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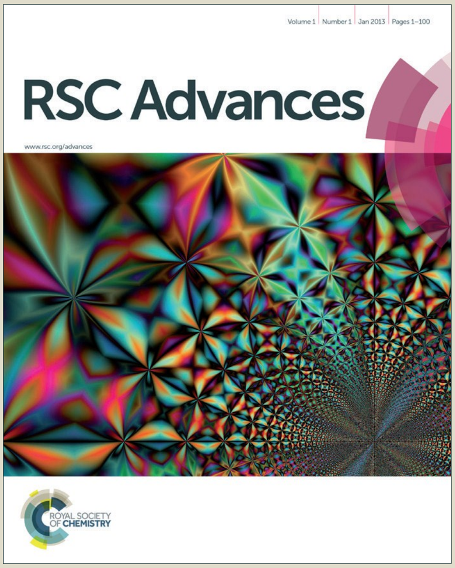

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Efficiency of biochar and compost (or composting) combined amendments for reducing Cd, Cu, Zn and Pb bioavailability, mobility and ecological risk in wetland soil

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Abstract: Biochar and compost are two inexpensive and effective *in situ* remediation materials for heavy metal contaminated soils. The interaction between biochar and compost (or composting) calls further studies to maximize potential benefits of both. In this study, we examined short-time efficiency of compost (C), biochar (B), mixture of compost and biochar (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting) for reducing bioavailability, mobility and ecological risk of Cd, Cu, Zn and Pb in wetland soil. Adding these amendment materials to the contaminated soil changed total organic carbon (TOC), water-extractable organic carbon (WEOC) and pH. All the materials decreased available Cd, Cu, Zn and Pb concentrations in soil (compost increased available Cu concentration) and Cd, Cu, Zn and Pb concentrations in pore water. As a whole, soil with Bced and BCing had the biggest decrease in these concentrations. These results indicated that all the materials reduced the bioavailability and mobility of heavy metals (compost improved bioavailability of Cu), and Bced and BCing had the greatest capacity for that. The materials improved soil microbial biomass and BCing created the biggest improvement, which suggested all the amendment materials reduced ecological risk of heavy metals and BCing had the greatest capacity for that.

Key words: Amendment; Heavy metal; Compost; Biochar; Soil microbial biomass

1. Introduction

Anthropogenic industrial and agricultural activities caused heavy metal (also is called potentially toxic metal) pollutants in extensive areas [1, 2]. Heavy metals are difficult to degrade or remove in the environment. Pollution of heavy metal in soils may cause long-term risks to ecosystems and humans [3, 4]. Accordingly, many techniques have been developed to remediate heavy metal polluted soils, including physical means, chemical means, incorporation of amendments, electrokinetic remediation, biological remediation and combined remediation technologies [3, 5]. Modern remediation approaches increasingly focus on *in situ* environmentally friendly techniques, such as assisted natural attenuation and phytostabilisation often primed by the addition of soil amendments [6, 7]. Compost (C), of the numerous amendment materials used for *in situ* stabilization of contaminants, has proven successful at binding heavy metals, rapid mobilization and vertical transport of trace metals [6, 8-10]. Biochar (B), produced by pyrolysis of biomass under low oxygen conditions, has caught more and more attention as a soil amendment material [8, 11]. Biochar has many favorable immobilization properties as a heavy metal modifier, such as a microporous structure, active functional groups, and high pH and cation exchange capacity (CEC) [12-14]. And it has been proved that biochar has a strong adsorptive power for heavy metals [15-17].

As two of the important and inexpensive soil amendment materials, biochar and compost (or composting) also had influences on each other’s properties. The interaction of biochar and compost (or composting) has been reported in the recent years [18, 19]. Addition of biochar could significantly influence the physic-chemical process and microbial community during the composting [20, 21], and also the composition and quality of the end product [18, 22, 23]. Surface of biochar is modified during the composting process due to the biotic and abiotic oxidation, and sorption of compost-derived organic compounds [24-26]. The changes of these properties may influence the effectiveness of biochar and compost amendment for soil heavy metals.

Interaction of biochar and compost (or composting) could provide a method for improving the effectiveness of biochar and compost amendment. Biochar and compost mixed amendment material (B+C) had been studied widely in recent years. Beesley et al. found that B+C had higher efficiency for reducing water-extractable As and Cd in soil than that of biochar or compost, and higher efficiency for reducing Zn and Cd in soil pore water than that of biochar or compost [8]. Other study also found that B+C did not have higher efficiency for reducing mobility of heavy metal and As in a naturally contaminated mine soil than that of biochar [27]. Borchard et al. reported composting increases the surface reactivity of biochars for Cu(Ⅱ) sorption in water due to their uptake of compost-derived organic matter [25]. The interaction between biochar and other organic amendment materials in soil should now be the focus of further study if we want to maximize the potential benefits of both [6]. However, little information is about the efficiency of composted biochar (Bced) or biochar-composting (BCing, biochar and biomass mixed before composting) on contaminated soil *in situ* remediation.

In this study, we examined soil properties, concentrations of Cu, Zn, Pb and Cd in soil pore water and available Cu, Zn, Pb and Cd in soil after addition biochar, compost, B+C, Bced and BCing to contaminated soil. Based on this work, the objectives of this study were: (1) to analyze the short-time efficiency of biochar and compost combined amendment materials for reducing heavy metals bioavailability and mobility; and (2) to examine the short-time efficiency of biochar and compost combined amendment materials for reducing heavy metals ecological risk, taking soil microbial biomass as an indicator.

2. Materials and methods

2.1. Soil and amendment materials

Soil (pH: 7.62; clay: 24.19 %, silt: 45.54 % and sand: 30.27 %) was sourced from beach of the Dongting Lake wetland. Dongting Lake, the second largest fresh lake in China, is located in the middle reach of Yangtze River region [1, 28]. The wetland is an important wintering habitat and pathway for East Asian migratory birds [29]. Because of the mining wastewater, industrial wastewater and natural sources, the soil of Dongting Lake wetland was polluted by heavy metals [28, 30-33]. The soil was collected from 10-20 cm soil depth on beach of the Dongting Lake wetland. The soil was air dried, sieved to a particle size of < 2 mm and biological debris was removed.

The whole procedure of the proposed method is shown in Fig. 1. Biochar was produced from corn cob at 450 ^{o}C using a slow pyrolysis method in a continuous flow

N_2 gas unit for a residence time of 1 h [34]. All used biochar in this study was sieved to make sure its grain size was 0.125 mm~1.000 mm. Compost was produced from rice straw according to a previous study [35]. B+C was made of the mixture of biochar and compost with the ratio of 1:1 (W/W). Bced was produced as follows: biochar was placed into mesh (0.125 mm) bags and then composted with rice straw, the bags filled with biochar was taken out after composting completed. BCing was produced as follows: biochar and rice straw were mixed (W/W: 1:1) and placed into the mesh bags and then composted with rice straw according above method. Chemical properties of these amendment materials are shown in Table 1.

2.2. Experimental design and procedure

The soil was thoroughly mixed with amendments in the following proportions, which constituted the treatments.

S: 500 g soil in each pot.

S + C: 500 g soil and 25 g compost in each pot.

S + B: 500 g soil and 25g biochar in each pot.

S + B + C: 500 g soil, 25 g B+C in each pot.

S + Bced: 500 g soil and 25 g Bced in each pot.

S + BCing: 500 g soil and 25 g BCing in each pot.

Finally, soil was placed into 1000 mL pots. Each treatment was implemented in triplicate.

One injection syringe of 15 mL was inserted into each pot, according to one previous study [27], to collect pore water. Deionized water was added to the soil of each pot to achieve a fixed moisture content of 60% water filled pore space. These pots were then placed in a controlled environment chamber with 28% relative humidity and at 25 ^{o}C for 60 days. The water content of the soil in each pot was adjusted weekly to maintain the water filled pore space of 60%. At day 7, 15, 30 and 60, pore water was collected by replace the injection syringe. At day 60, the soil in each pot was collected to analyses for soil properties, total metals, available heavy metals and soil microbial biomass.

2.3. Analytical procedures for soil characterization

Amended soil pH (water: soil ratio of 1:2.5) was tested by a digital pH meter. TOC of amended soil was examined by the loss-on-ignition method after ashing at 450 ^{o}C for 4 h [36]. Water-extractable organic carbon (WEOC) was obtained by aggressive aqueous extraction using a 1:10 soil to deionized water suspension (2.5 g soil: 25 g water), which was shaken for 3 h and centrifuged at 1408×g for 10 min, then filtered [6]. The filtered supernatant was determined using the Shimadzu TOC-V CPH analyser (Shimadzu, Tokyo, Japan). Total metal content in amended soil was determined by the AA700 atomic absorption spectrometry (PerkinElmer, USA) after HNO_3-HF-$HClO_4$ digestion process [1].

2.4. Measures of Cd, Cu, Zn and Pb bioavailability

Soil extraction method was evaluated using $CaCl_2$ solution as a surrogate measures of metal bioavailability and ecotoxicity [37]. Extraction with 0.5 M $CaCl_2$

solution was completed according to one previous study [38, 39]. Extracted metal content were measured using the above atomic absorption spectrometry.

2.5. Measures of Cd, Cu, Zn and Pb mobility

Metal content in pore water was determined as a surrogate measures of metal mobility [6]. Cd, Cu, Zn and Pb contents in pore water were measured by the above atomic absorption spectrometry.

2.6. Ecological risk

Soil microbial biomass was used to assess the ecological risk of metals in soil [40]. As a representative for soil microbial biomass, microbial biomass carbon (MBC) was determined by the fumigation-extraction method [41]. K_2SO_4-extracted C content was examined with the Shimadzu TOC-V CPH analyser (Shimadzu, Tokyo, Japan). The MBC was calculated as the difference in extractable C between fumigated and un-fumigated samples using a conversion factor of 0.37 [42].

2.7. Statistical analyses

One way analysis of variance (ANOVA), using Tukey test, was used to determine differences between each soil treatments. Correlation analysis was completed to determine the relationships between MBC and other examined parameters. All these analyses were conducted using SPSS (version 11.5).

3. Results

3.1. Effects of amendments on soil characteristics

TOC, WEOC and pH of each treatment are shown in Fig. 2. All amendment materials increased the TOC of soil. Compost had the smallest increase and biochar had the biggest increase. However, biochar had no significant effect on the WEOC and others caused obvious increase in that. The increase caused by B+C was the smallest increase and the increase caused by BCing was the biggest increase. The contrasting effects of amendments on the pH, compared to the TOC and WEOC, were B and B+C had no obvious change (S+B > S > S+B+C) on pH and others had a decrease.

Total heavy metals are shown in Fig. 3. And the total concentration of each metal of soil without amendment was almost the biggest one. Most of amendments had no significant effect on the total heavy metals. In stark contrast, these amendments had significant impact on the available heavy metals (Fig. 3). The available concentration of each element was far lower than its total concentration. Compost increased the concentration of available Cu and other amendments effectively reduced the concentration. All the amendments effectively decreased the concentration of available Zn, Cd and Pb. Bced and BCing had the highest efficiency in decreasing the concentration of available heavy metal. The efficiency of BCing was slightly above that of Bced. Among the different elements, available Zn had the biggest decrease and available Cd had the smallest decrease.

3.2. Effects of amendments on heavy metal concentration in pore water

All the amendments effectively decreased the concentration of heavy metals in pore water (Fig. 4). In 7th day, compost increased Cu concentration in pore water.

Other amendments reduced Cu concentration in pore water and BCing had the greatest magnitude of effect. The Cu concentration in pore water with addition of compost or BCing was decreased following time. The hierarchy in the effectiveness of amendments for decreasing Zn in pore water, comparing to the no amendment soil, was as follows: compost < biochar < B+C < Bced< BCing. The reduction in average of Cd concentration with addition of compost, biochar, B+C, Bced and BCing was 89.97%, 97.35%, 92.42%, 98.36% and 98.55%, respectively. Compost and biochar had similar efficiency in reducing Pb concentration in pore water, Bced and BCing had slightly higher efficiency.

3.3. Effects of amendments on soil microbial biomass

There was significant difference in soil microbial biomass between each treatment (Fig. 5). All the amendments had improved MBC of soil. MBC of soil with BCing had the biggest increase and that of soil with biochar had the smallest increase. The results of Pearson's correlation analysis are shown in Table 2. WEOC was strongly correlated ($P = 0.036$) with MBC. TOC ($P = 0.137$) and pH ($P = 0.153$) were not strongly correlated with MBC. There were significant negative correlations between MBC and available Zn ($P = 0.045$), Cd ($P = 0.021$) and Pb ($P = 0.048$), indistinctive negative correlation ($P = 0.348$) between MBC and available Cu. The order of correlation coefficients absolute value was: Available Cd > WEOC > Available Zn > Available Pb > TOC > pH > Available Cu.

4. Discussion

All the amendment materials, as organic amendment materials, improved TOC of the treated soil. Biochar increased more TOC because of its higher TOC, and compost increased less TOC because of its lower TOC. However, WEOC of soil with biochar increased less than that of others amendments, because that carbon pool of biochar is relatively stable and insoluble [6, 27]. Other studies [43, 44]also did not find an obvious change in concentration of WEOC caused by biochar. Effects of combined amendment (B+C, Bced and BCing) on soil TOC and WEOC were the results of combined impact of compost and biochar. Compost addition, whether alone or combined with biochar (B+C, Bced and BCing) resulted in reduction of soil pH. This is because of humic acids isolated from organic materials of compost. Other study [24] also found Bced reduced pH of neutral soil while biochar did not change pH. Total heavy metals of soil with all amendment materials almost were lower than that of soil without amendment materials. This could be attributed, in part to the dilution of the original contaminated substrate by amendment materials applied [6].

Comparison of available Cd, Cu, Zn and Pb, TOC and WEOC between each treatment showed that the organic amendment matetials can effectively reduce bioavailability of heavy metal (compost increase bioavailability of Cu) and improved TOC and WEOC. The reason for this was the phenomenon that amended soil with highly organic materials can generate large concentrations of WEOC to which free ions can complex with organic ligands [27, 45, 46]. Besides, heavy metals exchange with Ca^{2+}, Mg^{2+} and other cation associated [47, 48]. All these increase the concentrations of carbonate fraction, Fe and Mn fraction, organic matter bound fraction and residual fraction of heavy metal, and reduced available fraction. Bced and BCing have the greatest ability for improving WEOC of soil and also contain function of biochar. Therefore, Bced and BCing had the greatest efficiency for reducing bioavailability of heavy metal. Other study also found compost increased the concentration of available Cu and it because Cu was slightly mobilized by the humic acids[10].

Concentration of heavy metal in pore water suggested that all the amendment materials could stabilize heavy metal and reduced heavy metal mobility. Compost could stabilize heavy metal because heavy metals in compost amended soil was inextricably linked to organic carbon turnover [49]. Stabilization of heavy metals in soils with application of biochar could involve a number of possible mechanisms that could include (1) heavy metal exchange with Ca^{2+}, Mg^{2+} and other cations associated biochar, attributing to co-precipitation inner-sphere complexation with complexed humic matter and mineral oxides of biochar; (2) the surface complexation of heavy metals with different functional groups, and inner-sphere complexation with the free hydroxyl of mineral oxides and other surface precipitation; and (3) the physical adsorption and surface precipitation [47, 48]. According to this study, we could found that Bced and BCing had the greatest efficiency of reducing heavy metal mobility. This presumably is attributed to the phenomenon that composting process strongly increased biochar's CEC and O-content [50], which was related to sorption of heavy metal. The increase in CEC and O-content may be caused by biologically mediated oxidation of biochar surfaces [51-53] and/or strong sorption of organic matter during composting [50, 54].

Contaminants may affect the microbial processes in soil, thereby affect the nutrients cycling and the capacity to perform key ecological functions, such as mineralization of organic compounds and synthesis of organic matter [40]. Soil microbial biomass appears to be sensitive and responsive to changing environmental conditions [55, 56] and can be used as an indicator of ecological risk assessment of soil contamination [40]. In this study, we found that MBC was strongly negatively correlated with available Zn, Cd and Pb. The difference of MBC in this study means that BCing had the greatest efficiency for reducing ecological risk, and biochar had the weakest efficiency for that. Bced and BCing significantly changed soil microbial biomass and also affected soil microbial community structure, which played an important role in many ecological processes. How Bced and BCing affect soil microbial community structure needs further studies. Tripathy et al. found that MBC/OC was significantly and negatively correlated with water-soluble and exchangeable metals (Zn, Cu, Pb, Cr and Ni) and claimed that labile metal forms such as water-soluble and exchangeable fractions are the most important factors regulating microbial biomass in soil [57]. Besides, other studies found MBC was strongly correlated with TOC because organic matter was a substrate for microbial growth [55, 56, 58, 59]. However, in this study we found that MBC was strongly correlated with WEOC and was not strongly correlated with TOC. This is attributed to the fact that carbon pool of biochar was relatively insoluble and stable [6, 27], which could not be digested by soil microbes.

5. Conclusions

Adding compost (C), biochar (B), mixture of compost and biochar (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting) to contaminated soil changed soil physic-chemical properties, such as TOC, WEOC and pH. The changes of available Cd, Cu, Zn and Pb suggested that all the amendments reduced bioavailability of heavy metal (compost improved bioavailability of Cu), and Bced and BCing had the greatest capacity for that. The difference of Cd, Cu, Zn and Pb concentration in pore water between each treatment showed that all the amendments reduced mobility of heavy metal, and Bced and BCing had the greatest capacity for that. Comparison of MBC between each treatment declared amendments reduced ecological risk of heavy metal, and BCing had the greatest capacity for that. Influences of Bced and BCing on soil microbial community structure need further studies.

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Figure captions

Fig.1. The whole procedure of the proposed method.

Fig.2. TOC, water-extractable organic carbon (WEOC) and pH of soil with addition of nothing (S), compost (C), biochar (B), mixture of biochar and compost (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting). Error bars represent standard deviation (n = 3). Different letters indicate significant difference ($p<0.05$) between each treatment.

Fig.3. Concentration of total heavy metal and available heavy metal of soil with addition of nothing (S), compost (C), biochar (B), mixture of biochar and compost (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting). Error bars represent standard deviation (n = 3). Different letters indicate significant difference ($p<0.05$) between each treatment. Transverse lines were the local background values of total metals (According to the Environmental Quality Report (2011) of Hunan Province).

Fig.4. Heavy metal concentration in pore water from soil with addition of nothing (S), compost (C), biochar (B), mixture of biochar and compost (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting). Error bars represent standard deviation (n = 3). Different letters indicate significant difference ($p<0.05$) between each treatment in the same time.

Fig.5. Microbial biomass carbon (MBC) of soil with addition of nothing (S), compost (C), biochar (B), mixture of biochar and compost (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting). Error bars represent standard deviation (n = 3). Different letters indicate significant difference ($p<0.05$) between each treatment.

Table 1. Chemical properties (means±SD, n=3) of compost (C), biochar (B), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting).

Property	C	B	Bced	BCing
pH	6.72±0.02	9.98±0.01	7.13±0.04	7.04±0.01
TOC[a] (%)	30.25±1.02	55.97±2.41	57.16±3.63	51.80±0.73
WEOC[b] (g/kg)	28.77±3.56	1.84±0.23	6.91±0.20	31.27±1.34
CEC[c] ($cmol_c$/kg)	85.22±3.85	60.93±2.71	118.57±2.09	131.06±3.54
O-content (%)	15.36±0.23	9.64±0.19	12.59±0.17	13.98±0.19

[a] total organic carbon.

[b] water-extractable organic carbon.

[c] Cation exchange capacity.

Table 2. Pearson's correlation coefficients between MBC and other soil parameters.

Parameters	Correlations coefficient	P-value
pH	-0.661	0.153
TOC	0.681	0.137
WEOC	0.840	0.036
Available Cu	-0.469	0.348
Available Zn	-0.821	0.045
Available Cd	-0.879	0.021
Available Pb	-0.815	0.048

Fig.1

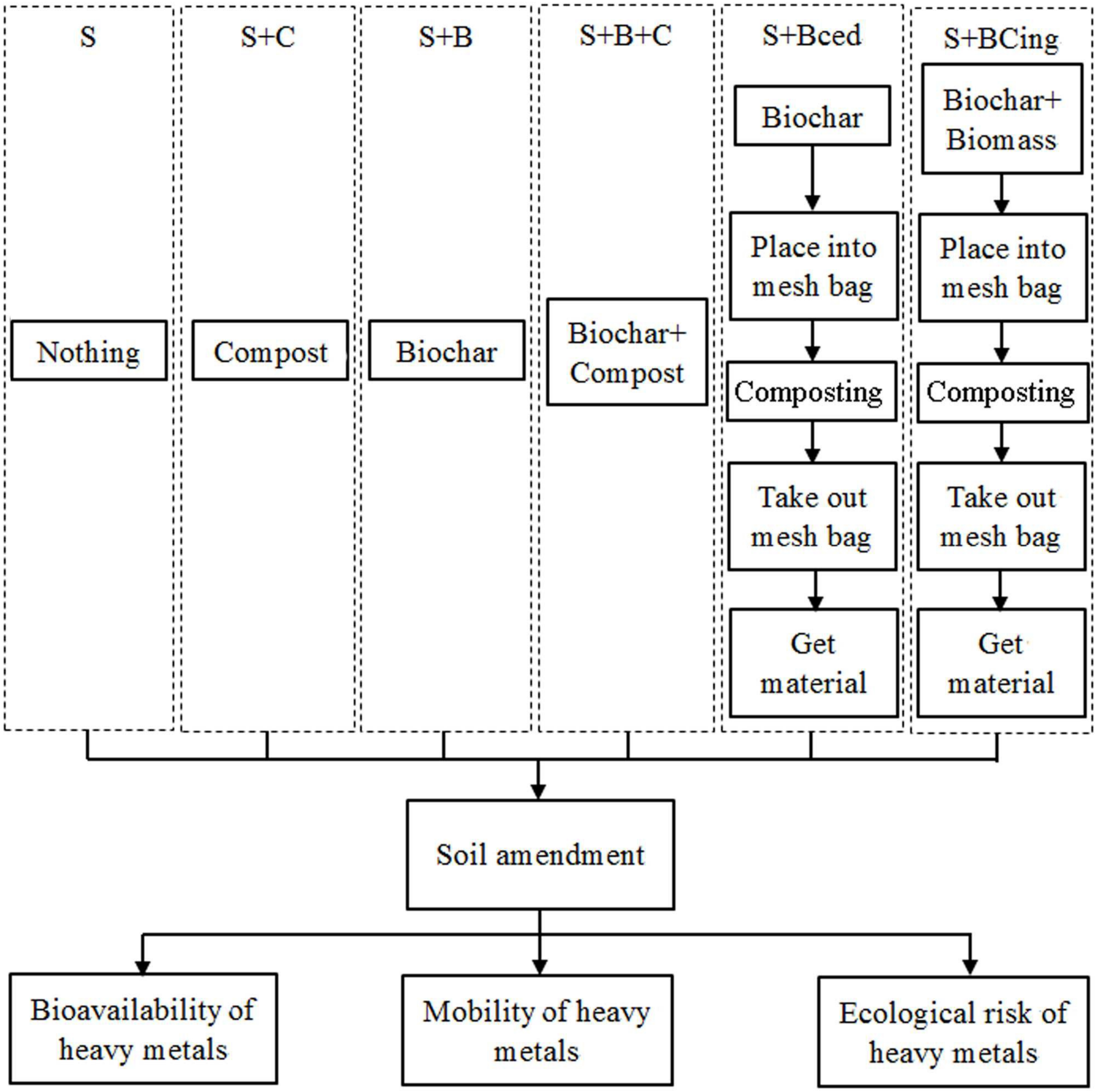
S
S+C
S+B
S+B+C
S+Bced
S+BCing
Nothing
Compost
Biochar
Biochar+ Compost
Biochar
Place into mesh bag
Composting
Take out mesh bag
Get material
Biochar+ Biomass
Place into mesh bag
Composting
Take out mesh bag
Get material
Soil amendment
Bioavailability of heavy metals
Mobility of heavy metals
Ecological risk of heavy metals

Fig.2

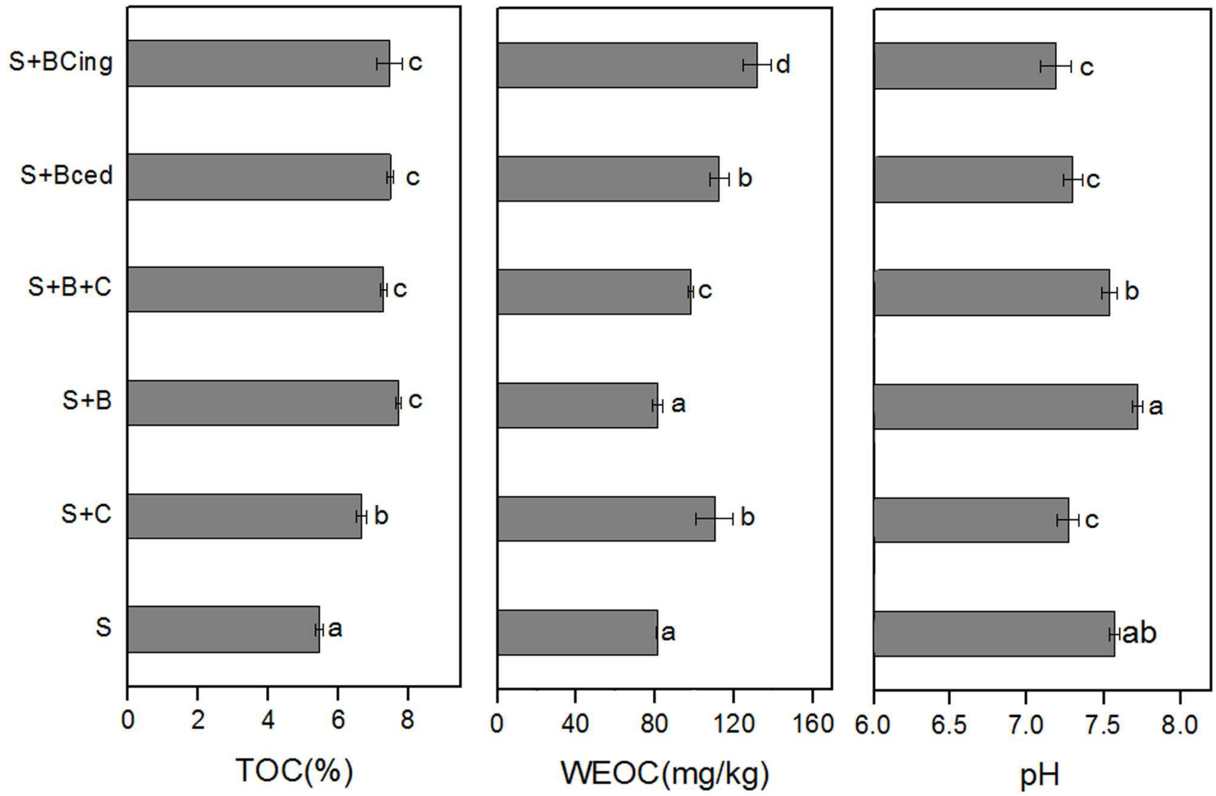
S+BCing
S+Bced
S+B+C
S+B
S+C
S
c
c
c
c
b
a
d
b
c
a
b
a
c
c
b
a
c
ab
0
2
4
6
8
TOC(%)
0
40
80
120
160
WEOC(mg/kg)
6.0
6.5
7.0
7.5
8.0
pH

Fig.3

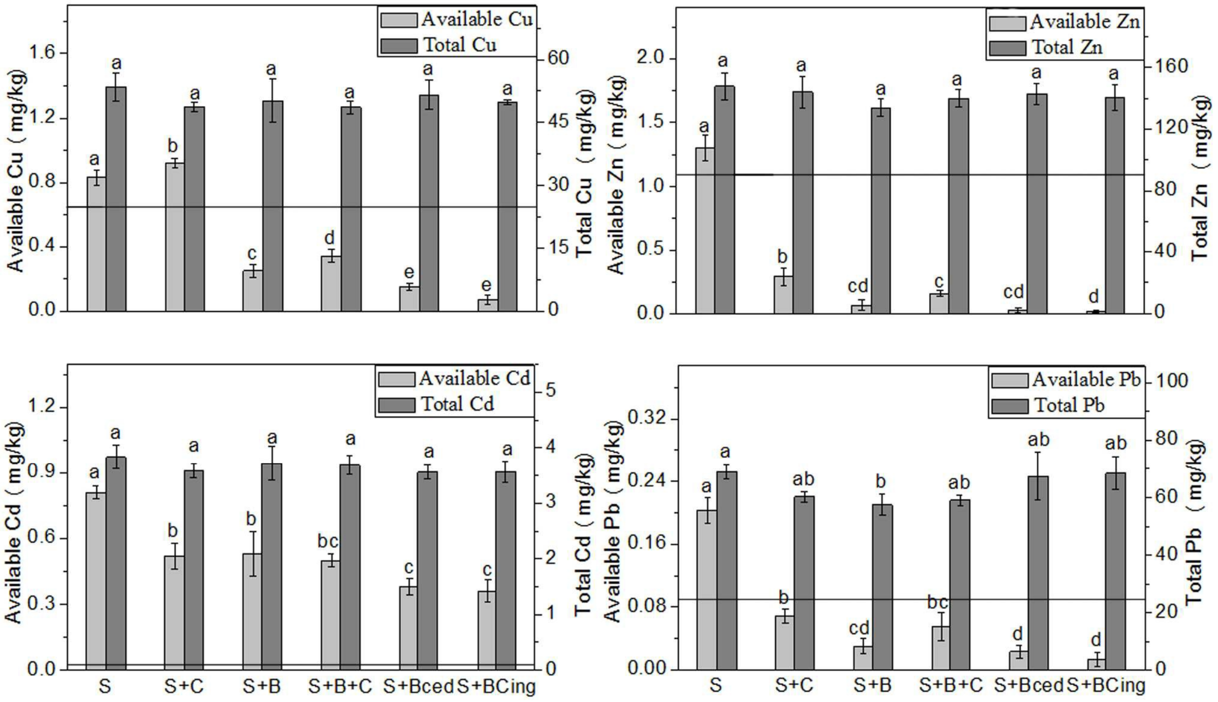
Available Cu
Total Cu
Available Cu（mg/kg）
Total Cu （mg/kg）
0.0
0.4
0.8
1.2
1.6
0
15
30
45
60
a
a
b
a
c
a
d
a
e
a
e
a
Available Zn
Total Zn
Available Zn（mg/kg）
Total Zn （mg/kg）
0.0
0.5
1.0
1.5
2.0
0
40
80
120
160
a
a
b
a
cd
a
c
a
cd
a
d
a
Available Cd
Total Cd
Available Cd（mg/kg）
Total Cd（mg/kg）
0.0
0.3
0.6
0.9
1.2
0
1
2
3
4
5
a
a
b
a
b
a
bc
a
c
a
c
a
Available Pb
Total Pb
Available Pb（mg/kg）
Total Pb （mg/kg）
0.00
0.08
0.16
0.24
0.32
0
20
40
60
80
100
a
a
b
ab
cd
b
bc
ab
d
ab
d
ab
S
S+C
S+B
S+B+C
S+Bced
S+BCing
S
S+C
S+B
S+B+C
S+Bced
S+BCing

Fig.4

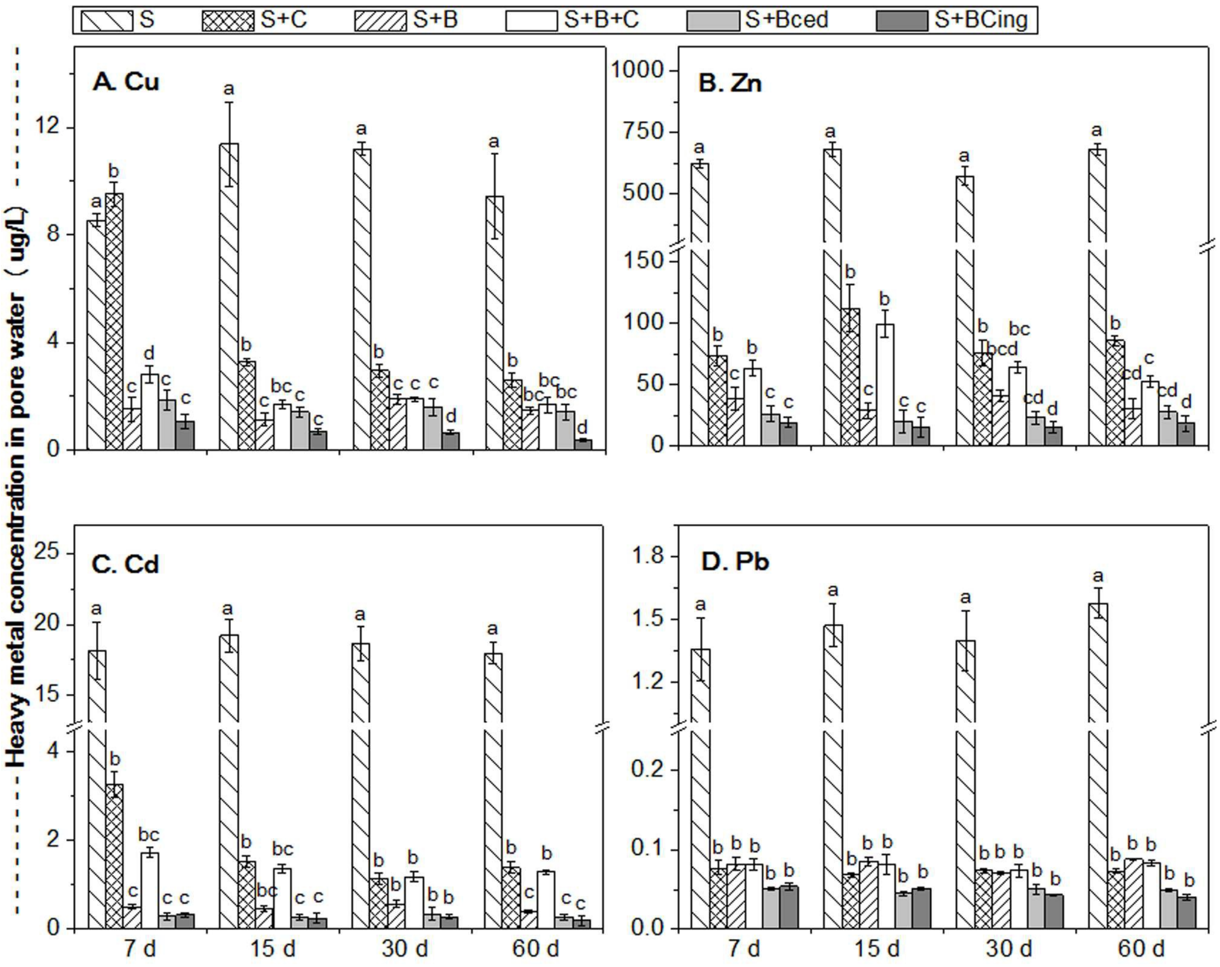
S
S+C
S+B
S+B+C
S+Bced
S+BCing
Heavy metal concentration in pore water （ug/L）
A. Cu
0
4
8
12
a b c d c c
a b c bc c c
a b c c c d
a bc bc bc bc d
B. Zn
0
50
100
150
500
750
1000
a b c b c c
a b c b c c
a b bcd bc cd d
a b cd c cd d
C. Cd
0
2
4
15
20
25
a b c bc c c
a b bc bc c c
a b b b b b
a b c b c c
D. Pb
0.0
0.1
0.2
1.2
1.5
1.8
a b b b b b
a b b b b b
a b b b b b
a b b b b b
7 d
15 d
30 d
60 d

Fig.5