Effects of Biochar on Heavy Metals Migration and Fractions Changes with Different Soil Types in Column Experiments

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Effects of biochar on different soil types were studied in soil column experiments. The results showed that the biochar decreased filtrate heavy metals concentration by 89.0% to 95.7% (Cd) and 93.2% to 99.3% (Pb) compared with the control. The biochar application changed 2.3% to 9.84% of the exchangeable Cd fraction Pb to residual fractions, so the bioavailable Cd and Pb were reduced by 4.48% to 10.69% (Cd) and 11.74% to 16.42% (Pb) in surface soil (0 to 4 cm). Through increasing the soil ratio, the concentration of bioavailability of Cd and Pb was decreased 13.84% to 16.15% and 4.02% to 13.40% in 4 to 8 cm soil. With sandy soil, the application of biochar effectively reduced the down migration of heavy metals, and accomplished the conversion of 100.0% and 95% exchangeable Cd and Pb fractions into 13.1 to 43.9% residual Cd and 11.6 to 100.0% residual Pb. The SOM and pH also increased 1.2 to 2.3 g kg^{-1} and 0.01 to 0.31 with biochar application. The biochar effectively increased the SOM content, and stabilized heavy metals, then reduced the migration of Cd and Pb.

Keywords: Soil column; Biochar; Heavy metals; Fractions; Vertical migration

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INTRODUCTION

Soil heavy metals pollution is a serious global environmental problem, which leads to human health risk through food chain transfer (Mitchell *et al.* 2017; Manzano *et al.* 2019; Saeedi *et al.* 2019). In field-contaminated soil in China specifically, the soil heavy metals pollution is linked to the rapid industrialization, urbanization, and subsequently widespread solid, liquid, and gaseous emissions from the factory and fertilizer use since 1970s (Cui *et al.* 2016; Fan *et al.* 2018a; Liu *et al.* 2018). Excessive accumulation of heavy metals in agricultural soils and products not only impairs soil quality and crop growth, but it also poses a threat to human and animals, which is necessitated to amend with using soil remediation practices (Yong *et al.* 2011; Fellet *et al.* 2014; Waqas *et al.* 2014). The 13.9% of grain production has been affected, due to the heavy metal pollution from mining and smelting, electroplate industry, sewage irrigation, urban development, and fertilizer application in farmland soil (Zhang *et al.* 2015). At present, the heavy metals fractions changes and migration prevention have contributed great benefits in reducing the plant uptake (Cui *et al.* 2017). Many conventional remediation techniques, such as replacement, electro-kinetics, soil flushing, and phytoremediation, have been developed to reduce the bioavailability and mobility of heavy metal in agricultural soils (Tang *et al.* 2016; Cui *et al.* 2017). The concentrations of bioavailable copper (Cu) and zinc (Zn) have been decreased by 48.9% and 16.4% in mature compost by adding calcium-based bentonite (10%, w:w) (Zhao *et al.* 2018). The soil Pb is mainly in surface soil (0 to10 cm) and associated with both carbonate-bound and metal-organic complex-bound fractions with natural leaching (Shangguan *et al.* 2015). The risk of rainfall-induced shallow groundwater heavy metals pollution (As, Cd, Cu, and Pb) is a small ratio among the natural factors (Wang *et al.* 2011).

Biochar is a carbon-rich byproduct of pyrolysis organic residues under anoxic oxygen conditions and low temperature (Fan *et al.* 2018b). The biochars feedstock derived from agricultural residue contains valuable macronutrients (especially N, P, Ca, *etc.*) and microelements (*e.g.*, Cu, Zn, *etc.*), which plays an important role in soil amendment and plant nutrition (He *et al.* 2017). In addition, amending soil with biochar has been paid more attention due to its great effects on soil quality and crop productivity improvement (Ahmad *et al.* 2014; Ling *et al.* 2017; Wu *et al.* 2017). The improved biochar (10%, w:v) effectively immobilizes 81.3% Cd after 28 days culture experiment, and the bioavailable Cd is reduced 80.0%, and the cabbage mustard belowground and aboveground parts showed decreased Cd uptake by 44.8% and 70.2%, respectively, which promotes cabbage mustard growth (Qiao *et al.* 2017). The 3% (w:w) biochar reduced porewater Cd in rhizosphere, resulting in 68% and 49% reduction in the root and grain Cd, which is efficient on Cd mobility in rice rhizosphere and transferring from soil (Yin *et al.* 2017). Biochar has great advantages and potential for soil amendment and polluted soil remediation. The experimental results have shown that wine lees-derived biochar efficiently increases soil pH and decreases the contents of soil exchangeable heavy metals, which then promotes heavy metals transformation to residual fractions (Anderson *et al.* 2011; Halim *et al.* 2015).

In heavy metals contaminated soils, the biochar can adsorb and immobilize metal ions due to its porous structure, large surface area, high surface charge density, and pH values (Hossain *et al.* 2010; Zwieten *et al.* 2010). The poultry manure compost (0 to 12%, w:w) significantly increases soil pH, and soil dissolves organic matters that may be complexed with Cd (50 mg Cd kg^{-1} soil), then transforms 47.8% to 69.8% soluble/exchangeable Cd to the organic-bound fraction, and consequently decreases Cd pakchoi uptake 56.2% to 62.5% compared with unamended soil (Chen *et al.* 2010). Wu *et al.* (2019) also found that the waste wheat straw biochar (*Hordeum vulgare* L.) application improved the qualities of heavy metal contaminated soil, and increased the soil microbial counts and soil enzyme activities in rhizosphere of vetiver grass (*Chrysopogon zizanioides* L.) and increased proportion of HOAc-extractable soil Cd and soil micro-ecology. It also significantly enhanced the vetiver grass Cd content and bioaccumulation factor (BCF) by 412% and 403% compared with control, respectively.

The biochar greatly modifies the degraded soils in the paddy soil and increases the utilization efficiency of the nutrient, the water retention of the soil, and affects the growth of the plant (Wang *et al.* 2018). Thus, it is necessary to amend the soil pollution, especially for the contaminated agricultural soil. Drake *et al.* (2016) found that *Acacia pycnantha* biochar (5 Mg ha^{-1}) decreases the concentration of Mn in plants and increases *Eucalyptus viminalis* height in the highly saline sodic soil (electrical conductivity: 49.4 dS m^{-1}, exchange sodium percentage: 45.1%). 150 g kg^{-1} sewage sludge (total Cd 3.47 mg kg^{-1}) treatment Cd concentration in leachates is almost four times higher than that of the unamended soil in the first leachate, which promotes the Cd downward movement, changes the Cd fractions in the surface soil decreased soil pH, and increases dissolved organic carbon (DOC) in saline-alkali soils (Gu and Bai 2018).

Biochar is widely used to mitigate the effects of Cd and Pb pollution in soils. There have been few studies on the long-term biochar effects on heavy metals fractions changes and vertical migration in contaminated soil. A hypothesis is proposed that the activity and migration of Cd and Pb in soil will be affected by the biochar application, soil types and the leachate solution that go through soil column. There are multiple effects of biochar amendment on the heavy metals polluted soil with leachate solution going through the column. On this basis, the effects of biochar on vertical migration of Cd and Pb under leaching solutions were studied in the authors' laboratory soil column, which provided a theoretical basis for promoting the application of biochar in soil improvement.

EXPERIMENTAL

Materials

Soil and biochar

The paddy soil was collected from the field surface (0 to 20 cm) in Yixing (31°24'27.01"N, 119°42'7.34"E), Jiangsu Province, China. The stones and litter were removed from soil, air-dried, passed through a 5-mm sieve, and prepared for the following soil column experiments.

Biochars were produced with wheat straw or branch-leaf under the anoxic pyrolysis at 450 ℃ in a continuous process using a vertical kiln that composed of refractory bricks at the Sanli New Energy Company, Henan Province, China. The basic biochar properties were determined following the methods recommended by the International Biochar Initiative (IBI, 2012) (Table 1). The biochars were made from wheat straw and branch-leaf were labeled as WSB, BLB respectively.

Table 1. Properties of Soil Sample and Biochars (g kg^{-1})

	pH	CEC (cmol kg^{-1})	EC (dS m^{-1})	SOC	Total N	Total P	Total K	Ash (%)
Soil	6.3	2.4	4.1	28.9	0.7	0.4	7.3	/
WSB	10.4	10.6	4.4	650.2	6.3	20.4	16.9	36.1
BLB	9.5	14.6	18.0	671.5	9.0	33.3	18.1	14.6

CEC: cation exchange capacity; EC: electrical conductivity; SOC: soil organic carbon

Column Tests

The diameter and height of each soil column were 5 and 20 cm (Fig. 1), in which 4 cm height of soil were fitted with biochar-contaminated soil/quartz sand (99%, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) composites. The contaminated soil was created by placing 100 g air-dried soil into a 500-mL glass beaker. Then, 45 mL of stock solution dissolved by 14 mg of Cd ($CdCl_2$, 99%, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) and 265 mg of Pb [$Pb(NO_3)_2$, 99%, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China] were added, thoroughly mixed, air dried, and then passed through a 2-mm sieve prior to placing at the top of the plot. This equated to 105 mg kg^{-1} of Cd and 2000 mg kg^{-1} of Pb for surface soil contamination. The biochar-to-soil/quartz sand ratios of 0 % (control), 1 % (w/w), and 5 % (w/w) were mixed uniformly with soil and then added to each soil column; the soil bulk density was similar to that in the field (~ 1.41 g cm^{-3}).

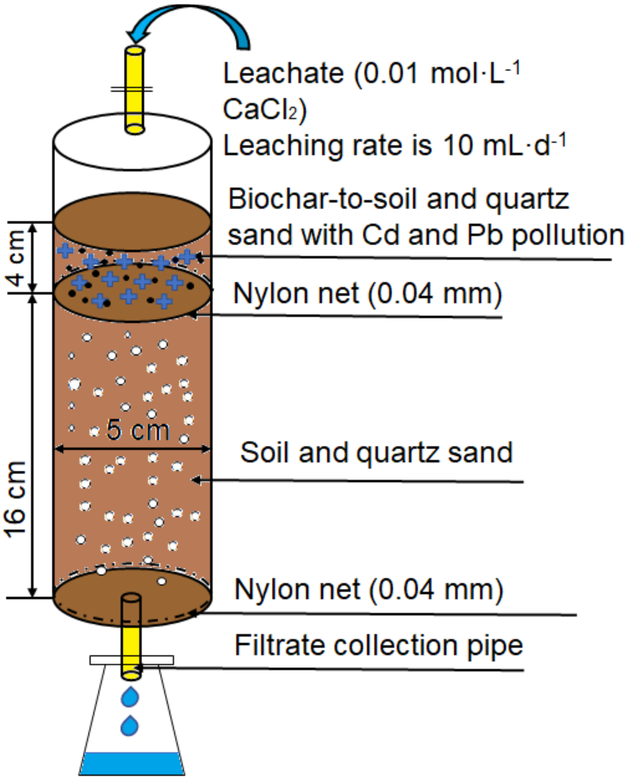

Fig. 1. Schematic of the research design

Soil/quartz sand composites were designed as follows (Table 2). The quartz sand (0.30 to 0.71 mm) was added into the paddy soil with ratios of 0%, 20%, 50%, and 100%. The 0.01 mol L^{-1} of $CaCl_2$ was used as leachate at 10 mL d^{-1} for the leaching test throughout the experiments. The treatments of 0%, 20%, 50%, and 100% quartz sand were simulated clay paddy soil, clay loam soil, sandy clay loam soil, and sand soil, respectively. The concentrations of heavy metals in filtrate were detected, and the leaching was stopped when the Cd concentration reached 10 μg L^{-1}. At the same time, the profile soil was divided into five parts every 4 cm and then air-dried. The soil organic matter (SOM), pH, and the speciation of heavy metals were analyzed for investigating of the leachability changes of soil heavy metals under the function of biochar addition.

Table 2. Soil Column Experiment Design with Biochar Application

Ad.	Treatments	Ad.	Treatments
Z1	100% Soil	bZ1	50% Quartz sand + 50% Soil
Z2	1% WSB + 99% Soil	bZ2	50% Quartz sand + 1% WSB + 49% Soil
Z3	5% WSB + 95% Soil	bZ3	50% Quartz sand + 5% WSB + 45% Soil
aZ1	20% Quartz sand + 80% Soil	cZ1	100% Quartz sand
aZ2	20% Quartz sand + 1% WSB + 79% Soil	cZ2	99% Quartz sand + 1% WSB
aZ3	20% Quartz sand + 5% WSB + 75% Soil	cZ3	95% Quartz sand + 5% WSB

Methods

Heavy metals fractions Analysis

The dried soil samples were crushed, mixed, homogenized, and passed through 2-cm sieve, then stored in air-tight polyethylene bags for analysis. The soil heavy metals fractions were extracted according to the European Community Bureau of Reference (BCR) continuous extraction method (Cui *et al.* 2019), such as exchangeable fraction (R1), reducible fraction (R2), oxidizable fraction (R3), and residual fraction (R4). The heavy metal fractions of soil samples were extracted according to the European Community Bureau of Reference (BCR) method, the brief process is shown in Table 3.

Table 3. Modified BCR Sequential Extraction

Abbr.	Extractant	Operation method
R1	0.11 mol L^{-1} HAc	Soil-liquid (1:40, m:v) was shook for 16h at 25°C
R2	0.5 mol L^{-1} $NH_2OH \cdot HCl$	Soil-liquid (1:40, m:v) was shook for 16h at 25°C
R3	8.8 mol L^{-1} H_2O_2 1 mol L^{-1} NH4OAc	Soil-liquid (1:10, m:v) was shook for 16h at 85°C, twice Soil-liquid (1:40, m:v) was shook for 16h at 25°C
R4	HNO_3:HF:$HClO_4$	The soil was digested with mixture of HNO_3,HF,$HClO_4$（10:10:3, v:v:v）

After every step of sequential extraction of BCR process was over, the leachate was then separated from the solid residue by centrifugation using a centrifuge instrument (Eppendorf 5417R, Eppendorf corporate, Hamburg, Germany) (Cui *et al.* 2016). The residue from the last step in the centrifuge tube was prepared for the next step, the solutions were transferred to 25-mL volumetric flask, and then filtered. The concentrations of heavy metals (Cd, Pb) in solution were detected by flame atomic absorption spectrometry (FAAS, Zeenit 700p; Analytik Jena AG, Jena, Germany). The quality control of soil heavy metal analysis was performed by standard addition recovery with national standard substances, and the recovery rates were 95.6 to 104.7% for Pb and 96.8 to 105.0% for Cd.

Statistical Analysis

All data were expressed as means plus or minus one standard deviation. Differences between the treatments were examined using a Student's t-test in a two-way analysis of variance (ANOVA), and considered significant when $p < 0.05$. All statistical analyses were performed using SPSS, version 22.0 (IBM Corporation, Chicago, Illinois, USA) and Origin 8.0 (Origin Lab, Northampton, MA, USA) software.

RESULTS AND DISCUSSIONS

Water-soluble Organic Carbon, pH, and Heavy Metals Change in Filtrate with Biochar Application

To detect the distribution of heavy metals in the soil column, the soil column was cut into four parts. However, cZ column had little interception effect on heavy metals, and then the leaching 20 mL of water solution was detected with higher content of heavy metals compared with Z, aZ, bZ treatments (Table 4). The biochar application (cZ2 and cZ3) decreased the concentrations of filtrate water-soluble heavy metals 89.0% to 95.7% (Cd) and 93.2% to 99.3% (Pb) compared with control (cZ1). The paddy soil column containing 50% and 20% quartz sand was leached with filtrate of 95.0 ± 4.6 mL and 253.0 ± 41.9 mL, respectively, with a small amount of Cd (~ 10 μg L^{-1}).

Table 4. Heavy Metal Content from Quartz Sand Treatment Filtrate

Treatment	Cd (mg L^{-1})	Pb (mg L^{-1})
cZ1	1.64 ± 0.21	1.52 ± 0.29
cZ2	0.18 ± 0.03	0.10 ± 0.01
cZ3	0.07 ± 0.09	0.01 ±0.01

The paddy soil column leached slowly with leachate (Cui *et al.* 2016), when the Cd or Pb concentration was over the detection limit. The changes of the water-soluble organic carbon (WSOC) concentration from filtrate are shown in Fig. 2(a, b). From the different soil columns treatments, the results showed that the leachate volume became smaller with lessening the ratios of quartz sand, so the quartz sand promoted the leaching speed and Cd transfer in the soil (Spokas *et al.* 2011). When 50 mL leachate leached in different treatments, the cZ, bZ, and aZ treatments took 1 min, 12 h, and 48 h, respectively. Most of the 50 mL leachate in paddy soil took three to four days, but the leaching rate was faster with the increase of ratio of quartz sand, so the whole soil column experiment lasted 305 days. The more quartz sand added resulted in lower WSOC content in the leachate. The more biochar added resulted in higher WSOC content in filtrate under the same quartz sand ratio. The leaching volume is shown in Fig. 2(b). The leaching speed was faster with biochar application, especially for the Z2 treatment with 1% wheat straw biochar compared with control, which was due to the increase of porosity in paddy soil with the porous structure of biochar application. The previous works also showed that biochar special basic physicochemical characteristics have great contribution in soil amendment (Quan *et al.* 2020). The WSOC content in the filtrate reached the maximum when 50 mL solution was added, and then it decreased to a stable concentration. After 5% wheat biochar treatment the WSOC concentration in the filtrate was the highest in 100 mL cumulative volume. The change tendency of filtrate pH was similar to that of filtrate WSOC, which was probably attributed to the influence of dissolved organic matter in the filtrate (Cui *et al.* 2019). From 50 to 100 mL cumulative volume, the leachate values of pH and WSOC increased at first and then decreased to a stable level (Jeffery *et al.* 2015).

The WSOC and pH changes are shown in Fig. 2. The content of WSOC in filtrates was 0.67 g kg^{-1}, which was 0.13 g kg^{-1} higher than that in Z1 treatment with 5% biochar application. The collected filtrate reached the maximum value in the second sampling point, and the WSOC content of the treatment with biochar (Z2 ~ 3) was increased 21.3% to 85.5% compared with Z1 treatment (Fig. 1a).

The WSOC content increased 0.024 g kg^{-1} to 0.58 g kg^{-1} in aZ experiments compared with bZ treatments (Fig. 2b). The WSOC contents in the filtrate of aZ treatments increased 0.10 g kg^{-1} to 0.70 g kg^{-1} compared with bZ2 and bZ3. The wheat biochar application effectively increased the content of WSOC in the filtrate under different soil types. The change tendency of pH was similar to the WSOC, which was one of the factors of the pH change in the filtrate. The pH value increased at first and then stabilized around 7.5. The change trend of pH was similar for all the filtrate pH changes of different soil treatments (Fig. 2c, d).

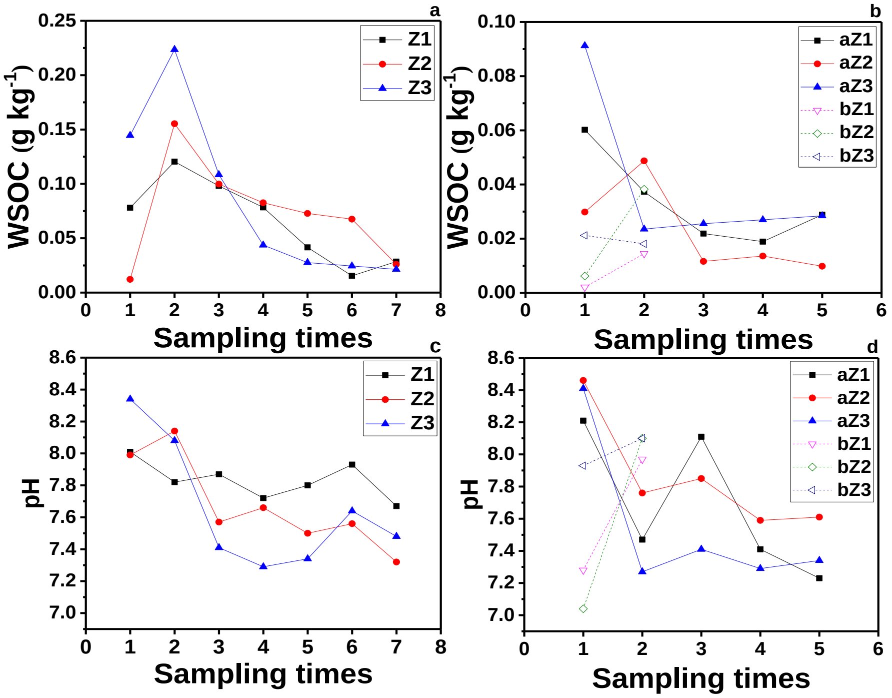

Fig. 2. Changes of pH and WSOC in filtrate (a, b: WSOC content change with the different ratio of sand; c, d: the pH value change with the different ratio of sand)

The filtrate WSOC content increased with biochar application, especially for 5% biochar ratio, which decreased with increased quartz sand ratio without biochar. The filtrate pH and WSOC decreased first and then stabilized after the biochar application. The filtrate pH was higher with wheat straw biochar treatment compared with the application of branch and leaf biochar, which was probably attributed to the different microstructure and functional groups on the biochars surface, this result was also shown by Abd-Alla *et al* (2012). With increasing biochar and paddy soil in different soil types, the content of WSOC was also increased and had great effects on immobilization of heavy metals, which made a contribution to changing heavy metals' fractions into low bioavailability fraction.

Effects of Biochars on Soil pH and Soil Organic Matter

The SOM was found only in surface soil (0 to 4 cm) under cZ treatment with biochar application and 20 mL leaching solution (Fig. 3a). However, the leachate solution leaching speeds were faster with bZ and aZ treatments compared with Z treatment, so part of the organic matter moved down with the leaching solution and the SOM increased 0.4 to 2.9 g kg^{-1} at 12 to 20 cm (bZ and aZ treatments) than that of the upper soil (0 to 4 cm) with no biochar application(bZ1, aZ1), respectively. The SOM cumulative effect was more obvious at bZ1 and aZ1 (Fig. 3b). With the increase of leaching volume and time, the SOM contents in the bottom layer (16 to 20 cm) were decreased compared with upper layer (0 to 4 cm). The accumulated SOM in the bottom layer 16 to 20 cm was also 0.5 to 3.1 g kg^{-1} higher than that in the upper layer applied with biochar (Z1). Among different biochar applications, SOM content was higher with branch and leaf biochar compared with wheat straw biochar, especially at 1% ratios (Z2). With the increase of paddy soil ratio in soil column, the SOM content was also increased, which was probably explained by the high SOM content in the paddy soil.

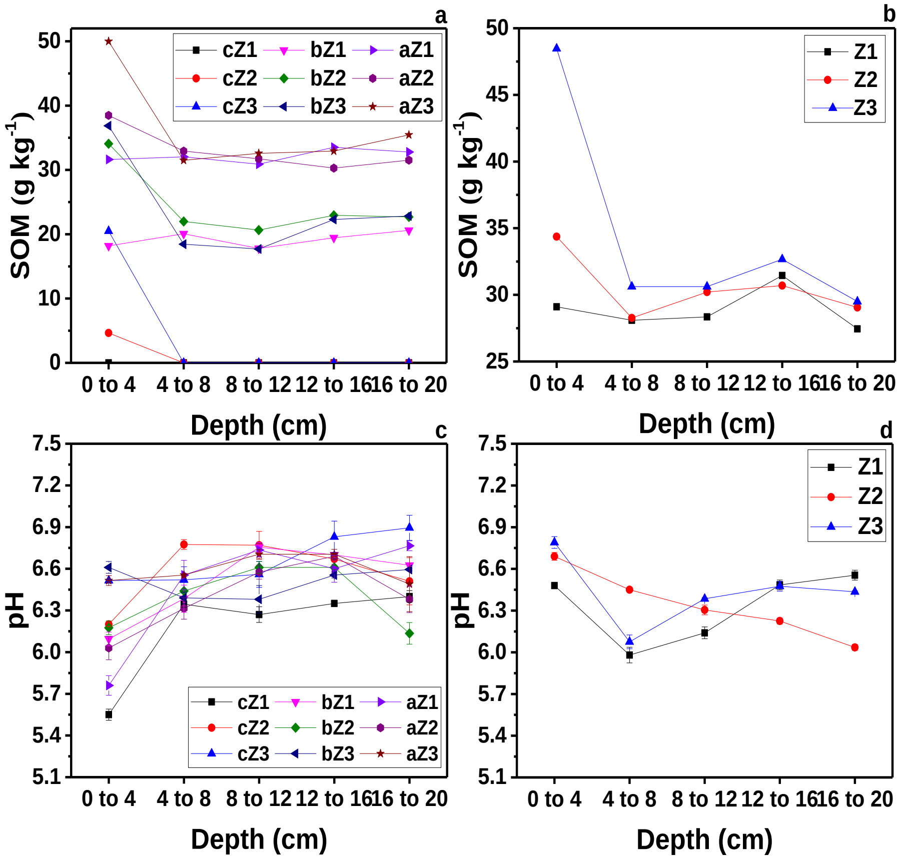

Fig. 3. Changes of SOM and pH in soil column after leaching (a, b: SOM content change with different ratios of sand; c, d: the pH change with different ratios of sand)

The SOM content of aZ treatments was 10.3 g kg^{-1} to 13.0 g kg^{-1}, which was higher than that of bZ treatments. The SOM value of the surface layer (0 to 4 cm) was decreased 0.42 g kg^{-1} to 1.89 g kg^{-1} than that of the bottom layer (4 to 8 cm) at the same quartz sand treatment, which was due to the leaching and downward migration of the leaching solution (Fig. 2a). The change trend of pH value under different biochar treatments was similar to that of SOM (Fig. 3b, d).

During the different biochar treatments, the leachate was weak alkaline, but the original paddy soil was weakly acidic (pH ~ 6.3), and the quartz sand was ~ 6.6, so the biochar played an important role on the soil pH change. The soil pH value of the surface layer (0 to 4 cm) were increased by 0.01 to 0.31 with 1% and 5% biochar used compared with no biochar, then the pH value was up to approximately 6.5 after 5% biochar treatment. The introduction of biochar increased the surface soil pH, so the organic matter was probably neutral or alkaline when it was removed by leaching solution (Rodríguez-Vila *et al.* 2016). Because the change trend of pH and SOM in the paddy soil was similar and the leaching time lasted a long time, the neutral or alkaline organic matter leached in the deeper layer, which caused the increase of the pH and led to the loss of organic matter at the bottom layer of 16 to 20 cm; this was also shown by others (Wu and Chen 2019). The branch-leaf biochar had greater ability on increasing the soil pH compared with wheat straw biochar.

Effects of Different Soil Types and Biochars on Vertical Migration of Heavy Metals

The heavy metals migrated fast from top to bottom with cZ treatments in the soil column, and the heavy metals completely passed through the column and were left in the leachate. With the increase ratio of biochar application, the heavy metals' migration rate was slowed down compared with control (Fig. 4). The higher the ratio of biochar, the slower rate that the soil heavy metals migrated from top to bottom, which was attributed to the microstructure and functional groups of biochar. The Pb migration was different from Cd, even on cZ treatment, which was below detectable limit, indicating that the migration and fractions of Pb was more complex comparing with Cd (Zhan *et al.* 2019).

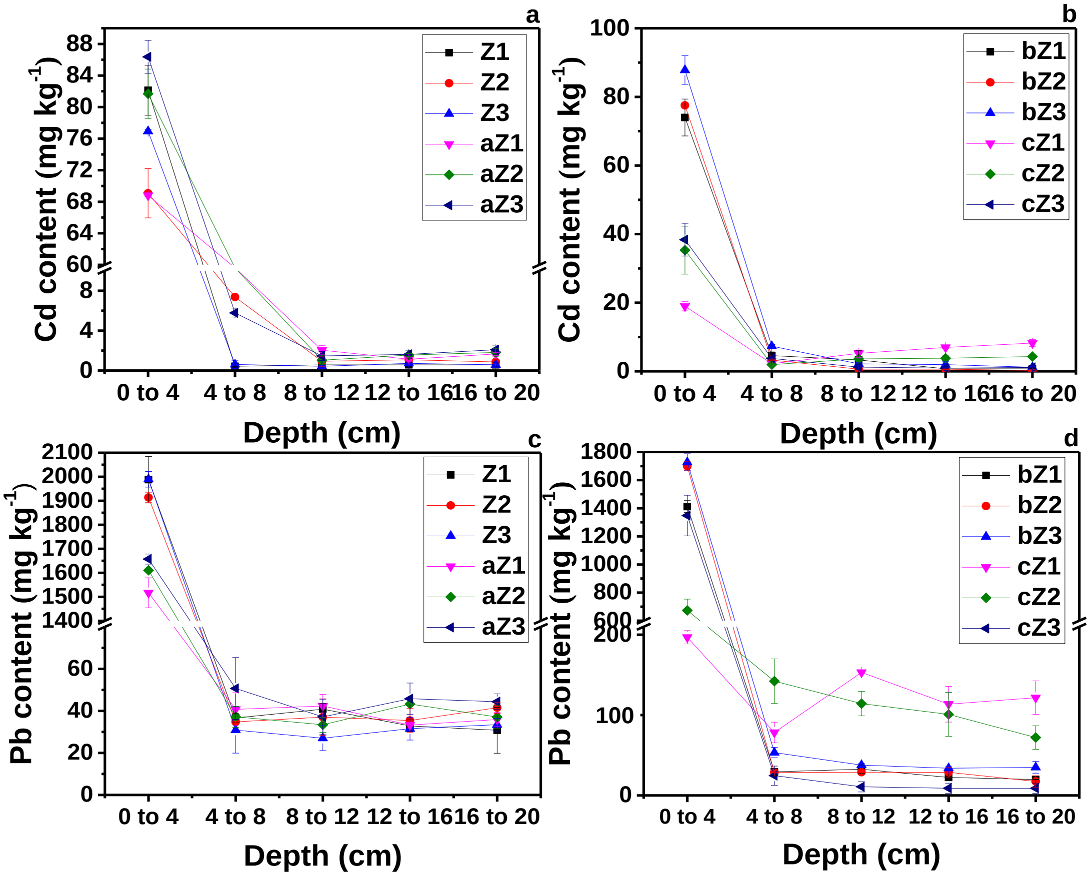

Fig. 4. Changes of Cd and Pb contents in soil columns with different soil textures and biochars (a, b: Cd content change with different ratio of sand; c, d: Pb content change with different ratio of sand)

For 4 to 20 cm layer soil, the Pb content with branch and leaf biochar treatment increased 6.07 to 38.65 mg kg^{-1} compared to wheat biochar application (Fig. 4c, d). The results showed that the ability of wheat biochar to retain Pb and Cd was stronger than that of branch and leaf biochar. The leachates were leaching faster in Z2 treatment compared to Z1 with different biochars during 305 d (Fig. 4a, c). The Cd concentration was up to 7.37 mg kg^{-1} in the Z1 sub soil (4 to 8 cm), which was higher in comparison with other treatments. In this experiment, the wheat straw biochar probably offered more micropore structures in the paddy soil compared with branch and leaf biochar, which allowed the leaching solution to more easily migrate into the groundwater, and increased the risk of underground water pollution. The Pb is mainly residual fraction in the surface soil, and difficult to migrate from surface soil to bottom soil in the column (Zhan *et al.* 2019).

With the increased quartz sand ratio, the ability of heavy metal retention in soil decreased. Furthermore, the content of bioavailable heavy metals was decreased by 11.6% to 68.6% with biochar added. With the increase of paddy soil ratio, the concentration of heavy metals in 4 to 8 cm decreased 21.2% to 30.3%, indicating that the heavy metals downward migration to the deep layer decreased.

The Cd migrated downward rapidly during cZ treatment in the leachate, but the Pb was slow in downward migration. This may have been because it was present in the form of compounds in the soil (Table 5). Without biochar application, the Cd and Pb were detected as the exchangeable (> 90%) and residue fractions. At 12 to 20 cm depth, Cd fraction was mainly present as exchangeable Cd, and the same to Pb that amounted to over 90% proportion. The exchangeable fractions of Cd and Pb in the surface layer were converted to reducible fractions under 1% biochars application. While the exchangeable fractions of Cd and Pb in the surface layer were converted to reducible and exchangeable fractions under 5% biochar application. The biochar application effectively reduced the ratio of exchangeable heavy metals and the vertical heavy metals migration. In the treatment of cZ2 and cZ3, the exchangeable Cd and Pb increased in the deep layer. The loss of heavy metals in cZ1 surface soil came into the deep layer and the leaching solution, indicating that the quartz sand had poor ability on keeping the heavy metals without biochar and organic matter application.

Table 5. Cd Fraction and Distribution in Soil Column with cZ Treatment (R1: Exchangeable Cd and Pb, R2: Reducible Cd and Pb, R3: Oxidizable Cd and Pb, R4: Residual Cd and Pb)

		cZ1				cZ2				cZ3			
	Depth (cm)	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
Cd	0 to 4	97.2	1.3	/	1.5	96.6	2.4	/	1.0	88.6	8.31	1.0	2.0
	4 to 8	90.3	/	/	9.7	94.4	/	/	11.2	82.7	3.99	/	13.3
	8 to 12	94.8	/	/	5.2	78.5	/	/	21.5	78.5	7.77	/	13.8
	12 to 16	100	/	/	0	86.9	/	/	13.1	69.6	/	/	30.4
	16 to 20	100	/	/	0	85.8	/	/	14.3	56.1	/	/	43.9
Pb	0 to 4	95.5	/	/	4.5	95.7	3.4	/	0.9	82.7	14.9	0.4	2.0
	4 to 8	87.4	/	/	12.6	96.8	/	/	3.2	71.1	/	/	28.9
	8 to 12	95.1	/	/	4.9	90.6	/	/	9.4	/	/	/	100
	12 to 16	96.1	/	/	3.8	88.5	/	/	11.6	/	/	/	100
	16 to 20	92.2	/	/	7.9	81.4	/	/	18.6	/	/	/	100

Changes of Heavy Metals Fractions during Vertical Migration

For the top two layers (0 to 4 and 4 to 8 cm), the soil heavy metals fractions were analyzed (Fig. 5). The Cd and Pb ions during bZ and aZ treatments (0 to 12 cm) were difficult to migrate, and Pb ion mainly was present as residual fraction (~ 90%) and reducible fraction (~ 10%). The Cd and Pb fractions and distribution in surface within different soil types are shown in Fig. 5(a, b, c, d). For bZ treatments, the oxidizable Cd fraction increased 1.03% to 1.21% after biochar application compared with Z1. The biochar application in paddy soils (4 to 8 cm) increased the oxidizable Cd fraction and reduced the exchangeable Cd fraction by 13.8% to 16.2% in contrast to control (Z1). The biochar improved the Cd fractions and restrained the downward migration. Moreover, the proportion of exchangeable Cd fraction increased, but the residual Cd fraction decreased due to the downward migration. In this experiment, the downward migration of Cd was mainly as exchangeable fraction in the soil. With the ratio of paddy soil increased in the soil column, the exchangeable Cd fraction decreased 17.25% ± 9.36%, but the ratio of other forms increased (Vivas *et al.* 2005).

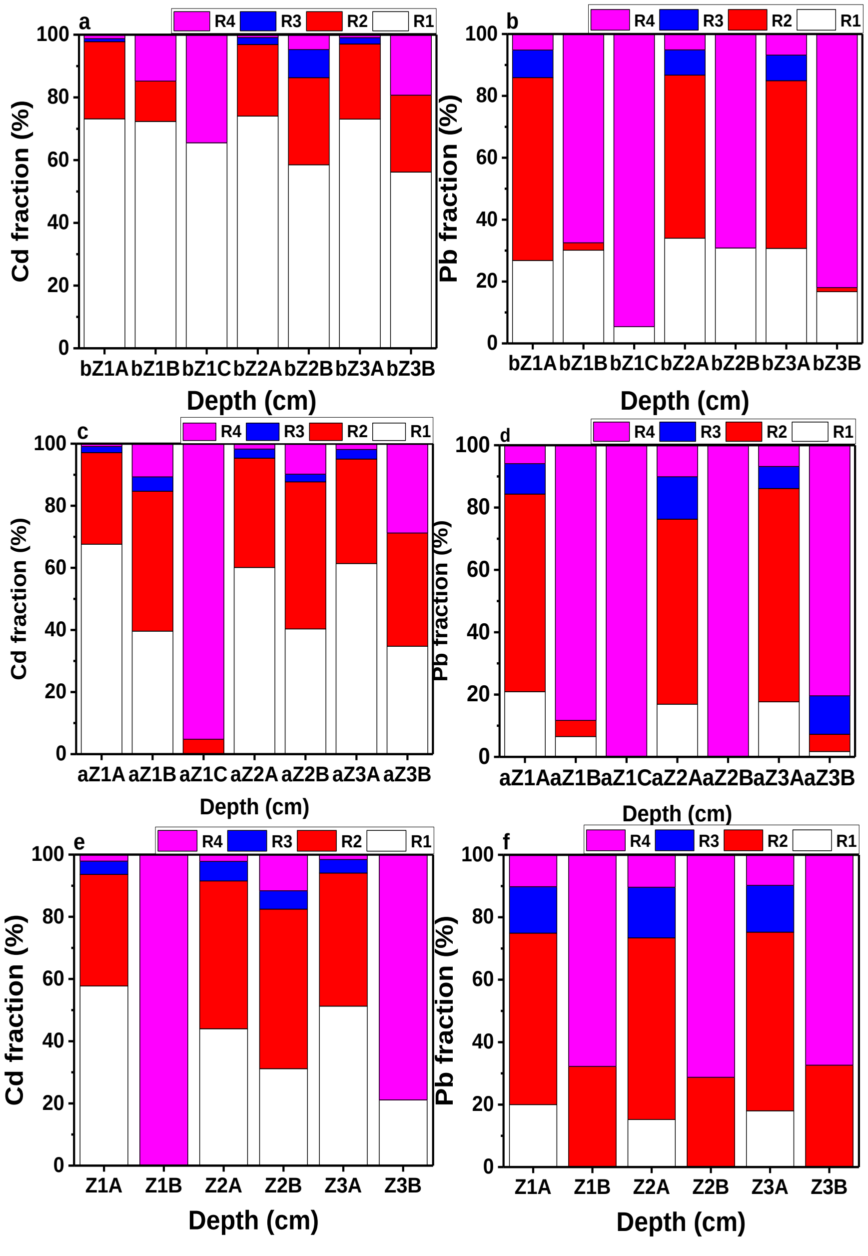

Fig. 5. The Cd and Pb fractions and distribution in soil column (a, c, e: the Cd content under different ratios of sand; b, d, f: the Pb content under different ratios of sand. The capital letters in the x axes presents the different depth, A: 0 to 4 cm, B: 4 to 8, and C: 8 to 12 cm) (R1: Exchangeable Cd and Pb, R2: Reducible Cd and Pb, R3: Oxidizable Cd and Pb, R4: Residual Cd and Pb)

The Pb was difficult to migrate and transfer fractions in the soil column and the natural environment (Yin *et al.* 2016). Thus, the Pb was difficult to transfer from the top to the bottom layer, but the addition of biochar changed the form of Pb in the deeper layer. In the bottom soil, low proportions of quartz sand treatment increased the proportion of residual Pb fraction, which prevented the downward migration of Pb. Because the biochar added in the paddy soil, the residual fraction Pb was increased and difficult to migrate in the soil column. During the leaching process, the low ratio of residual Pb fraction was transformed into other forms. The exchangeable Pb fraction was under the detection limit in 20% and 50% quartz sand treatment in 4 to 20 cm soil, while the Pb was transformed from exchangeable fraction into oxidized fraction in the deep layer. When the biochar was applied to the surface soil, the exchangeable Pb fraction was decreased, due to the biochar effectively changing the Pb existing form and reducing the Pb bioavailability. The ratio of oxidizable and reducible Pb fractions in the 4 to 20 cm soil was increased; the soluble organic matter in leaching solution was responsible for this. Qiao *et al.* (2017) found that the Chinese herb medicine residue biochar immobilizes 81.3% Cd in contaminated soil during 28 days incubation, which shifted Cd ion from exchangeable fraction (*i.e.*, bioavailable fraction, which was easy uptake with root.) into residual fraction and organic bound phases (*i.e.*, stable fraction, which was difficult uptake with root.).

The fractions distribution of amended soil column profile with different biochars content are shown in Fig. 5(e, f). The Cd was the mainly residual fraction at 8 to 20 cm soil. After leaching for 305 d, the Cd was mainly on the surface soil (0 to 4 cm). Paddy soil had a good protective effect on Cd contaminated infiltration compared with quartz sand treatment. The Z1 treatment leached only 307 ± 93 mL solution due to its good water retention compared with quartz sand added treatment. The soil bioavailable Cd was leached to the deep layer (4 to 8 cm) and it mainly became residual fraction. The leaching solution was 1233 ± 254 mL in Z2 treatment with 1% wheat straw biochar application, which may be attributed to the increasing of voids created by biochar and resulting in Cd downward migration to 4 to 8 cm soil (7.37 mg Cd kg^{-1}). The leaching volume of Z3 treatment was 421 ± 190 mL. Although the leaching volume increased a little in the Z treatment, Cd was difficult to migrate down. The distribution of different Cd fractions in the deep layer was changed. The reducible Cd fractions (22.2%) were transformed into other forms, probably because the leaching also brought the change of soluble organic matter from biochar and changed the Cd fraction (Qiao *et al.* 2017). The 1% and 5% branch and leaf biochar treatments decreased the ratio of exchangeable Cd fractions in the surface layer by 4.5% and 10.7%, and increased reducible Cd fractions by 4.8% and 8.7% compared with the control, respectively. The leaching solution volume of 1% and 5% branch and leave biochar treatments were 250 ± 120 mL and 744 ± 220 mL, respectively. With the increase of biochar, the leaching rate and leachate volumes were also increased. This indicated that the mixing of biochar improved the soil properties and increased the leaching rate. The Pb mainly existed as residual (~ 85%) and reducible (~ 15%) fractions, except for surface soil (0 to 4 cm, Fig. 4d). When biochar was applied with 1% and 5% treatment, the exchangeable Pb decreased by 0.53% to 4.79%, and the reducible Pb increased by 2.30% to 9.84% and the oxidizable fraction increased 0.11% to 1.35%, respectively. However, the exchangeable Pb fraction was under the detection limit at 4 to 8 cm, indicating that the fluidity of Pb was poor and usually existed as a compound in the soil. The heavy metals transfer order and approximate removal rates were similar, as reported by Saeedi *et al.* (2019) for the same heavy metals-contaminated clay mineral mixtures. The Pb had great affinity for adsorption onto soil particles and organic matter, which was probably because of the smaller hydrated radius (Li 2006). The reducible Pb fractions treated with wheat straw biochar and branch-leaf biochar application increased 2.3% to 3.3% and 7.1% to

9.8% for 0 to 4 cm layer soil, respectively, indicating that the reducible functional groups of branch-leaf biochar that can combine with Pb were more than that of wheat straw biochar (Fan *et al.* 2018b). The residual Pb fraction increased 0.4% to 3.5% and 11.7% to 16.4% in 4 to 8 cm layer soil for wheat straw and branch-leaf biochar (such as Z2, Z3), respectively, indicating that branch-leaf biochar had better ability to convert Pb into residual fraction than wheat straw biochar.

With increased paddy soil ratio, the bioavailable heavy metals were mainly transferred to the insoluble fractions in the surface layer, which was difficult to move down in the soil column. The heavy metal forms in the surface layer were changed from exchangeable and reducible fractions to oxidizable and residual fractions, which was due to the biochar application. The added biochar effectively increased the SOM and provided more functional groups and heavy metal complexation reaction sites, which also effectively reduced the bioavailability of heavy metal concentration in the surface layer. Additionally, the pollution caused by the downward movement of heavy metals was relieved by the biochar application. The original heavy metals form in soil is mainly as residual fraction, after being leached by leachate solution with biochar amendment, the leachate solution had more organic matter and intermediate compared with no biochar application, which led to the transfer of the heavy metals from residual fraction to other fractions.

Correlation Analysis

Correlation was used to analyze the relationships of different source factors, especially for the experiment affected by many factors. The high value of correlation coefficient among them indicated they were similar sources. In this research, principal component analysis (PCA) was used to analyze the correlation of SOM, pH, Cd, and Pb with different fractions (Table 6).

Table 6. Correlation Coefficient Value with Different Factors

	SOM	pH	Cd1	Cd2	Cd3	Cd4	Pb1	Pb2	Pb3	Pb4
SOM	1	0.08	0.27^{*}	0.47^{**}	0.46^{**}	0.61^{**}	0.06	0.45^{**}	0.37^{**}	0.59^{**}
pH		1	-0.09	0.06	0.14	0.21	-0.03	0.02	0.04	0.06
Cd1			1	0.86^{**}	0.76^{**}	0.16	0.82^{**}	0.91^{**}	0.78^{**}	0.68^{**}
Cd2				1	0.97^{**}	0.35^{**}	0.57^{**}	0.98^{**}	0.91^{**}	0.89^{**}
Cd3					1	0.36^{**}	0.53^{**}	0.93^{**}	0.88^{**}	0.89^{**}
Cd4						1	0.01	0.30^{*}	0.28^{*}	0.51^{**}
Pb1							1	0.63^{**}	0.53^{**}	0.43^{**}
Pb2								1	0.91^{**}	0.85^{**}
Pb3									1	0.79^{**}
Pb4										1

**p at 0.01 level; *p at the 0.05 level; The Cd1, Cd2, Cd3, Cd4, Pb1, Pb2, Pb3, and Pb4 represent the R1, R2, R3, and R4 fractions of Cd and Pb, respectively

Except for there being no correlation found with exchangeable Pb, the SOM was significantly correlated with exchangeable Cd ($p < 0.05$), and the others (the reducible fraction, oxidizable fraction and residual fraction of Cd and Pb) were significantly correlated with other forms of Cd and Pb ($p < 0.01$), which was also shown by Zhan *et al.* (2019). There was no significant correlation between pH and other factors, which was probably attributable to the fluctuant change with different sampling time. The relationship among them indicated that the SOM was an important factor to influence the changes of heavy metals factions, such as the transfer of oxidizable heavy metals fractions, reducible fractions, and residue fractions Cd and Pb (Deng *et al.* 2014). Most of the different Pb and Cd fractions were significantly correlated, except for pH. The residual Cd, exchangeable Cd, and exchangeable Pb were significantly correlated ($p < 0.01$). The residual Cd was not significantly related with exchangeable Cd and Pb (Guo *et al.* 2017).

The correlations between soil properties and heavy metals fractions are listed in Table 7, of which two principal components PC1 and PC2 were extracted, whose eigenvalues were higher than 1. The cumulative variance contribution rate of these two principal components was 77.0%. The PC1 and PC2 contributed 61.4% and 13.6%, respectively. The main contributions for first principal component PC1 were four forms of Cd and Pb, among which Cd1, Cd2, Cd3, Pb2, Pb3, and Pb4 were high load as 0.88, 0.98, 0.95, 0.98, 0.91, and 0.91 (Table 7). The Cd4 and Pb1 had medium load, 0.43, 0.64, indicating that were similar sources. While PC2 mainly included SOM and Cd4 with moderate loads of 0.63 and 0.73, which were essentially natural sources.

Table 7. Loadings for PCA of Heavy Metals in Study

Variables	Principal Component												
	SOM	pH	Cd1	Cd2	Cd3	Cd4	Pb1	Pb2	Pb3	Pb4	EV	CR	CCR
PC1	0.53	0.05	0.88	0.98	0.95	0.43	0.64	0.98	0.91	0.91	6.14	61.4	61.4
PC2	0.63	0.30	-0.37	-0.03	0.03	0.76	-0.53	-0.11	-0.09	0.22	1.56	13.6	77.0

EV: Eigenvalue; CR: Contribution rate (%); CCR: Cumulative Contribution rate (%)

The Cd1, Cd2, Cd3, Pb2, Pb3, and Pb4 accounted for 14.3%, 16.0%, 15.5%, 15.9%, 14.9%, and 14.8% of the principal components in PC1, respectively. Heavy metal fractions changes were mainly affected by heavy metal factors, while the scores of SOM and Cd4 in PC2 were 40.2% and 49.0%, respectively, indicating that soil physical and chemical properties were the main factors. The main factors influencing heavy metal migration were heavy metal factors, followed by soil physical and chemical factors, such as the content of SOM, pH, WSOC and Cd/Pb fractions in our experiment, and the soil types, the abundance of functional groups, microorganism (*e.g.*, Cui *et al.* 2013, 2016).

CONCLUSIONS

1. The biochar application had great efficiency on reducing the Cd and Pb downward migration, and effectively decreased the ratio of bioavailable Cd and Pb. The ratio of exchangeable Cd and Pb fractions also decreased in the surface layer with biochar application.
2. The leaching solution also changed the Cd and Pb fractions in the deep layer through binding the soluble organic matter from the soil and/or biochar, which promoted the

transformation of residual Cd and Pb into reducible and oxidizable fractions. The biochar improved the forms of Cd and Pb and also accelerated the Cd and Pb leaching rate in soil column. The wheat biochar (1%) showed great effects on Cd and Pb mobility.

3. The different soil types demonstrated good influence on the heavy metal's migration and transfer fractions. The sand soil (namely the high quartz sand ratio) promoted the Cd and Pb transfer compared with the paddy soil, which also increased the soil water permeability. The biochar and quartz sand treatment increased the water holding capacity compared with control, and more leaching solution led to the migration of Cd and Pb to the deep layer, which then partly increased the risk of Cd and Pb pollution to groundwater. The biochar application also improved the soil properties, such as increasing the SOM and pH, which played an important role on soil amendment and environment.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundations of China (21677119, 41501339), the Postgraduate Research & Practice Innovation Program of Jiangsu Province (SJCX17-0687), and the Natural Science Foundation of Jiangsu Province (BK20170475).

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Article submitted: December 27, 2019; Peer review completed: March 27, 2020; Revised version received and accepted: April 20, 2020; Published: April 23, 2020.
DOI: 10.15376/biores.15.2.4388-4406