set for food (0.2 µg g⁻¹ FW) by 0.1%–23.2%. However, RSB addition successfully decreased the Cd level in lettuce shoots to an acceptable level (<0.2 µg g⁻¹ FW) when applied using both band and broadcast methods. In addition, concentrations of other nutrient elements such as K, P, Ca, Mg, Zn, and Fe were also determined in lettuce shoots (Table S3). Shoot concentrations of P, Ca, Mg, Zn and Fe were not significantly influenced by RSB addition ($p < 0.05$). However, RSB addition caused a significant increase in K concentration of up to 27% in G18 and 18–26% in G5, G6, G20 and G21 ($p < 0.05$). Thus, there was an improvement in the quality of lettuce in response to elevated K level after RSB addition. The observation of no clear effect on Fe concentrations in lettuce shoot, despite the reduction in Fe extractability (Table S2), following RSB additions may be attributed to the mechanisms of plants for acquiring Fe and the initially high Fe availability (27.7–39.2 µg g⁻¹) (Marschner and Rohmheld 1994; Zheng et al. 2015b).

Shoot Cd concentration exceeded the maximum allowable limit for lettuce grown in control soils (G18 and G20), whereas Cd concentrations in lettuce shoots grown in G6 soil was close to the limit. Moreover, Cd concentrations in lettuce shoots grown in the other control soils were below the regulatory limit, although the soils in these greenhouses had higher Cd concentrations that were above the regulatory limit for soils (Table 2). This was expected because the bioaccumulation factor (BF) of Cd, determined as a ratio of Cd

concentration in lettuce shoot (edible parts) to that in soil, is an intricate function of the genotype of the plant and the soil properties such as pH, organic carbon content, cation exchange capacity, and clay content (Gaw et al. 2008; Zhang et al. 2014; Mombo et al. 2016). The BFs changed in accordance with shoot Cd concentrations and ranged from 0.04 to 0.11 (Table S2). These are similar to results reported in other studies (Yang et al. 2009; Zhang et al. 2014; Mombo et al. 2016).

The observation of HQs with values less than 1 indicate daily intake of Cd to be less than the reference dose, implying an absence of unacceptable risk to human health. The EDI and HQ values of Cd among the experimental greenhouses were decreased by up to 57.2% and 57.2%, respectively, after RSB additions (Table 5). The most notable result was that RSB addition decreased Cd HQ for G20 from an initial 1.11 to an acceptable level (<1). The results obtained in the present study are significant as they showed potential for biochar amendment to improve vegetable safety through the sequestration of bioavailable Cd from greenhouse soil to edible parts of the vegetable. Consequently, the EDIs and HQ associated with vegetable consumption were reduced.

Other studies investigating effects of biochar on lettuce growth and/or Cd accumulation in pot experiments support our results (Gunes et al. 2014; Upadhyay et al. 2014; Kim et al. 2015; Ye et al. 2016). Biochar derived from paper mill sludge significantly ($p < 0.05$) decreased Cd concentration in lettuce leaves by 26%–71% and increased lettuce biomass (Kim et al. 2015). Additions of poultry manure biochar (PMB) resulted in significant ($p < 0.05$) increases in shoot K concentration of lettuce (Gunes et al. 2014), which supports our results. However, the reported increases in shoot concentrations of P and reductions in Fe and Zn concentrations after PMB addition disagreed with the present study. This may be attributed to the initially high available P level (0.31–0.48 mg g⁻¹) in greenhouse soils in the present study and the much higher concentrations of available Fe and Zn in greenhouse soils than that in Gunes et al. (2014) (27.7–39.2 vs. 6.4 µg g⁻¹ for Fe and 9.9–12.9 vs. 0.72 µg g⁻¹ for Zn). Further studies have indicated that biochars, such as gasification and fast-pyrolysis pine and poplar wood chars, inhibited lettuce growth (Marks et al. 2014), and coffee husk char induced an increase of extractable Cd in a sandy loam soil despite a decrease in Cd concentration in lettuce (Woldetsadik et al. 2016). Such variability in outcome might relate to the distinct properties (such as nutrient composition, surface area, and volatile matter content) of biochars made from diverse materials or under different pyrolysis conditions, which cause varying effects on Cd immobilization and lettuce growth performance (Marks et al. 2014; Woldetsadik et al. 2016).

Regarding the economic cost and benefits of biochar, it was cost effective to add biochar, by virtue of saving potassic fertilizer and increasing vegetable yield. The band application

of biochar provides an opportunity to halve biochar cost. Thus, we suggest biochar application in bands as a recommended remediation approach for vegetable greenhouses contaminated by Cd.

Conclusions

Rice straw biochar addition effectively reduced Cd concentrations in lettuce shoots under greenhouse vegetable production primarily through immobilization of soil Cd rather than biomass dilution. Shoot Cd concentrations in lettuce were decreased by up to 57.2% with increased RSB addition. Whether RSB was amended using broadcast or band application, Cd concentrations in lettuce shoots were reduced. Despite soil Cd concentrations being above guideline values for agricultural land, the levels of Cd in lettuce were reduced to a level below the Chinese regulation limit set for food (0.2 µg g⁻¹ FW) in all RSB-treated soils. Lettuce yields were increased significantly by 8.2%–14.8% along with decreases in SBD, increases in WHC, and available nutrient (K and P) concentration following RSB addition.

Biochar application in bands is a recommended method to constrain biochar costs, to immobilize soil Cd, and to reduce Cd accumulation in edible parts of vegetables. Given that rice straw is a readily available agricultural residue, we suggest that its conversion to biochar and incorporation into greenhouse soil contaminated by Cd, especially in band application, could be an achievable and cost-effective approach to mitigate Cd exposure associated with vegetable consumption.

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