The effects of biochars from rice residue on the formation of iron plaque and the accumulation of Cd, Zn, Pb, As in rice (*Oryza sativa* L.) seedlings

Rui-Lun Zheng^a, Chao Cai^b, Jian-Hong Liang^a, Qing Huang^b, Zheng Chen^a, Yi-Zong Huang^a, Hans Peter H. Arp^c, Guo-Xin Sun^{a,*}

^a State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

^b Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361003, China

^c Department of Environmental Engineering, Norwegian Geotechnical Institute, Ullevål Stadion, P.O. Box 3930, 0806 Oslo, Norway

HIGHLIGHTS

- Rice biochar reduced Cd, Zn and Pb transport to rice shoots, though increased As.
- Biochars derived from rice straw were more influential than from bran and husk.
- Mechanisms for these effects were identified.
- Biochar increased iron plaque formation, and its capacity to retain Cd and Pb.
- Biochar also influenced the soil pore water solubility of Cd, Zn, Pb and As.

ARTICLE INFO

Article history:

Received 17 January 2012

Received in revised form 7 May 2012

Accepted 7 May 2012

Available online 2 June 2012

Keywords:

Biochar

Heavy metal

Accumulation

Rice

Iron plaque

ABSTRACT

A historically multi-metal contaminated soil was amended with biochars produced from different parts of rice plants (straw, husk and bran) to investigate how biochar can influence the mobility of Cd, Zn, Pb and As in rice seedlings (*Oryza sativa* L.). Rice shoot concentrations of Cd, Zn and Pb decreased by up to 98%, 83% and 72%, respectively, due to biochar amendment, though that of As increased by up to 327%. Biochar amendments significantly decreased pore water concentrations (C_{pw}) of Cd and Zn and increased that of As. For Pb it depended on the amendment. Porewater pH, dissolved organic carbon, dissolved phosphorus, silicon in pore water and iron plaque formation on root surfaces all increased significantly after the amendments. The proportions of Cd and Pb in iron plaque increased by factors 1.8–5.7 and 1.4–2.8, respectively; no increase was observed for As and Zn. Straw-char application significantly and noticeably decreased the plant transfer coefficients of Cd and Pb. This study, the first to investigate changes in metal mobility and iron plaque formation in rice plants due to amending a historically contaminated soil with biochar, indicates that biochar has a potential to decrease Cd, Zn and Pb accumulations in rice shoot but increase that of As. The main cause is likely biochar decreasing the C_{pw} of Cd and Zn, increasing the C_{pw} of As, and increasing the iron plaque blocking capacity for Cd and Pb.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The use of biochar for soil amendment is becoming increasingly popular because of its potential benefits of soil carbon sequestration (Lehmann, 2007), improvement of soil quality (Novak et al., 2009), increase of crop yield, mitigation of nutrient leaching (Steiner et al., 2007) and organic contaminant remediation (Yang et al., 2006). In addition, a number of studies indicate that biochar could significantly contribute to the immobilization of certain heavy metals such as Cd, Zn and Pb in the soil (Beesley et al., 2010; Cao et al., 2011); however, biochar can also increase the mobility of

metalloids that form for oxy-anions, like As. Addition of hard-wood derived biochar to a multi-metal polluted soil resulted in a 10-fold decrease of Cd and more than 30-fold increase of As in pore water (Beesley et al., 2010). Cao et al. (2011) showed that dairy-manure biochar could immobilize Pb in soil via both adsorption and precipitation, which reduced the uptake of Pb up to 79% by earthworms. However, it remains unknown to what extent the original biomass material and the particle size of biochar can influence the effect on immobilization and phytoavailability of contaminant metals in soils, and what the predominating mechanisms are for such processes. To address this, this study investigates the influence and mechanisms of biochar amendments on the phytoavailability of contaminant metals to rice-plants grown in a historically contaminated soil.

* Corresponding author. Tel.: +86 10 6284 9328; fax: +86 10 6292 3563.

E-mail address: gxsun@rcees.ac.cn (G.-X. Sun).

Iron plaque is usually formed on the surface of rice roots owing to the release of oxygen or oxidants from roots into the rhizosphere (Chen et al., 1980). This iron plaque exhibits a high capacity to sequester certain metals. The proportion of As, Cd, and Pb distributed in iron plaque on rice root surface could reach 88%, 43% and 33% respectively (Lei et al., 2011). The presence of iron plaque on root surfaces has been reported to reduce the uptake of metals by plants because of the high adsorption capacity of its iron hydroxide functional groups (Greipsson and Crowder, 1992; Batty et al., 2000; Liu et al., 2011). The formation of iron plaque and its ability to sequester contaminant metals is influenced by soil contents and soil amendments. It was reported that both formation of iron plaque and iron plaque adsorbed Cd increases with increasing Fe amendment, though the proportion of iron plaque adsorbed Cd decreases when Cd levels in soil are enhanced (Liu et al., 2008). Sequestration of Zn in iron plaque was reported to be significant under field conditions, but not in nutrient solution cultures (Ye et al., 1998). It is expected that biochar-amendments may also influence iron plaque formation and thereby the sorption, mobility and plant translocation of heavy metals. This present study is the first to investigate this potential influence of biochar.

Herein, using a historically contaminated soil, we present the results of experiments designed to test: (i) the influence of biochar parent materials and particle sizes on immobilizing heavy metals in soil and reducing their accumulation in rice plants; and (ii) the effects of these additions on the iron plaque sequestrations of Cd, Zn, Pb and As. Rice was chosen as the test species here, as biochar amendments are expected to be commonly applied to rice fields.

2. Materials and methods

2.1. Biochar, soil and rice culture

Biochar used here was made from three parts of rice plants (*Oryza sativa* L.): straw, husk and bran. These are referred to as straw-char, husk-char and bran-char, respectively. Each type of biomass was charred in a pyrolyzer under a stream of N_2 at 500 °C for 4 h. Following charring, biochars were ground and sieved, yielding fine (<0.18 mm) and coarse (0.5–2 mm) size fractions. Some physicochemical properties of biochars are reported in Table S1 and component difference arising from different particle sizes and parent materials of biochars are listed in Table S2.

The test soil is a historically multi-metal contaminated soil collected from farmer fields near a mining area in Zhuzhou city, Hunan province, south China. The soil was air-dried, crushed and passed through sieves with 2-mm mesh. Physicochemical properties of the soil are presented in Table S3.

Rice seeds disinfected in 30% H_2O_2 (w/w) solution for 15 min were thoroughly washed with deionized water and germinated in moist perlite. After growing for 5 weeks, rice seedlings were transplanted to soils with or without biochar. The seedlings grew under flooded conditions with a layer of water about 2 cm above the soil surface. The rice was cultured in a green house set to 14 h daytime with a light intensity of $350 \mu\text{mol m}^{-2} \text{s}^{-1}$, 28 and 20 °C day and night temperatures, and a relative humidity of 60–70%.

2.2. Treatment

Fine and coarse straw-, husk- and bran-char were thoroughly homogenized with the contaminated soils (5% biochar by weight), flooded with deionized water and incubated for two weeks. An addition of 5% was chosen because it is a typical addition based on previous work on biochar. Pore water was collected with a soil moisture sampler (Rhizosphere Research Products, Netherlands) inserted into each pot prior to transplanting rice seedlings. Basal

fertilizers were added to the soil as 200 mg N (as $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), 180 mg P (as KH_2PO_4) and 60 mg Mg (as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) kg^{-1} (based on air-dry weight). Soils without biochar addition (0%) were designated as the control. There were seven treatments and each treatment was replicated four times containing 400 g soil and 2 seedlings per pot. Plants were harvested after 4-weeks growth. Shoots were cut at the soil surface, washed with deionized water, dried at 70 °C for 48 h, and then weighed. Roots were taken from soils by gentle sieving for further measurements.

2.3. Analytical methods

Iron plaque was extracted from fresh root surfaces using dithionite-citrate-bicarbonate (DCB) solution containing 0.03 M sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), 0.125 M sodium bicarbonate (NaHCO_3) and 0.06 M sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). The whole root system per pot was dipped into DCB solution (40 mL) at 25 °C for 60 min, and then rinsed three times with deionized water. Rinsed water was poured into the DCB extracts and the total volume was made up to 50 mL with deionized water. The final solution was passed through a 0.45- μm nylon filter and refrigerated at 2 °C for subsequent analysis (Liu et al., 2004).

After extraction by DCB solution, fresh roots were scanned into the WinRhizo software for the estimation of root length, and then oven dried at 70 °C for 48 h.

Each dried shoot and root sample were ground and weighed (0.1 g) into 50 mL polyethylene centrifuge tubes and digested with concentrated HNO_3 (2 mL) in a microwave digester (Sun et al., 2008). The pH value of pore water was determined using a glass electrode immediately after sampling with the soil moisture sampler. The other part of pore water was acidified with nitric acid (GR grade) to pH 2 and stored for further analysis of elements. All solutions used were passed through a 0.45- μm nylon filter. Measurements of elemental concentrations were carried out using inductively coupled plasma mass spectroscopy (ICP-MS, 7500a, Agilent Technologies, USA) for Cd^{114} , Zn^{66} , Pb^{208} , As^{75} and indium isotopes (In^{115}) as internal standards ($10 \mu\text{g L}^{-1}$), and ICP optical emission spectroscopy (ICP-OES, Optima 2000, PerkinElmer Co., USA) for P, Si and Fe. Certified reference material (CRM) GBW07603 (Bush Twigs and Leaves), spikes and blanks were used for quality control. The recovery ratios of the elements determined were from 89% to 120% throughout the analysis procedures. A Liqui TOC II analyser (Elementar Analysensysteme GmbH, Germany) was used for measuring dissolved organic carbon.

2.4. Data analysis

Percentage (%) of Cd, Zn, Pb and As in shoot, root and iron plaque were based on the total amount of Cd, Zn, Pb and As measured.

All data were subjected to two-way analysis of variance (ANOVA) to test for significant differences resulting from the biochar type and particle size treatments. Comparisons between means were made using the Tukey-test ($p < 0.05$). Multiple linear regressions were used to examine relationships between heavy metal concentrations in shoot and plant transfer coefficient, root length, heavy metal concentrations in pore water (C_{pw}) and in iron plaque. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., USA).

3. Results

3.1. Properties of biochars

The produced biochars exhibited a wide range of pH (8.1–11.3) and water extractable DOC concentrations ($0.04\text{--}0.36 \text{ mg g}^{-1}$) in

the sequence of bran < husk < straw (Table S1). All biochars contained negligible amounts of water-extractable metals. The highest levels of P and Si were measured in bran-char (3.6 mg g⁻¹) and straw-char (4.6 mg g⁻¹), respectively (Table S2).

3.2. pH, DOC and heavy metals in pore water

Biochar soil additions increased the pore water pH from 7.1 to up to 8.2 ($p < 0.05$) (Table 1). With the exception of husk-char, additions of straw-char and fine bran-char increased the initial DOC C_{pw} (37.2 mg L⁻¹) by 61–112%. Fine biochars were generally more effective at enhancing the pH and DOC C_{pw} ($p < 0.001$) (Table 1).

Initial C_{pw} of Cd (107 µg L⁻¹) and Zn (2.1 mg L⁻¹) decreased significantly ($p < 0.001$) by 43–89% and 55–95%, respectively, as a result of biochar additions; however, the C_{pw} of As (0.05 mg L⁻¹) increased by up to a factor 14.2 (Fig. 1). Additions of husk- and bran-char significantly decreased Pb C_{pw} (27 µg L⁻¹ initially) by up to 26%, however straw-char resulted in a significant increase of 23% (Fig. 1). Overall, straw-char addition caused the largest decrease of Cd and Zn C_{pw} (to factors 0.1 and 0.05 of the original, respectively); bran-char addition resulted in the largest decrease of Pb C_{pw} (to a factor 0.7), and the greatest increase of As C_{pw} .

Regarding the size fraction, for Cd and Zn C_{pw} fine bran-char caused 32% and 24% more of a decrease than the coarse one, though fine husk- and straw-char performed resulted in a slight, though significant decrease compared to the coarse varieties ($p < 0.01$). For As, fine bran-char caused a 290% increase in C_{pw} compared to the coarse fraction, though fine husk-char and straw-char performed similarly to coarse. There were no significant differences in Pb C_{pw} between coarse and fine particle sizes for any biochar treatment.

3.3. Iron plaque and its heavy metals content

Iron plaque on root surface (DCB-Fe) ($p < 0.01$) increased as a result of biochar amendments. The maximum increase was a factor 2.1 when using fine straw-char (Table 1). Fine straw-char application resulted in more DCB-Fe (1.6 vs 1.2 mg g⁻¹) than the coarse fraction, though for bran-char the fine fraction produced less than the coarse fraction (0.8 vs 1.0 mg g⁻¹).

The influence of biochar amendments on concentrations of Cd, Zn, Pb and As in iron plaque was various (Fig. 2). Addition of straw-char significantly ($p < 0.05$) decreased concentrations of Cd and Zn in iron plaque by up to 75% and 79%, respectively, while

it enhanced Pb concentration by 136–163%, and for As it made no significant change. Concentration of DCB-Cd was 27% lower in fine sized straw-char treatment than in the coarse one ($p < 0.05$). Addition of husk- and bran-char in many cases resulted in non significant or slight changes in DCB concentrations of Cd, Zn, Pb and As. Exceptions were the husk-char addition significantly reducing DCB-As by up to 72%, and bran char-addition significantly reducing DCB-Zn by 59%.

3.4. Plant growth and heavy metal accumulations

No significant change in shoot biomass existed after additions of any of the biochars. Statistical decreases of root biomass (29%) and root length (35%) were observed only in fine bran-char treatment but not other biochars (Table 1).

Translocation of Cd, Pb and As from root to shoot in rice plant were significantly different ($p < 0.001$) in the presence of various types of biochars, though for Zn no significant change was observed (Table S4). The most substantial change was for straw-char amendment, which significantly decreased transfer coefficients of Cd and Pb by up to 88% and 55%, respectively, and enhanced that of As by up to 658%. The influence of husk and bran biochars on the transfer coefficients were generally within a factor 2.6. Biochar size fraction had no significant influence on plant transfer coefficients for Cd, Pb and As, though for Zn the fine fraction resulted in significantly higher coefficients.

Fig. 3 presents shoot concentrations measured after different biochar treatments. Biochar addition decreased initial shoot concentrations of Cd, Zn and Pb (0.2, 1.1 and 0.02 mg g⁻¹ DW) by up to 98%, 83% and 72%, respectively, while they increased As concentration (1.1 µg g⁻¹ DW) by up to 327% (Fig. 3). Straw-char additions caused the greatest decrease of Cd, Zn and Pb concentrations in shoot, despite raising the Pb C_{pw} (Fig. 1). Fine biochars were generally more effective on reducing concentrations of Cd and Zn ($p < 0.001$) in rice shoots than coarse chars, but not on that of Pb ($p = 0.16$). This was especially the case for fine bran-char application that resulted in lower shoot Cd (44.7 vs 95.4 µg g⁻¹) and Pb (8.4 vs 15.5 µg g⁻¹) concentrations and higher shoot As concentration (4.9 vs 3.2 µg g⁻¹) than the coarse bran-char treatment. For straw-char, the Cd and Zn shoot concentrations were also significantly lower for the fine-fraction than coarse-fraction (2.9 vs 5.0 µg g⁻¹ and 0.2 vs 0.3 mg g⁻¹, respectively).

Additions of biochar could have affected concentrations of Cd, Zn, Pb and As in rice shoots through altering their C_{pw} , iron plaque

Table 1
pH and DOC concentration of soil pore water, shoot biomass, root biomass, root length and iron plaque of rice seedlings under different biochar additions. Values are given as mean ± standard deviation for four measurements.

Biochar addition	Biochar type	Particle size	Pore water		Shoot biomass (g DW pot ⁻¹)	Root biomass (g DW pot ⁻¹)	Root length (m pot ⁻¹)	DCB-Fe (mg g ⁻¹ RDW)
			pH	DOC (mg L ⁻¹)				
0% ^a			7.1 ± 0.02	37.2 ± 6.5	0.53 ± 0.01	0.03 ± 0.01	4.5 ± 0.8	0.7 ± 0.2
5%	Straw ^b	Coarse	8.1 ± 0.06	63.1 ± 2.0	0.51 ± 0.04	0.03 ± 0.00	5.3 ± 1.0	1.2 ± 0.2
5%		Husk	7.2 ± 0.04	23.2 ± 3.6	0.51 ± 0.05	0.03 ± 0.01	4.7 ± 0.5	1.3 ± 0.3
5%	Bran	Coarse	7.3 ± 0.05	41.0 ± 3.7	0.52 ± 0.07	0.03 ± 0.00	3.5 ± 0.6	1.0 ± 0.1
5%		Straw	8.2 ± 0.02	79.0 ± 9.4	0.49 ± 0.06	0.03 ± 0.00	4.4 ± 0.4	1.6 ± 0.2
5%	Husk	Fine	7.4 ± 0.07	23.6 ± 3.0	0.52 ± 0.02	0.03 ± 0.00	4.3 ± 0.4	1.3 ± 0.2
5%		Bran	7.4 ± 0.03	59.8 ± 9.1	0.49 ± 0.04	0.02 ± 0.00	2.9 ± 0.4	0.8 ± 0.1
Significance of ^c								
Biochar addition			*	ns	ns	ns	ns	**
Biochar type (T)			***	***	ns	*	***	***
Particle size (S)			***	***	ns	*	*	ns
T × S			ns	*	ns	ns	ns	*

^a 0% and 5% represent soils without biochar addition and with additions of biochar in an 1:20 ratio (w/w), respectively.

^b Straw, husk and bran represent soils with additions (5%) of biochars derived from rice straw, husk and bran, respectively.

^c Levels of significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns, not significant.

Fig. 1. The influence of biochar produced from different tissues of rice plant on the concentrations of Cd, Zn, Pb and As in pore water at day 14 after biochar application before rice (*O. sativa* L.) planting. Control, straw, husk and bran represent soils with additions of no char, 5% straw-char, husk-char and bran-char, respectively. Different letters above columns (a or b) indicate significant difference at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * Indicates significant difference at $p < 0.05$ comparing the control with other treatments.

Fig. 2. The influence of biochar produced from different tissues of rice plant on concentrations of Cd, Zn, Pb and As in iron plaque of rice (*O. sativa* L.). Control, straw, husk and bran represent soils with additions of no char, 5% straw-char, husk-char and bran-char, respectively. Different letters above columns (a or b) indicate significant difference at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * Indicates significant difference at $p < 0.05$ comparing the control with other treatments.

concentrations and their root-shoot transfer coefficients. In Table 2, multiple linear regressions are presented that correlate shoot concentrations with the most significant of these variables (with $p < 0.05$) (Table 2). DOC was found to linearly correlate with C_{pw} of Cd, Zn, Pb and As (with r^2 of 0.39, 0.27, 0.38 and 0.43, respectively) ($p < 0.01$), making it difficult to differentiate the roles of these two factors by correlation analysis. Thus, DOC concentrations in pore water were not included in this statistic analysis.

3.5. Plant distribution of heavy metals

As evident in Table S5, due to increased iron plaque formation (Table 1), additions of biochar increased proportions of Cd and Pb in iron plaque ($p < 0.05$) by factors ranging 1.8–5.7 and 1.4–2.8, respectively, corresponding to the reduced proportions of Cd and Pb in shoots by up to factors 0.3 and 0.3, respectively. For Zn, iron plaque and shoot proportions remained consistent. For As, the iron

Fig. 3. The influence of biochar produced from different tissues of rice plant on concentrations of Cd, Zn, Pb and As in shoot of rice (*O. sativa* L.). Control, straw, husk and bran represent soils with additions of no char, 5% straw-char, husk-char and bran-char, respectively. Different letters above columns (a or b) indicate significant difference at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * Indicates significant difference at $p < 0.05$ comparing the control with other treatments.

Table 2

Multiple linear regressions between heavy metal concentrations in rice shoot (s) vs pore water concentrations (pw), iron plaque concentrations (ip), plant transfer coefficients (TCs), and root length (RL) ($n = 28$) using a stepwise method. Variables in regression equations were all significant ($p < 0.05$).

Model	<i>r</i>	Sig.
$[Cd]_s = -15.21 + 1.716 \cdot [Cd]_{pw} + 133.039 \cdot TC_{Cd}$	0.961	$p < 0.001$
$[Zn]_s = 178.608 + 0.245 \cdot [Zn]_{pw} + 0.196 \cdot [Zn]_{ip}$	0.930	$p < 0.001$
$[Pb]_s = -1.671 + 19.407 \cdot TC_{Pb} + 3.407 \cdot RL - 0.039 \cdot [Pb]_{ip}$	0.877	$p < 0.001$
$[As]_s = 0.562 + 0.006 \cdot [As]_{pw} + 0.195 \cdot TC_{As}$	0.936	$p < 0.001$

plaque proportion decreased to factors ranging from 0.5 to 0.8, and the shoot proportions correspondingly increased by factors ranging from 1.6 to 2.3.

Biochar's influences on root proportions were mixed. For Cd and As there were generally no significant changes, except for straw char, increasing the root proportion of Cd by up to a factor 2.5 and decreasing the As proportion to a factor 0.2. For Pb and Zn, root proportions generally decreased to a factor 0.6, except when straw char was used which resulted in no significant change.

Particle size of biochar had no significant effect on distributions of Cd, Pb, Zn and As in iron plaque and shoot. However, percentages of Pb and Zn in root were generally ($p < 0.01$) lower in the fine sized biochar treatment than in the coarse one.

4. Discussion

To our knowledge, the above observations are the first to investigate the influence of biochar additions on heavy metal accumulations in rice plant grown in a historically multi-metal contaminated soil under flooded conditions, and the first to account for the potential role of iron plaque. As presented in Fig. 3, the biochar additions resulted in a decrease of Cd, Pb and Zn concentrations in rice shoots, especially the straw-derived biochar. However, biochar also increased the rice shoot concentration of As. Further, the type of biochar source material and grain size made widely varying effects on rice shoot concentrations.

The various biochar additions decreased the C_{pw} of Cd and Zn, generally decreased the C_{pw} of Pb (except straw-char), and increased that of As (Fig. 1). This alteration of Cd, Zn and As in pore water is in agreement with the biochar amendment study by Beesley et al. (2010). The greatest decrease of Cd and Zn C_{pw} was from straw-char and for Pb it was bran-char. These may be attributed to the increased soil pH for straw-char amendment (Table 1) and enhanced available phosphorus in soil due to bran-char addition (Fig. S1 and Table S6). Soil pH is an important parameter influencing soil-surface chemistry. The number of negatively charged surface sites in soil increases with increasing pH, and the sorption capacity of soil to cationic metals correspondingly increases (Bradl, 2004). Increased pH values could also result an increase in the C_{pw} of Si (Korndörfer et al., 2005; Oliveira et al., 2007), which is supported by a significant correlation ($p < 0.001$) existed here between pH and the C_{pw} of Si (Fig. S2). Thus, the decrease of the C_{pw} of Cd, Zn and Pb could be accountable via the formation of silicate precipitates (Neumann and Nieden, 2001; Gu et al., 2011). In addition, phosphorus in biochars made from rice straw, husk and especially bran (which had the most phosphorous, see Table S2) can also play a contributing role in immobilizing Cd and Zn (McGowen et al., 2001; Cao et al., 2003).

The enhanced C_{pw} of Pb after straw-char amendment is consistent with the results by Beesley and Dickinson (2011), and may be attributable to high C_{pw} of DOC from the straw-char addition (Table S6). Lead can be activated when binding with dissolved organic matter, although it can also be immobilized via specific adsorption reactions by organic matter (Adriano, 2001).

Thus, altogether, biochar could have decreased C_{pw} of Cd, Zn and Pb by a combined effect of increasing the pH, and the C_{pw} of silica, phosphate (Table S6), complexation to biochar derived-DOC, as well as other effects to be discussed in further detail below.

Regarding As, an increase in pH and the C_{pw} of phosphate ions increases As mobilization (Sadiq, 1997). Phosphorus and silicon compete with both arsenate and arsenite for binding sites at Fe-oxide surfaces in soils to influence As solubility in pore water (Jain and Loeppert, 2000; Waltham and Eick, 2002). Further, as the pH increases, the number of positively charged sites on

minerals decreases, which lowers the sorption capacity of negatively charged oxy-anions of As (Wilson et al., 2010). Thus, it is reasonable that the arsenic C_{pw} increased (Fig. 1) after additions of straw-char and bran-char which greatly enhanced soil pH or soil-P level (Table 1 and Fig. S1).

More iron plaque was formed on root surface after additions of biochar ($p < 0.01$) than the control (Table 1). However, this extra iron plaque sequestered more Pb but not Cd, Zn and As (Fig. 2), despite the general consensus that iron plaque has a high adsorption capacity for all metals (Liu et al., 2008; Lei et al., 2011). For Cd and Zn, this is likely attributable to their C_{pw} decreasing after biochar additions (Fig. 1), thus undergoing less transport to the roots. Though there is a lower DCB-Cd in biochar amended soils, sorption affinity to root plaque is still higher, as indicated by the increased proportion in root plaque compared to shoots (Table S5) and decreased transfer coefficients (Table S4), as was also observed for Pb. For Zn, however, the root plaque seemed to have no influence on proportions or plant transfer coefficients (Tables S4 and S5).

The transfer coefficients of Cd and Pb decreased most by straw-char amendments, and not by husk- and bran-char amendments. One possible explanation is the increased silicon content by straw-char could block the translocation of Cd and Pb through depositing metals-bound with silica in the endodermis (Shi et al., 2005), the root pericycle (Cunha and Nascimento, 2009) or by increasing Si-induced apoplastic binding in roots (Wang et al., 2004). Similar results were reported by Gu et al. (2011), which showed that application of steel slag enriched in silicon decreased the uptake of Cd and Pb by rice and their translocations in plant. Unaffected Zn transfer coefficients by biochar additions may be ascribable to the Zn metabolism of rice, as rice contains an abundance of Zn transporters dedicated to Zn uptake and translocation in rice plants depending on external Zn levels (Ishimaru et al., 2011).

Regarding As, the elevated Si and P in pore water (Fig. S1) may have out-competed As for adsorption onto the iron plaque after additions of straw- or bran-char, which would account for why DCB-As did not increase despite higher As C_{pw} values (Fig. 2). It was reported that ratios of shoot to root As concentrations in rice plants in hydroponic culture were 0.01–0.97 (Marin et al., 1992; Raab et al., 2007). The unusually higher translocation of As from roots to shoots in the biochar amended soils 1.6–7.3 (Table S4) compared to other studies is hard to explain. The pathway of As translocation in rice plant is poorly understood but likely involves the mechanisms of As sequestration in the vacuoles, As loading and unloading in xylem and phloem, and enzymes responsible for arsenate reduction and methylation (Zhao et al., 2010). Competition of sorption sites in root tissues between Si and As may explain the increase of the As transfer coefficient due to biochar additions. Lending some support to this hypothesis, there were significant negative linear correlations ($p < 0.001$) between transfer coefficients of Cd, Pb and Si concentrations in pore water, and positive correlation between As transfer coefficient and Si C_{pw} (Fig. S3).

One unusual observation is that the husk-char significantly decreased the DOC concentration ($p < 0.05$) in pore water, contrary to straw-char and bran-char (Table 1) and contrary to biochar containing DOC. This is difficult to account for because many factors such as soil pH, ionic strength, cation exchange capacity (CEC) and microbial activity can influence DOC content in soil. Biochar amendment can also influence the cation exchange capacity and microbial activity (Liang et al., 2006; Steinbeiss et al., 2009). Though this influence was not quantified in the present study, the observed changes in metal mobility could also have been correlated to such changes.

Root biomass and root length decreased significantly ($p < 0.05$) after fine bran char application moreso than any other biochar application (Table 1). The highest water extractable-P levels (Table

S2) and highest P C_{pw} levels (Fig. S1) occurring for the fine bran char may be the primary reason. Increased bioavailable P can lead to shorter root lengths (Zheng et al., 2011). Root length was also one of considerable factors to influence metal concentrations in rice shoot (Table 2).

Generally there was more decrease of Cd and Zn concentrations in pore water and rice shoot in fine biochar treatment than the coarse one (Figs. 1 and 3), though this was generally slight. This could be attributable to a larger specific surface area of fine biochar. In addition to the influences of pH, chemical precipitation in soil and blocking of iron plaque, direct sorption of biochar also could be one of mechanisms by which metals are retained (Beesley and Marmiroli, 2011). However, in this study, straw-char that made the greatest effect on stabilization of Cd and Zn in soils had the lowest surface area (Table S1). Further study is need to understand the mechanisms that biochar immobilize heavy metals in soils.

5. Conclusions

Using a historically multi-metal polluted soil, additions of biochar produced from different tissues of rice plant reduced accumulations of Cd, Zn and Pb in rice shoot to various extents, while they greatly increased shoot As concentration. Straw-char had the biggest influence on the immobilization of Cd and Zn and the reduction of Cd, Zn and Pb concentrations in rice shoots. Straw-char amendments also caused a substantial decrease in the transfer coefficient of Cd and Pb as well as an increased distribution of these metals in iron plaque. The increased capacity of iron plaque for retaining Pb is considered the main mechanism for reducing Pb accumulation in rice shoots. Increased pH and phosphorus levels in soil pore water owing to biochar additions were also correlated with changes in the mobilization of Cd, Zn, Pb and As. A decrease in biochar particle size slightly increased the reductions in Cd, Zn and Pb accumulation in rice shoot, most noticeably in the case of bran-char. In summary, the main cause of reduction in the Cd, Zn and Pb accumulations in rice shoot was a decreasing the C_{pw} in the case of Cd and Zn due to increased pH, surface sorption and precipitation of phosphates, whereas the blocking capacity of root iron plaque is important for Cd and Pb. Nevertheless, the mechanisms by which biochar amendment reduces mobility of Cd, Zn and Pb and enhances the mobility of As may vary from soil to soil, where changes in the surface functional groups, surface area, porous structures of biochars, CEC, pH, C_{pw} content of P, Si or DOC of soils, changes in microbial activity, or growth of iron plaque, could all vary. This study indicates a favorable potential using biochar to remediate soils contaminated with Cd, Zn and Pb, especially in areas where rice grown on such soils is being consumed. However, in areas where there is As contamination and rice is not being consumed, biochar and rice may have some application as a phytoremediative strategy, where the As rich rice plants are disposed of after harvesting.

Acknowledgments

This work was financially supported by the National Water Pollution Control and Management Technology Major Projects (2009ZX07212-001-05), and the Natural Science Foundation of China (41090284).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2012.05.008>.

References

- Adriano, D.C., 2001. Trace Elements in Terrestrial Environments, second ed. Springer-Verlag, New York.
- Batty, L.C., Baker, A.J.M., Wheeler, B.D., Curtis, C.D., 2000. The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex. Steudel. Ann. Bot. 86, 647–653.
- Beesley, L., Dickinson, N., 2011. Carbon and trace element fluxes in the pore water of an urban soil following greenwaste compost, woody and biochar amendments, inoculated with the earthworm *Lumbricus terrestris*. Soil Biol. Biochem. 43, 188–196.
- Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480.
- Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287.
- Bradl, H.B., 2004. Adsorption of heavy metal ions on soils and soils constituents. J. Colloid Interface Sci. 277, 1–18.
- Cao, R.X., Ma, L.Q., Chen, M., Singh, S.P., Harris, W.G., 2003. Phosphate-induced metal immobilization in a contaminated site. Environ. Pollut. 122, 19–28.
- Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45, 4884–4889.
- Chen, C.C., Dixon, J.B., Turner, F.T., 1980. Iron coatings on rice roots: morphology and models of development. Soil Sci. Soc. Am. J. 44, 1113–1119.
- Cunha, K.P.V., Nascimento, C.W.A., 2009. Silicon effects on metal tolerance and structural changes in maize (*Zea mays* L.) grown on a cadmium and zinc enriched soil. Water Air Soil Pollut. 197, 323–330.
- Greipsson, S., Crowder, A.A., 1992. Amelioration of copper and nickel toxicity by iron plaque on roots of rice (*Oryza sativa*). Can. J. Bot. 70, 824–830.
- Gu, H.H., Qiu, H., Tian, T., Zhan, S.S., Deng, T.H.B., Chaney, R.L., Wang, S.Z., Tang, Y.T., Morel, J.L., Qiu, R.L., 2011. Mitigation effects of silicon rich amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on multi-metal contaminated acidic soil. Chemosphere 83, 1234–1240.
- Ishimaru, Y., Bashir, K., Nishizawa, N.K., 2011. Zn uptake and translocation in rice plants. Rice 4, 21–27.
- Jain, A., Loeppert, R.H., 2000. Effect of competing anions on the adsorption of arsenate and arsenite by ferrihydrite. J. Environ. Qual. 29, 1422–1430.
- Korndörfer, G.H., Nolla, A., Ramos, L.A., 2005. Available silicon in tropical soils and crop yield. in: Korndörfer, G., Coelho, L. (Eds.), Silicon in Agriculture. Elsevier, Brazil, pp. 22–26.
- Lehmann, J., 2007. A handful of carbon. Nature 447, 143–144.
- Lei, M., Tie, B., Williams, P.N., Zheng, Y., Huang, Y., 2011. Arsenic, cadmium, and lead pollution and uptake by rice (*Oryza sativa* L.) grown in greenhouse. J. Soils Sediment 11, 115–123.
- Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70, 1719–1730.
- Liu, H.J., Zhang, J.L., Christie, P., Zhang, F.S., 2008. Influence of iron plaque on uptake and accumulation of Cd by rice (*Oryza sativa* L.) seedlings grown in soil. Sci. Total Environ. 394, 361–368.
- Liu, J.G., Leng, X.M., Wang, M.X., Zhu, Z.Q., Dai, Q.H., 2011. Iron plaque formation on roots of different rice cultivars and the relation with lead uptake. Ecotoxicol. Environ. Saf. 74, 1304–1309.
- Liu, W.J., Zhu, Y.G., Smith, F.A., Smith, S.E., 2004. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? J. Exp. Bot. 55, 1707–1713.
- Marin, A.R., Masscheleyn, P.H., Patrick, W.H., 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil 139, 175–183.
- McGowen, S.L., Basta, N.T., Brown, G.O., 2001. Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. J. Environ. Qual. 30, 493–500.
- Neumann, D., Niesen, U.Z., 2001. Silicon and heavy metal tolerance of higher plants. Phytochemistry 56, 685–692.
- Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., Niandou, M.A.S., 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Sci. 174, 105–112.
- Oliveira, L.A., Korndörfer, G.H., Pereira, A.C., 2007. Silicon accumulation in rice in different rhizosphere pH conditions. Rev. Bras. Cienc. Solo 31, 685–690.
- Raab, A., Williams, P.N., Meharg, A., Feldmann, J., 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. Environ. Chem. 4, 197–203.
- Sadiq, M., 1997. Arsenic chemistry in soils: an overview of thermodynamic predictions and field observations. Water Air Soil Pollut. 93, 117–136.
- Shi, X.H., Zhang, C.C., Wang, H., Zhang, F.S., 2005. Effect of Si on the distribution of Cd in rice seedlings. Plant Soil 272, 53–60.
- Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41, 1301–1310.
- Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macedo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291, 275–290.
- Sun, G.X., Williams, P.N., Carey, A.M., Zhu, Y.G., Deacon, C., Raab, A., Feldmann, J., Islam, R.M., Meharg, A.A., 2008. Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. Environ. Sci. Technol. 42, 7542–7546.
- Waltham, C.A., Eick, M.J., 2002. Kinetics of arsenic adsorption on goethite in the presence of sorbed silicic acid. Soil Sci. Soc. Am. J. 66, 818–825.
- Wang, Y.X., Stass, A., Horst, W.J., 2004. Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize. Plant Physiol. 136, 3762–3770.
- Wilson, S.C., Lockwood, P.V., Ashley, P.M., Tighe, M., 2010. The chemistry and behaviour of antimony in the soil environment with comparisons to arsenic: a critical review. Environ. Pollut. 158, 1169–1181.
- Yang, Y.N., Sheng, G.Y., Huang, M.S., 2006. Bioavailability of diuron in soil containing wheat–straw-derived char. Sci. Total Environ. 354, 170–178.
- Ye, Z.H., Baker, A.J.M., Wong, M.H., Willis, A.J., 1998. Zinc, lead and cadmium accumulation and tolerance in *Typha latifolia* as affected by iron plaque on the root surface. Aquat. Bot. 61, 55–67.
- Zhao, F.J., McGrath, S.P., Meharg, A.A., 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Ann. Rev. Plant Biol. 61, 535–559.
- Zheng, R.L., Li, H.F., Jiang, R.F., Römhild, V., Zhang, F.S., Zhao, F.J., 2011. The role of root hairs in cadmium acquisition by barley. Environ. Pollut. 159, 408–415.