Effect of Biochars from Rice Husk, Bran, and Straw on Heavy Metal Uptake by Pot-Grown Wheat Seedling in a Historically Contaminated Soil

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The effect of biochar amendment of a multi-element contaminated soil on the transfer and accumulation of Cd, Zn, Pb, and As in wheat was investigated in this study. Addition of biochars from rice residues (straw, husk, and bran) significantly decreased shoot Cd, Zn, and Pb concentrations by up to 71%, 37%, and 60%, respectively, but increased As by up to 199%. Biochar additions decreased the NH_4NO_3-extractable concentrations of Cd, Zn, and Pb in soil by 23 to 81%, 29 to 94%, and 31 to 92%, respectively, especially straw-char treatment, though biochar treatment increased the concentration of As by 64 to 2650%. A decrease in biochar particle size generally favored the immobilization of Cd, Zn, and Pb in soil and reductions in their accumulation in wheat shoot, but this was reversed for As. Increases of up to 21%, 70%, 59%, and 40% in shoot biomass, root length, and shoot P and K levels, respectively, of wheat seedlings were caused by biochar amendments. Biochar has the potential to reduce accumulations of Cd, Zn, and Pb in wheat shoot and improve its growth.

Keywords: Biochar; Heavy metal; Contaminated soil; Soil amendment; Wheat

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INTRODUCTION

The potential benefits of using biochar for agriculture and the environment have received significant attention from researchers in recent years. In addition to mitigation of global warming (Molina *et al.* 2009), improvement of soil fertilities (Fellet *et al.* 2011), enhancement of plant growth (Zhang *et al.* 2012), and the increase of the nutrient retention capacity of soil (Laird *et al.* 2010a), biochar is also drawing increasing interest for its ability to remediate organic and inorganic contaminants (Chai *et al.* 2012; Uchimiya *et al.* 2012).

The impacts of biochar addition on the mobility and bioavailability of metal(loid)s in soils have been reported in recent years. Incorporation of biochars made from wheat straw was found to greatly reduce Cd concentrations in rice grain and wheat grain in field experiments (Cui *et al.* 2011, 2012). Decreases in Cd, Zn, and Pb concentrations and an increase in As concentration in rice plants grown in a multi-element contaminated soil under flooded conditions after biochar addition were also observed in previous studies by the authors (Zheng *et al.* 2012). Studies showed that heavy metals were immobilized and As was mobilized in soils after biochar addition (Beesley *et al.*

2010; Zheng *et al.* 2012). Concentrations of Cd and Zn in soil pore water decreased significantly, whereas that of As increased markedly in a multi-element polluted soil amended with biochar under various soil moisture conditions (Beesley *et al.* 2010). Biochar-induced increases in soil pH, the number of oxygen functional groups, and/or phosphorous levels caused the formation of ligands, which contribute greatly to the immobilization of toxic metals in soils and may result in As activation (Cao *et al.* 2011; Uchimiya *et al.* 2010; Uchimiya *et al.* 2012; Sadiq 1997). In addition to changing the mobility of metal(loid)s in soils, the blocking capacity of iron plaque, root-to-shoot translocation in the plant, and the accumulation of metal(loid)s in rice shoot were influenced by biochar amendments (Zheng *et al.* 2012).

Different types of biochars from various parent materials exhibit quite different properties (Kasozi *et al.* 2010). However, it remains unknown to what extent different biochars can influence translocation and accumulation of metal(loid)s in wheat (an upland crop) grown in a historically contaminated soil containing multiple metals and As. Particle size of utilized biochar is a key factor that affects its specific surface area and the further immobilization of heavy metals. The mechanism by which different biochar particle sizes affect the mobility of heavy metals in soil and their accumulation in plants is still unknown.

The present study was carried out using a historically multi-metal and As contaminated soil to investigate the effects of biochar additions on the following: i) the mobility of metals and As in aerobic soils, ii) the translocation and accumulation of Cd, Zn, Pb, and As in wheat seedlings, iii) and the wheat growth and root elongation. In addition, the potential mechanisms were analyzed.

EXPERIMENTAL

Materials

Biochars used in this study were made from three parts (straw, husk, and bran) of rice plant grown in Xiamen city, China and are referred to as straw-char, husk-char, and bran-char, respectively. Each type of dried biomass without crushing was heated up to 500 °C in a pyrolyzer under a stream of N_2. After pyrolysis for 4 h at 500 °C, the biochars were cooled under the N_2 gas stream to room temperature. Following charring, biochars were ground and sieved, yielding fine (< 0.18 mm) and coarse (0.5 to 2 mm) fractions. Detailed information about biochar preparation has been described in our previous report (Zheng *et al.* 2012).

The soil (clay loam) in the study location is a historically multi-element contaminated soil collected from the farmland near a mining area in Zhuzhou city, Hunan province, south China. The soil was air-dried and was crushed and sieved with 2-mm mesh. Some physicochemical properties of the soil and biochars were analyzed, and the results are presented in Table 1.

Wheat seeds disinfected in 30% H_2O_2 (w/w) solution for 15 min were thoroughly washed and submerged in deionized water and were kept in an incubator at 30°C. After germination, seeds with uniform appearance were chosen and planted in soils with or without biochar additions (5%). Eight germinated seeds were planted in each pot (a diameter of 7 cm and a height of 15 cm); after emergence, the pots were thinned to four seedlings. Deionized water was added as required to maintain soil moisture content at 20% by regular weighing. All pots were kept in a greenhouse set to 14 h daytime with a light intensity of 350 µmol m^{-2} s^{-1}, 28 and 20 °C day and night temperatures, respectively, and a relative humidity of 60% to 70%.

Table 1. Physicochemical Properties of Soil and Biochars Used

Properties	Soil	Biochar		
		Straw-char	Husk-char	Bran-char
pH	7.0	11.3	10.0	8.1
Total carbon (%)	2.4	47.8	59.3	63.4
Total nitrogen (%)	0.14	1.7	1.0	4.4
Carbonate (cmol kg^{-1})	—	113.4	20.8	9.7
CEC* (cmol kg^{-1})	11.0	38.2	3.6	4.4
Cd (µg g^{-1})	23.5	0.14	0.13	0.48
Zn (µg g^{-1})	2216	262.4	77.7	218.6
Pb (µg g^{-1})	717	14.4	9.1	5.3
As (µg g^{-1})	77	0.77	0.24	0.74
P (%)	—	0.49	0.2	4.7
K (%)	—	7.9	0.8	4.0
Ca (%)	—	0.74	0.30	0.21
Mg (%)	—	0.46	0.13	1.9
Si (%)	—	8.3	12.7	0.43
WOC* (mg g^{-1})	—	0.36	0.12	0.04
BET surface area (m^2 g^{-1})	—	12.7	71.3	18.2

* WOC, water soluble organic carbon; CEC, cation exchange capacity.

Treatments

Fine- and coarse-sized straw-, husk-, and bran-char were added to the contaminated soil at a 5% ratio based on dry weight, together with basal fertilizers. Soils unamended with biochar (0%) but with basal fertilizers were designated as the control. After fertilizers and biochars were amended, the mixture was thoroughly homogenized and left to equilibrate for two weeks, maintaining 20% moisture content. Basal fertilizers were 200 mg N (as $Ca(NO_3)_2·4H_2O$), 180 mg P (as KH_2PO_4), and 60 mg Mg (as $MgSO_4·7H_2O$) kg^{-1} based on air-dry weight. There were seven treatments, each of which was replicated 4 times using 400 g of soil and 4 seedlings per pot. Plants were harvested after 4 weeks of growth. Shoots were cut at the soil surface, washed with deionized water, dried in an oven at 70 °C for 48 h, and then weighed. Roots were taken from soils by gentle sieving and were cleaned with deionized water for further measurements. Soils from each pot were air dried and passed through a 2-mm sieve for the determination of NH_4NO_3-extractable elements and pH values.

Analytical Methods

Water extracts of fine-sized biochar were made using 1:10 suspensions (w/v), shaken for 3 h, and centrifuged for 10 min at 3000 rpm. The supernatant was then filtered through a 0.45-µm pore-size Millipore filter. The pH of the supernatant was measured using a glass electrode, and the values were recorded as the pH value of biochar. Water-extractable organic carbon (WOC) was determined using a Liqui TOC II analyzer. The BET surface area of biochars was determined using a BET meter (ASAP2000, Micromeritics, USA). Soil pH was measured in a suspension of soil and water (1:2.5 w/v). NH_4NO_3 extracts of soil were carried out using 1 M NH_4NO_3 (1:2.5 w/v) and shaking for 2 h. The cation exchange capacity (CEC) was determined referring to a modified barium chloride compulsive exchange method (Lee *et al.* 2010). Total C and N analyses of soil and biochar were conducted on a solid TC/TN analyzer (Vario EL III, Elementar Analysensysteme GmbH, Germany). Finely ground (< 0.149 mm) soil and biochar samples were digested with HNO_3-HCl-$HClO_4$ (3:1:1).

Fresh roots were scanned into WinRhizo software for the estimation of root length and were then oven dried at 70 °C for 48 h. Each dried shoot and root sample of the wheat plant was ground and weighed (0.2 g) and was then digested with concentrated HNO_3 in a microwave digester (Sun *et al.* 2008).

Elements in dissolved samples and extracts were determined using inductively coupled plasma mass spectroscopy (ICP-MS, 7500a, Agilent Technologies, USA) for Cd, Zn, Pb, and As with indium isotopes (In^{115}) as internal standards (10 µg L^{-1}), and ICP optical emission spectroscopy (ICP-OES, Optima 2000, PerkinElmer Co., USA) was used to determine P and K. Certified reference material (CRM) GBW07603 (Bush Twigs and Leaves), GBW07406 (GSS-6 Yellow Red Soil), spikes, and blanks were used for quality control. The recovery ratios of the elements determined were from 85% to 102% throughout the analytical procedures.

Data Analysis

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 the heavy metal concentrations in shoot and shoot biomass, NH_4NO_3-extractable concentrations in soil, and plant transfer coefficients of heavy metals. Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., USA).

RESULTS AND DISCUSSION

Characteristics of Biochars

Biochars derived from rice straw, husk, and bran contained mainly carbon, a smaller proportion of nitrogen, and a certain amount of trace elements and water-soluble organic carbon (WOC). All biochars showed alkaline pH, varying CEC, and considerable surface area. Properties of biochar were highly varied and depended on the parent material (Table 1). Straw-char had the highest pH (11.3), which was 3.2 and 1.3 units higher than that of bran- and husk-char, respectively. The total nitrogen percent of bran-char (4.4%) was 166% and 338% higher than that of straw- and husk-char, respectively. The total carbon percents were in the sequence of straw-char < husk-char < bran-char. However, the carbonate concentrations showed a contrary order: bran-char < husk-char < straw-char (Table 1). Particularly, the CEC (38.2 cmol kg^{-1}) and WOC (0.36 mg g^{-1}) of straw-char were much higher than that of other biochars, with CECs < 4.4 cmol kg^{-1} and WOCs < 0.12 mg g^{-1}. Husk-char had the largest surface area (71.3 m^2 g^{-1}) compared to that of other biochars (< 18.2 m^2 g^{-1}). Though bran-char had the highest level of total carbon, the contents of carbonate and WOC in it were lowest. For trace elements, straw-char had the highest concentrations of Zn, Pb, and As, and bran-char had the highest concentration of Cd. However, concentrations of the four heavy metals in biochars were all much lower than the Chinese regulation limit of pollutants for urban waste or sludge to be used for agriculture (GB8172-87, GB18918-2002). This was because the rice plant from Xiamen prepared for biochar production here contained low concentrations of heavy metal. Concentrations of Cd, Zn, Pb, and As in rice straw were lower than 0.05, 89, 5.0, and 0.3 µg g^{-1}, respectively. All biochars had higher pH values than the soil (pH 7.0). Total carbon (2.4%) and nitrogen concentrations (0.14%) in soil were 95 to 96% and 86 to 97% lower than that in biochars, respectively (Table 1).

Plant Growth and Heavy Metal Accumulations

Biochar additions significantly increased ($p < 0.01$) the shoot biomass of wheat seedlings by up to 21% on average, and there were no significant differences in the enhancing effects of biochars from different parent materials and with different particle sizes (Table 2). The effects of biochar addition on root biomass and the ratio of shoot to root biomass (S/R) were biochar type-dependent ($p < 0.01$). The root length of wheat seedlings with all biochar amendments increased by 40 to 70% from the initial 11.8 m pot^{-1}. There were no statistical differences in root length between biochar types or between particle sizes for any biochar treatment. Biochar additions increased shoot P and K concentrations by up to 59% and 40%, respectively, depending on the raw materials or particle sizes of biochar (Table 2). Bran-char had the highest P level, which caused the biggest increase in shoot P concentration, and straw-char likewise resulted in the highest shoot K concentration.

Table 2. Soil pH, Growth, and Nutrition Status of Wheat Seedlings with Different Biochar Additions

Biochar addition	Biochar type	Particle size	Soil pH	Shoot biomass (g DW pot^{-1})	Root biomass (g DW pot^{-1})	S/R	Root length (m pot^{-1})	[Shoot P] (mg g^{-1} DW)	[Shoot K] (mg g^{-1} DW)
0% [a]			7.0 ± 0.03	0.30 ± 0.02	0.11 ± 0.01	2.8±0.0	11.8 ± 0.5	7.0±0.1	58±2.2
5%	straw[b]	coarse	8.4 ± 0.06	0.36 ± 0.05	0.11 ± 0.02	3.5±0.5	16.6 ± 2.4	8.2±0.4	81±1.3
5%	husk	coarse	7.2 ± 0.02	0.34 ± 0.01	0.13 ± 0.01	2.7±0.3	17.1 ± 1.2	7.0±0.4	67±0.5
5%	bran	coarse	7.4 ± 0.03	0.36± 0.01	0.13 ± 0.01	2.7±0.2	18.5 ± 1.4	10.7±0.2	70±1.6
5%	straw	fine	8.5 ± 0.01	0.37 ± 0.01	0.10 ± 0.01	3.8±0.1	17.0 ± 0.6	8.4±0.5	81±2.2
5%	husk	fine	7.2 ± 0.02	0.36 ± 0.04	0.13 ± 0.01	2.7±0.2	20.1 ±1.4	7.7±0.3	69±3.1
5%	bran	fine	7.5 ± 0.02	0.35 ± 0.03	0.11 ± 0.01	3.0±0.1	19.3 ± 1.7	11.1±0.5	72±1.3
Significance of [c]									
Biochar addition			*	**	ns	ns	***	*	***
Biochar type (T)			***	ns	**	***	ns	***	***
Particle size (S)			ns	ns	ns	ns	ns	*	ns
T×S			ns	ns	ns	ns	ns	ns	ns

Values are given as mean ± standard deviation for four measurements.

[a] 0% and 5% represent soils without biochar addition and with additions of biochar at 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.

Biochar addition significantly ($p < 0.05$) decreased shoot Cd, Zn, and Pb concentrations (16.5, 550, and 0.5 µg g^{-1} DW, respectively) by up to 71%, 37%, and 60%, respectively, but increased shoot As concentration (1.1 µg g^{-1} DW) by up to 199% (Fig.

1). Straw-char addition caused the largest decrease in shoot Cd and Zn concentrations, but bran-char addition caused the largest decrease in shoot Pb concentration and the largest increase in shoot As concentration. Fine bran-char addition resulted in lower shoot Cd (5.6 *vs*. 6.8 µg g^{-1} DW) and lower shoot Pb (0.2 *vs*. 0.4 µg g^{-1} DW) concentrations than coarse bran-char treatment, but particle size played only a small role in the case of straw- and husk-char amendments. The shoot As concentration was slightly higher in the fine-fraction than coarse-fraction for straw-char treatment (1.4 *vs*. 1.2 µg g^{-1} DW) (Fig. 1).

Soil pH and NH_4NO_3-Extractable Heavy Metals

Biochar addition increased soil pH to different degrees depending on biochar feedstocks (Table 2). Soil pH increased from 7.0 to 8.5, straw-char playing the biggest role. The particle size of biochar had no significant effect on soil pH (Table 2).

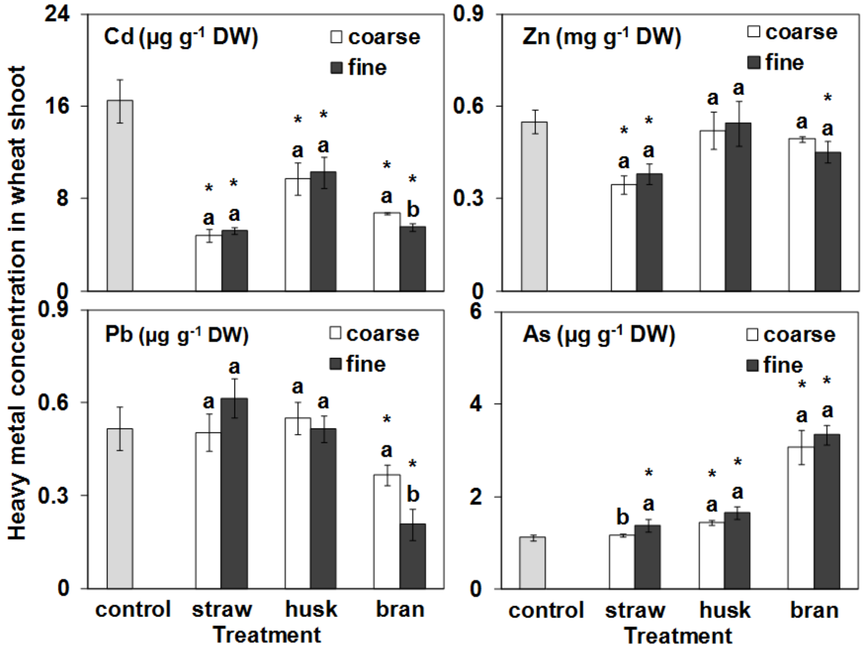

Fig. 1. The influence of biochar produced from different rice plant tissues on concentrations of Cd, Zn, Pb, and As in shoots of wheat (*Triticum aestivum*). Control, straw, husk, and bran represent soils with additions of no char and 5% straw-char, husk-char, and bran-char, respectively. Different letters above columns (a or b) indicate significant differences at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * indicates a significant difference at $p < 0.05$ compared to the control.

Initial NH_4NO_3-extractable concentrations of Cd, Zn, and Pb in soil decreased ($p < 0.05$) by 23 to 81%, 29 to 94%, and 31 to 92%, respectively, though that of As increased by 64 to 2650% due to biochar amendment (Fig. 2). Straw-char addition caused the greatest decreases in NH_4NO_3-extractable Cd, Zn, and Pb concentrations, though husk-char caused the minimum increase in that of As.

Regarding the particle-size fraction, fine husk- and bran-char resulted in 28% and 28% decreases in NH_4NO_3-extractable Cd concentration and 38% and 22% decreases in NH_4NO_3-extractable Zn concentration compared to the coarse ones, respectively. There were no significant differences in soil NH_4NO_3-extractable Cd and Zn concentrations between coarse- and fine-sized straw-char treatments.

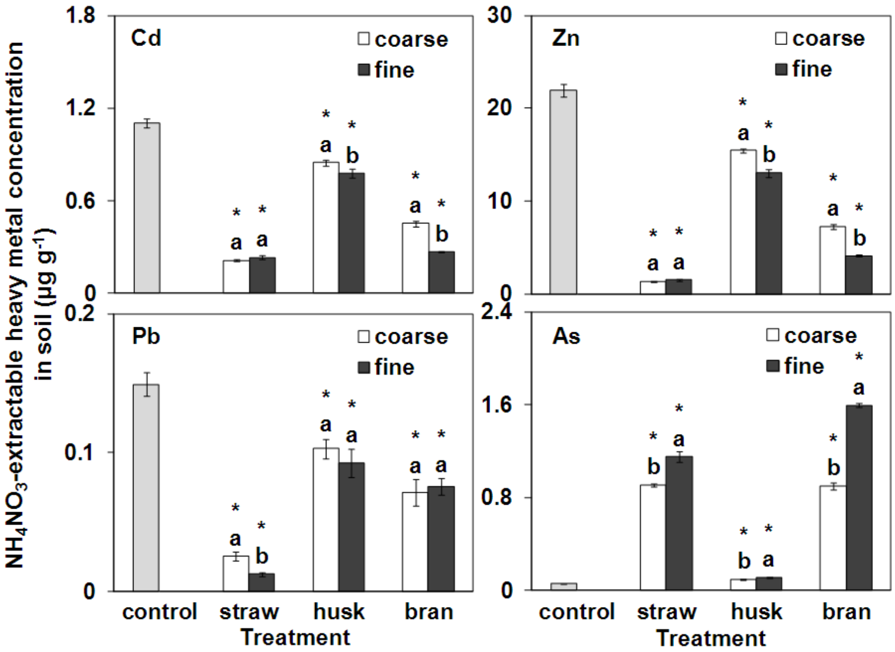

Fig. 2. The influence of biochar produced from different tissues of rice plant on the NH$_4$NO$_3$-extractable concentrations of Cd, Zn, Pb, and As in soil. Control, straw, husk, and bran represent soils with additions of no char and 5% straw-char, husk-char, and bran-char, respectively. Different letters above columns (a or b) indicate significant differences at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * indicates a significant difference at $p < 0.05$ compared to the control.

Table 3. Plant Transfer Coefficients of Cd, Zn, Pb, and As of Wheat Seedlings Grown with Different Biochar Additions

Biochar addition	Biochar type	Particle size	Transfer coefficient			
			Cd	Zn	Pb ($\times 10^{-3}$)	As
0% [a]			0.11 ± 0.01	0.26± 0.03	4.5 ± 0.2	0.14 ± 0.03
5%	straw [b]	coarse	0.10 ± 0.01	0.23 ± 0.01	4.5 ± 1.0	0.07 ± 0.01
5%	husk	coarse	0.12 ± 0.02	0.28 ± 0.02	4.6 ± 0.4	0.13 ± 0.01
5%	bran	coarse	0.10 ± 0.01	0.26 ± 0.02	3.6 ± 0.3	0.23 ± 0.03
5%	straw	fine	0.10 ± 0.01	0.24 ± 0.03	7.0 ± 1.2	0.10 ± 0.01
5%	husk	fine	0.13 ± 0.01	0.32 ± 0.05	6.2 ± 0.8	0.21 ± 0.03
5%	bran	fine	0.08 ± 0.01	0.19 ± 0.02	2.0 ± 0.7	0.20 ± 0.04
Significance of [c]						
Biochar addition			ns	ns	ns	ns
Biochar type (T)			***	***	***	***
Particle size (S)			ns	ns	*	*
T×S			*	**	***	**

Values are given as mean ± standard deviation for four measurements.

[a] 0% and 5% represent soils without biochar addition and with additions of biochar at 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.

The NH_4NO_3-extractable Pb concentration in the soil of the fine straw-char amendment was 51% lower than in the coarse one, though the particle size of husk- and bran-char had no significant effects on the soil NH_4NO_3-extractable Pb concentration. Additions of fine-sized biochar made from any material caused a greater increase in soil NH_4NO_3-extractable As concentration than the coarse fraction, especially for bran-char, which had an 83% higher increase with fine particle size treatment than the coarse one.

Translocation of Heavy Metals in Plant

The plant transfer coefficients of Cd, Zn, Pb, and As from roots to shoots varied significantly depending on biochar type ($p < 0.001$), and that of Pb and As were influenced by the particle size of biochar as well ($p < 0.05$) (Table 3). The largest decrease in transfer coefficients for Cd, Zn, and Pb occurred in the case of fine bran-char amendment and were up to 34%, 28%, and 55%, respectively; yet there was no significant impact on the transfer coefficients of Cd and Zn in the case of straw- and husk-char amendments or coarse bran-char treatment. Addition of fine straw- and husk-char significantly increased the plant transfer coefficient of Pb by 55% and 39%, respectively, but coarse char treatments had no effect. For As, the coarse fraction of straw- and bran-char caused the largest decrease (by 49%) and increase (by 68%) in the transfer coefficients, respectively.

Biochar amendments could have affected the concentrations of Cd, Zn, Pb, and As in wheat shoots by altering their NH_4NO_3-extractable concentrations in soil and their root-to-shoot transfer coefficients in plants. Dilution from increased shoot biomass was not a significant influencing factor. Table 4 shows multiple linear regressions that correlate the shoot concentrations of heavy metals with the most significant of the variables (with $p < 0.05$).

Table 4. Multiple Linear Regressions between Heavy Metal Concentrations in Wheat Shoot ($_s$) *vs*. Shoot Biomass, NH_4NO_3-extractable Concentrations in Soil ($_{NN}$), and Plant Transfer Coefficients (TCs) of Heavy Metals (n = 28) using a Stepwise Method

Model	r	sig
$[Cd]_s = 2.13 + 11.3 \cdot [Cd]_{NN}$	0.929	$p < 0.001$
$[Zn]_s = 204.5 + 6.54 \cdot [Zn]_{NN} + 803.6 \cdot TC_{Zn}$	0.837	$p < 0.001$
$[Pb]_s = 0.13 + 73.0 \cdot TC_{Pb}$	0.868	$p < 0.001$
$[As]_s = -0.39 + 0.91 \cdot [As]_{NN} + 10.8 \cdot TC_{As}$	0.941	$p < 0.001$

Variables in regression equations were all significant ($p < 0.05$).

DISCUSSION

Cd and Zn concentrations in wheat shoot decreased to different extents, depending mostly on biochar type, whereas shoot As concentrations increased after biochar addition to the soil. Only bran-char addition reduced the shoot Pb concentration significantly (Fig. 1). Shoot concentrations of Cd, Zn, Pb, and As were influenced by their mobility in the soil and root-to-shoot translocation in plants (Table 4). Unlike that in bran-char addition, shoot Pb concentrations in straw- and husk-char treatments did not decrease, although NH_4NO_3-extractable Pb concentration in soil decreased significantly

(Fig. 2). The most probable reason for this is that bran-char contains a much higher P concentration than in the others (straw- and husk-char), causing more $Pb_5(PO_4)_3OH$ and $Pb_5(PO_4)_3Cl$ precipitates. This was thought to be the main pathway by which bran-char immobilized Pb in soil. Chemical precipitates such as $Pb_5(PO_4)_3OH$ and $Pb_5(PO_4)_3Cl$ with much larger pK_{sp} (> 82) values than $PbSO_4$, $Pb(OH)_2$, and $PbCO_3$ (pK_{sp} < 15) are stable enough that the plant root cannot readily move and absorb them via proton and organic acid root exudates (Cao *et al.* 2011; Ma *et al.* 1995). However, little $Pb_5(PO_4)_3OH$ and $Pb_5(PO_4)_3Cl$ precipitates can be formed in straw- and husk-char treatment due to much lower P levels. Lead immobilized in soils through an increase in soil pH, precipitation of $Pb(OH)_2$ and $PbCO_3$, or surface adsorption can still be activated easily in the rhizosphere. Development of lead phosphate precipitates with higher stability may be more conducive to the decrease of plant uptake than other precipitates and surface adsorption by functional groups.

All biochar amendments significantly ($p < 0.05$) decreased the NH_4NO_3-extractable concentrations of Cd, Zn, and Pb in soil. Straw- and bran-char additions caused larger decreases than husk-char (Fig. 2), most likely due to the higher pH of straw-char and the higher P concentration (47.2 mg g^{-1}) of bran-char (Table 1). Increased soil pH due to biochar addition, especially in the case of straw-char, can regularly increase the number of negatively charged surface sites in soil and correspondingly increase the sorption capacity of soil for cationic metals such as Cd, Zn, and Pb (Bradl 2004). Phosphorus contained in the biochar, especially in the case of bran-char, can induce the formation of metal-phosphate precipitates with low solubility products, and these are responsible for soil Cd, Zn, and Pb immobilization (McGowen *et al.* 2001; Cao *et al.* 2003a). These results have also been supported by other studies, although different biochars were used (Uchimiya *et al.* 2010; Park *et al.* 2011; Cao *et al.* 2011). In addition to increasing soil pH and developing phosphate precipitates, surface adsorption by biochar was also thought to be one of the mechanisms for stabilizing Cd, Zn, and Pb in soil. Biochar normally has a large specific surface area due to its porosity and abundant functional groups, which are helpful in the adsorption of metallic elements (Uchimiya *et al.* 2011; Uchimiya *et al.* 2012). A general decrease in NH_4NO_3-extractable Cd, Zn, and Pb concentrations in soil occurred in fine biochar treatments, rather than in coarse treatments, due to higher specific surface area in fine biochar compared with the corresponding coarse biochar (Fig. 2). However, direct surface adsorption of biochar made minor contributions to metal stabilization in this study, with straw-char having the largest effect on stabilization of Cd, Zn, and Pb in soils yet having the lowest surface area (Table 1). These results implied that the mechanism of metal stabilization may vary with biochar made from different parent materials.

The NH_4NO_3-extractable concentration of As in soils increased after biochar addition, especially with straw- and bran-char treatments (Fig. 1). This increase can be attributed to the increase in soil pH and/or soil P level due to biochar amendments (Table 1, 2). The sorption capacity of soils to negatively charged oxy-anions of As decreases with increasing soil pH, which causes the number of positively charged sites on minerals to decrease (Klitzke and Lang 2009; Wilson *et al.* 2010). The increased phosphate anion as an analog to arsenate competes for binding sites at iron oxide surfaces in soils with arsenate and accordingly results in a desorption of As retained in soils (Cao *et al.* 2003b; Jain and Loeppert 2000). Thus, it is reasonable that As was mobilized after biochar addition. Accordingly, a reduction in the particle size of biochar favoring the increase of soil pH and oxygen-containing functional groups further aided As mobilization. Because all cations contained are involved in the competitive interactions with anions and adsorption sites, the content of common exchangeable cations (K^+, Ca^{2+}, Mg^{2+}) in soil and biochars was added as supplementary data (Table S1 in the Appendix). Among the three types of biochar, straw-, husk-, and bran-char contained the highest level of exchangeable K^+, Ca^{2+}, and Mg^{2+}, respectively.

The transfer coefficients of Cd, Zn, and Pb decreased with fine bran-char treatment, but not in others. This may be attributable to higher phosphorus levels of bran-char and higher concentrations of water-extractable phosphorus for fine fractions (3.6 mg/g) of bran-char than for coarse ones (2.7 mg/g) (Zheng *et al.* 2012). Fine bran-char supplied the most available phosphorus for the plant. Phosphorus and heavy metals may form a deposit in plant roots that is able to tolerate the toxicity and restrict the translocation of heavy metals (Brennan and Shelley 1999; Yu and Zhou 2009). This finding was supported to some extent by the increase in root P concentration and the decrease in root-to-shoot translocation of P in the fine bran-char treatment (Fig. S2). Reductions in the root-to-shoot translocation of heavy metals in the plant after phosphorus-containing amendment additions were also observed in other studies (Zhu *et al.* 2001; Chen *et al.* 2009). It is hard to explain the increase of Pb transfer coefficients in fine straw- and husk-char treatments. Once it has penetrated into the plant root, Pb may undergo stabilization by negatively charged pectins within the cell wall, accumulation in plasma membranes, complexation by phytochelatin, sequestration in the vacuoles of cells, and precipitation of insoluble salts in intercellular spaces (Pourrut *et al.* 2011). Biochar addition, which influenced the soil pH, ionic strength, cation exchange capacity (CEC), nutrient level, and microbial activity, can play a key role in the plant's physiological metabolism indirectly and can subsequently alter Pb translocation.

For As, arsenate entering into the root is reduced to arsenite, which is ready for complexation by phytochelatins (PCs); then the PCs-As complexes are sequestered in vacuoles of roots, and only a small part of As absorbed is transported to the shoots (Zhao *et al.* 2009; Duan *et al.* 2011). The formation of As-PCs complexes is As concentration-dependent (Raab *et al.* 2007; Mishra *et al.* 2011). The decrease in the transfer coefficient of As can likely be attributed to the increase in available As in the soil around the roots after straw-char addition. These conditions may upregulate the detoxification system, which increases phytochelatin synthesis and limits the root-to-shoot translocation of As. The higher P levels in soil that resulted from bran-char addition may partially account for the increase in the transfer coefficient of As with bran-char treatment. It has been reported that increasing the P supply increased the root-to-shoot translocation of As in two cultivars of winter wheat (Geng *et al.* 2006). However, this explanation is not sufficient because the pathway of As translocation in higher plant functions involves As sequestration in the vacuoles and As loading and unloading in the xylem and phloem; enzymes responsible for arsenate reduction and methylation are as yet poorly understood (Zhao *et al.* 2010).

All biochar additions increased the shoot biomass and root length of wheat seedlings (Table 2). This may be attributed to reduced metal toxicity through immobilization, supply of nutrients, and improvement of the soil physical properties (Ahmad *et al.* 2012; Park *et al.* 2011; Rajkovich *et al.* 2012; Laird *et al.* 2010b). Although there were no significant differences of increases in shoot biomass among biochar types (Table 2), feedstock type of biochar caused an eight times greater effect on corn biomass production than pyrolysis temperature (Rajkovich *et al.* 2012). Nutrient-rich biochars made from switchgrass, corn, and rice husk increased corn growth, but nutrient-low biochars from materials like hardwood did not have the same effect (Rajkovich *et al.* 2012). The increase in maize yield in a field experiment was also attributed to the increased nutrient availability due to biochar addition (Zhang *et al.* 2012). However, it is considered to be very good news that biochar from rice husk had lower concentrations of nutrients (N, P, K, and Mg) than others, though wheat biomass in husk-char treatment was not lower than that in other char treatments. Biochars utilized in the present study had high concentrations of N, P, and K up to 4.4%, 4.7%, and 7.9%, respectively, depending on the feedstock. These values were equal to those with soils that had additional fertilizers of N, P, and K (as 2.1, 2.2, and 3.8 mg g^{-1} dry weight soil, respectively) along with biochar incorporation. On the other hand, considering that plant available nutrients in biochars and soil could give positive contributions to the plant growth within a short period, plant available nutrients concentrations in soil and biochars represented by exchangeable and water soluble nutrients, and Olsen-P were supplied as supplementary data (Table S1, S2). All biochars had higher exchangeable K and Mg levels and Olsen-P than the soil. Especially, Olsen-P in bran-char was up to 9 mg g^{-1} versus 0.03 mg g^{-1} in soil. Much higher water soluble P and Mg concentrations were contained in bran-char than in other biochars. Straw-char had the highest concentrations of K and Ca extracted by water. Bulk density, which is one of the important indicators of soil's physical properties, can be reduced significantly after biochar addition (Laird *et al.* 2010b; Zhang *et al.* 2012). Although not tested here, biochar incorporation into soil is thought to decrease bulk density and increase soil porosity (Jones *et al.* 2011; Atkinson *et al.* 2010), thereby potentially promoting root elongation and providing more essential nutrients for mass growth.

Regarding the property of biochar, the difference of pH between various types of biochar was attributable to the organic anions, carbonates, and other alkaline substances contained in the biochar. And, carbonates were likely to be the main alkaline components of the biochar produced at the relatively high temperature of 500 ºC in the present study (Yuan *et al.* 2011). Higher pH of biochar corresponding to higher level of carbonate (Table 1) partly supported this interpretation. The ample amounts of oxygen-containing functional groups on the biochars indicated the abundant organic anions on biochar surface (Fig. S3, supplementary data). In addition, pyrogenic amorphous carbon (lignin residues, aliphatics, and small (poly)aromatic units, *etc.*) was supposed to be the main carbon form for biochars utilized here according to Keiluweit *et al.* (2010). The components of amorphous carbon of biochar controlled by charring conditions and parent biomass components (hemicelluloses, cellulose, and lignin, *etc.*) may also influence sorption behavior of biochar (Keiluweit *et al.* 2010; Melo *et al.* 2013).

Increasing the yield of crops and also reducing the accumulations of Cd, Pb, and excessive Zn in crops such as wheat grain (Cui *et al.* 2012) through soil amendment with biochars is a win-win situation. However, the increase in As accumulation after biochar addition should be monitored. A combination of plant and biochar may be developed as a phytoremediative strategy for As.

CONCLUSIONS

1. Incorporation of biochars significantly reduced the concentrations of Cd, Pb, and Zn extracted by a NH_4NO_3 solution to different extents in a historically multi-element polluted soil, although the As concentration increased.
2. Biochar additions decreased the shoot concentrations of Cd, Zn, and Pb, but the results mainly depended on biochar feedstocks. Straw-char caused the largest decrease in shoot Cd and Zn concentrations, and bran-char brought the lowest shoot Pb concentration and highest shoot As concentration. There is a potential risk of the increase in As uptake by crops when using biochar with high P levels as amendment.
3. A decrease in biochar particle size generally favored the immobilization of Cd, Zn, and Pb in soil and reduced their accumulation in wheat shoot, but these results were reversed for As.
4. Biochar addition promoted the shoot growth and root elongation of wheat seedlings and increased the concentrations of shoot P and K.

ACKNOWLEDGMENTS

This work was financially supported by the Foundation for Young Scientists of the Beijing Academy of Agriculture and Forestry Sciences (QNJJ201314) and the National High Technology Research and Development Program of China (863 Program, 2013AA06A209, 2012AA06A204).

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Article submitted: June 10, 2013; Peer review completed: August 8, 2013; Revised version received: September 23, 2013; Accepted: September 26, 2013; Published: October 2, 2013.

APPENDIX

Supplementary Material

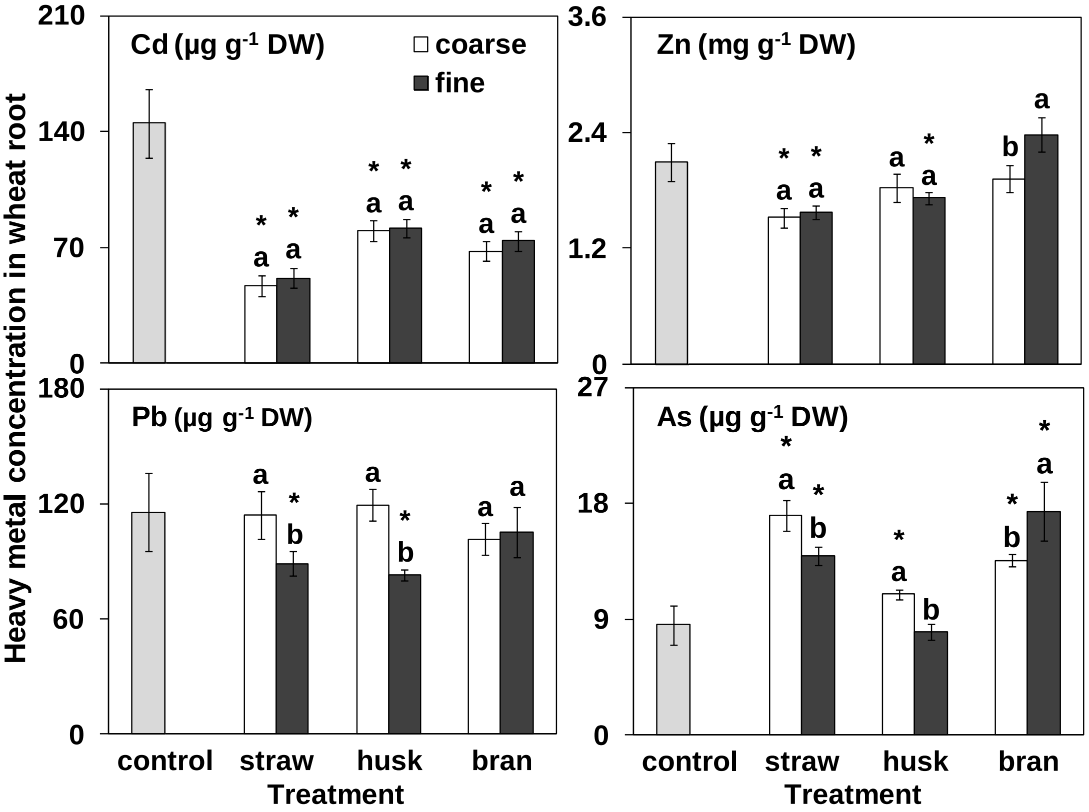

Fig. S1. The influence of biochar produced from different rice plant tissues on concentrations of Cd, Zn, Pb, and As in roots of wheat (*Triticum aestivum*). Control, straw, husk, and bran represent soils with additions of no char and 5% straw-char, husk-char, and bran-char, respectively. Different letters above columns (a or b) indicate significant differences at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * indicates a significant difference at $p < 0.05$ compared to the control.

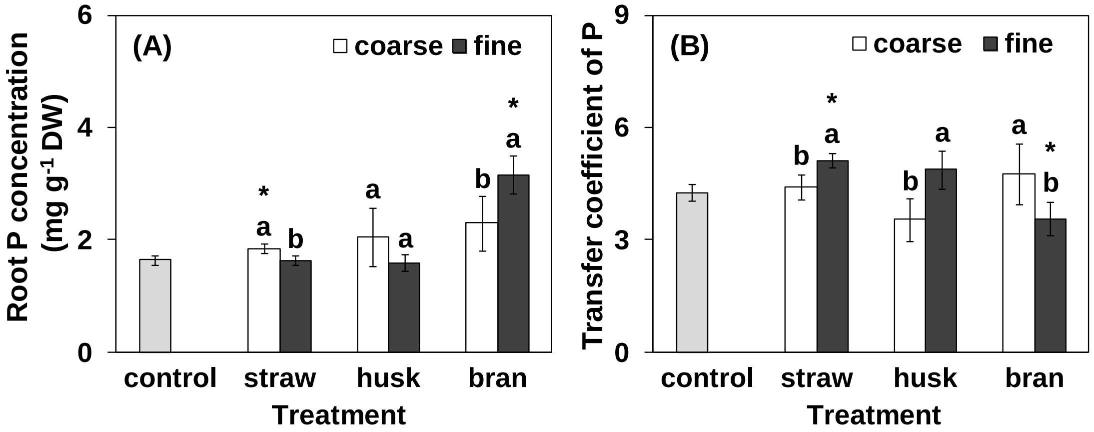

Fig. S2. The influence of biochar produced from different rice plant tissues on P concentrations in roots of wheat (*Triticum aestivum*) (A) and transfer coefficient of P of wheat seedlings (B). Control, straw, husk, and bran represent soils with additions of no char and 5% straw-char, husk-char, and bran-char, respectively. Different letters above columns (a or b) indicate significant differences at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar. * indicates a significant difference at $p < 0.05$ compared to the control.

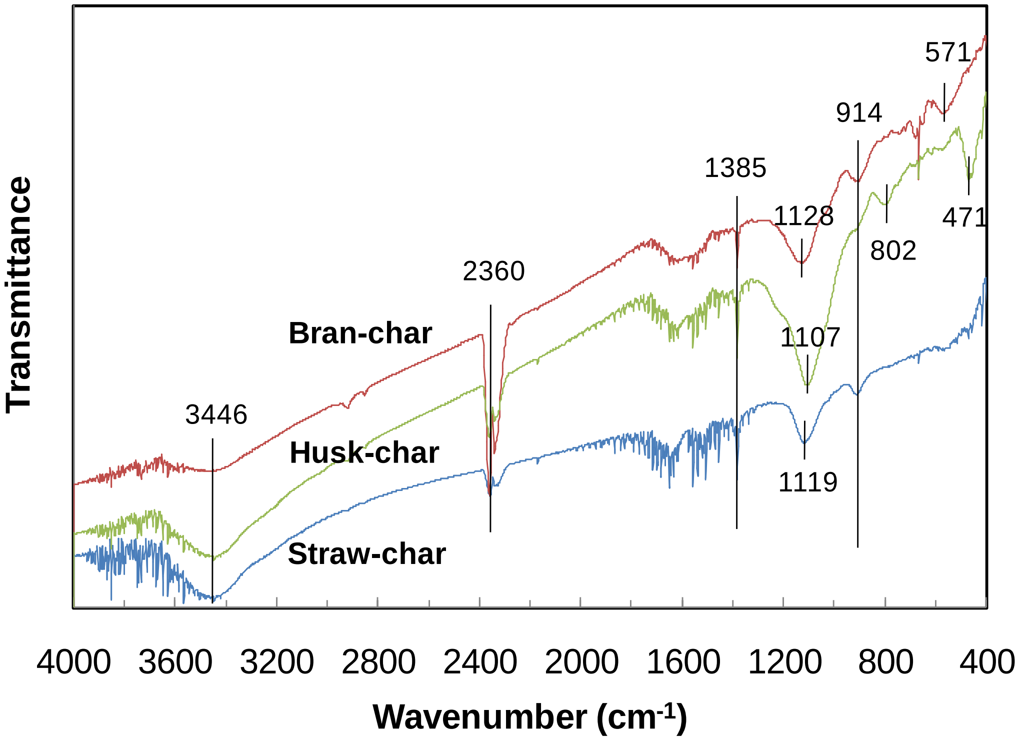

Fig. S3. The FTIR spectra of biochars produced from rice straw, husk and bran

Table S1. Concentrations of Exchangeable Cations (K, Ca, Mg), Olsen-P, and Carbonates in the Soil and Biochars

Biochar/soil	Exchangeable Cation (cmol$_c$ kg^{-1})			Olsen-P (mg g^{-1})	Carbonate (cmol kg^{-1})
	K	Ca	Mg		
straw-char	196.9±3.0	3.6±0.1	8.8±0.1	0.9±0.03	113.4±0.5
husk-char	15.4±1.0	4.1±0.6	3.1±0.1	0.5±0.00	20.8±1.4
bran-char	80.9±1.1	0.7±0.0	15.4±0.5	9.0±0.19	9.7±1.2
soil	0.4±0.0	18.9±1.4	1.4±0.2	0.03±0.00	——

Values are given as mean ± standard deviation for three measurements.

Exchangeable cations were extracted with 1 M ammonium acetate (pH 7) and K, Ca, and Mg in the extracts were analysed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy). Available P (Olsen-P) was extracted with 0.5 M $NaHCO_3$ (pH 8.5) (1:20 w/v for 60 min) (Lu 2000a) and P was analysed by ammonium molybdate-ascorbic acid method on a spectrophotometer (HACH - DR 5000). The carbonate (CO_3^{2-}) content in biochar was determined by a modified capacity titration method. The biochar was immersed in 0.5 M HCl (1:10 w/v), and the carbon dioxide liberated was analyzed by titrating with a standard 0.25 M NaOH solution (Lu 2000b).

Table S2. Concentrations of P, K, Ca, Mg of Straw-, Husk- and Bran-char Extracted by Water

Biochar type	Particle size	P (mg g^{-1})	K (mg g^{-1})	Ca (μg g^{-1})	Mg (μg g^{-1})
straw	coarse	0.73±0.02 a	50±0.24 b	19.8±0.4 a	68.2±2.1 b
	fine	0.76±0.07 a	53±0.77 a	21.4±2.7 a	74.3±1.2 a
husk	coarse	0.14±0.01 b	1.5±0.04 b	7.9±0.1 b	50.7±1.4 a
	fine	0.23±0.01 a	2.3±0.05 a	12.7±0.7 a	51.7±0.3 a
bran	coarse	2.72±0.12 b	3.7±0.21 b	14.6±1.1 a	1303.1±87.3 b
	fine	3.63±0.30 a	4.6±0.44 a	16.3±1.5 a	1694.1±41.3 a

Note: Different letters (a or b) equal significant differences at $p < 0.05$ between two particle sizes (coarse and fine) for the same type of biochar.
Values are given as mean ± standard deviation for three measurements.

Biochars were ground and sieved, yielding fine (< 0.18 mm) and coarse (0.5 – 2 mm) size fractions. Water extracts were carried out using 1:10 suspensions, shaken for 3 h, centrifuged for 10 min at 3000 rpm, the supernatant were filtered through a 0.45 µm pore-size Millipore filter and then concentrations of P, K, Ca, Mg were determined using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy).

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