



Influence of alkaline silicon-based amendment and incorporated with biochar on the growth and heavy metal translocation and accumulation of vetiver grass (*Vetiveria zizanioides*) grown in multi-metal-contaminated soils

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Received: 25 September 2018 / Accepted: 5 December 2018 / Published online: 14 December 2018
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Abstract

Purpose This study investigated the effects of alkaline silicon-based amendment (A) and incorporated with biochar (B) on the growth, heavy metal translocation, and accumulation of vetiver grass (*Vetiveria zizanioides*) grown in multi-metal-contaminated soils.

Materials and methods Soil incubation for 6 months and pot experiments for 6 months were performed to investigate the growth and the translocation factor (TF) and bioaccumulation factor (BCF) values of heavy metals in vetiver grown in As-, Cd-, Cr-, and Pb-contaminated soils amended with A (0.5%) and AB (0.5% A; 1.5% B).

Results and discussion The vetiver could grow in multi-metal-contaminated soils due to the restriction of metals translocation in vetiver. Vetiver could be the plant for phytostabilization of As, Cr, Cd, and Pb because As, Cr, Cd, and Pb accumulated by the vetiver were largely retained in the roots, as the TF values for As, Cr, Cd, and Pb were < 1; the BCF values for As, Cr, Cd, and Pb were obviously greater in roots than shoots. Application of A and AB markedly improved vetiver growth due to A- and AB-induced accumulation of Cd, Pb, and Cr in the roots, and then enhanced phytostabilization of Cd, Pb, and Cr in roots based on the BCF values, and the immobilization of Cd and Pb in soils based on increase of soil pH and Si-induced accumulation of Cd, Pb, and Cr in the roots.

Conclusions Vetiver represents a candidate for revegetation of multi-metal-contaminated soils amended by A or AB. Further investigations are required to determine the feasibility of revegetation in multi-metal-contaminated soils at the field scale.

Keywords Alkaline silicon-based amendment · Biochar · Multi-metal-contaminated soils · Revegetation · Vetiver grass

Responsible editor: Jianming Xue

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11368-018-2219-5>) contains supplementary material, which is available to authorized users.

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1 Introduction

Industrial activity is a major cause of farmland heavy metal contamination; the contaminated farmlands have reached 20 Mha in China over the past several decades (Wei and Yang 2010). The heavy-metal-contaminated sites are primarily caused by five key industries, including lead battery manufacturing, electronic component manufacturing, metal surface and heat treatment processing, non-ferrous metal smelting, and raw chemical material manufacturing (Song et al. 2014). Heavy metal contamination restricts plant growth and results in air and water pollution. Moreover, metal pollution can be distributed across large distances as a result of wind transfer, surface erosion, and leaching (Radziemska et al. 2017). Therefore, the remediation of heavy-metal-contaminated soil has become a major and urgent task for environmental protection in China.

The remediation of multi-metal-contaminated soils is difficult because the diverse properties of metals and metalloids, such as As, Cd, Pb, Cr, Cu and Zn, can be immobilized in the soil by sorption, precipitation, and complexation reactions, although these elements can also be removed from soil by plant uptake, leaching, and volatilization (Bolan et al. 2014). Phytoremediation can be used as a practical, economical, and eco-friendly alternative for revegetation of multi-metal-contaminated soils. However, phytoremediation also has challenges, such as several years needed for remediation; phytoextraction efficacy of most hyperaccumulators is restricted by the low biomass and slow growth (Mahar et al. 2016). Revegetation can assist in stabilizing heavy metals and minimizing the wind dispersion of heavy metals and the migration of heavy metals through soil via water (Radziemska et al. 2017). However, revegetation requires the plant to be tolerant to extreme environmental conditions, low soil fertility, and heavy metal toxicity (Zhang et al. 2016). Thus, the success of revegetation should overcome two major problems: the selection of appropriate plant species and soil amendment.

Vetiver grass (*Vetiveria zizanioides*) is a promising plant for revegetation of multi-metal contaminated soils. This plant is perennial and tall (at a maximum of 2 m) and displays rapid growth, high-biomass production, and a long root system (up to 4.6 m) (Chen et al. 2004). Furthermore, vetiver is tolerant to high levels of heavy metals, for example Cd, Cu, Pb, and Zn (Banerjee et al. 2016). Vetiver has been applied for the phytostabilization of metal pollution and to prevent contaminants from leaching into groundwater and running off into surface water bodies. Ghosh et al. (2015) used vetiver for phytoremediation of soils contaminated with fly ash. Meeinkuirt et al. (2013) planted vetiver for phytostabilization of Pb mine tailings. Mirza et al. (2017) used vetiver for phytoremediation of As contamination. Abaga et al. (2014) demonstrated the ability of vetiver for Cd phytoremediation in agriculture soils. However, the effects of vetiver on multi-metal-contaminated soils remain poorly investigated.

Soil amendments are necessary for the establishment of vegetation in metal-contaminated soils (Yang et al. 2003), especially amendments that can improve revegetation and productivity of plants by decreasing the solubility, leaching, and bioavailability of heavy metals (Mahar et al. 2016; Wen et al. 2016) by regulating soil pH and increasing nutrient availability. Soil amendments are divided into two categories: pH-change-inducing immobilizers and sorption agents (Yao et al. 2017). A readily available alkaline silicon slag containing abundant Ca, Fe, and Si appears to be a promising amendment for heavy metal immobilization of multi-metal-contaminated soils, due to the pH-adjusting and strong adsorption capacity. Si has been proven to be an essential mineral element for plant growth and beneficial for increase the resistance to metal toxicity (Adrees et al. 2015). Si-based amendment has the potential for use as an alternative method of

remediating contaminated soil. The application of Si-based amendment has been shown to decrease the phytoavailability of heavy metals and suppress metal uptake by rice planted on multi-metal-contaminated soil (Gu et al. 2011).

The effect of alkaline amendment on contaminated soils often diminished with the growth of plants because of the decreasing of pH. Furthermore, raising soil pH to even higher values may not practice in alkaline soils (pH > 8). Biochar acts as a sustainable remedial material as it boosts the intrinsic sorption capacity of the soil. Biochar has large surface area, highly porous structure, and various functional groups (e.g., carboxyl, hydroxyl, phenolic groups), which has a strong adsorption capacity for heavy metals. The type of metal ions present plays an important role in sorption, different mechanisms dominate the sorption of Cd and Pb (complexation, cation exchange, and precipitation), Cr (electrostatic interactions, reduction, and complexation), and As (complexation and electrostatic interactions) by biochar (Xu et al. 2016; Li et al. 2017). Additionally, the micropores of biochar also responsible for the sorption of dissolved organic matter (DOM) and promoted microbial activity (Zama et al. 2018). Individual application of biochar for phytoremediation and revegetation of contaminated soil was reported to have some disadvantages. The biochar decreased the availability of nutrients (such as P and N) due to stronger adsorption and then inhibited plant growth (Beesley and Marmiroli 2011). Melo et al. (2016) reported that binding reactions on biochar surfaces are reversible; the adsorbed heavy metals are easily desorbed. These disadvantages of biochar could be offset by combining amendment with biochar. Therefore, combined application of alkaline amendment and biochar could achieve long-term remediation.

The objectives of this study are to evaluate (1) the potential of vetiver for phytoremediation of multi-metal-contaminated soils and (2) the effects of alkaline silicon-based amendment and amendment incorporated with biochar on the growth and translocation and accumulation of heavy metals in vetiver. This study could provide a practical basis for field-scale remediation and thereby realize revegetation and sustainable remediation in multi-metal-contaminated soils.

2 Materials and methods

2.1 Materials

The contaminated soil samples were collected from farmland near the vicinity of a former battery factory of approximately 60,000 m² in Fuyang City, Anhui Province, China, in May 2017, which was originated from a lead smelting industry with approximately 150 years of history.

The top soil layer (0–20 cm) was sampled for the present soil incubation and for greenhouse pot experiments. The soil was

thoroughly mixed, air dried, and passed through a 2-mm sieve. The soil was sandy loam soil (40.4% sand, 41.5% silt, and 18.1% clay). The soil properties include pH, 7.9; organic matter, 18.5 g kg⁻¹; cation exchange capacity (CEC), 25.0 cmol kg⁻¹; total N, 1.2 g kg⁻¹; Olsen-P, 21.2 mg kg⁻¹; available K, 120.0 mg kg⁻¹; citric acid-extractable Si, 868.2 mg kg⁻¹; total SiO₂, 68.8%; and total As, Cd, Cr, Cu, Pb, and Zn, 104.3, 2.65, 148.3, 28.54, 76.06, and 91.19 mg kg⁻¹, respectively. The soil Cd and As exceeded class III, and soil Cr and Pb exceeded class I of the Environmental Quality Standards for Soils of China (GB15618-1995).

The alkaline silicon-based amendment (A) was derived from CaO-activated acid-leached-Cu tailings. The amendment was grounded to a fine powder and sieved at < 0.18 mm. The amendment properties included pH, 12.25 (1: 5 H₂O); citric acid-extractable Si, 611 mg kg⁻¹; CaO, 25.83%; Fe₂O₃, 19.81%; specific surface area, 1.08 m² g⁻¹; total pore volume, 0.008 cc g⁻¹; average pore size, 309 nm. Cd was not detected in the amendment. The total As, Cr, Cu, Pb, and Zn contents were 41.56, 59.65, 564.8, 30.09, and 228.0 mg kg⁻¹, respectively. The contents of As, Cr, Cd, and Pb were less than the standard for fertilizers in China (GB/T 23349–2009). Trace amount of As (0.01–0.05 mg L⁻¹), Cr (0.01 mg L⁻¹), and Cu (0.01 mg L⁻¹) was released from the amendment, but no Cd and Pb were released from the amendment based on water leaching and soil suspension experiments for 9 weeks (Table S1, Table S2 in the Electronic Supplementary Material - ESM).

The biochar (B) prepared from maize straw through pyrolysis at a temperature of approximately 500–600 °C for 2 h. The biochar was grounded to a fine powder and sieved at < 0.18 mm. The biochar contained total C, 60.49%; total P, 335.9 mg kg⁻¹; pH, 9.23 (1:1.25 H₂O); specific surface area, 346.89 m² g⁻¹; total pore volume, 0.273 cc g⁻¹; average pore size, 3.15 nm. As was not detected in biochar, the total Cd, Cr, Cu, Pb, and Zn contents were 1.72, 48.53, 48.01, 152.8, and 347.25 mg kg⁻¹, respectively. The contents of As, Cr, Cd, and Pb were less than the standard for fertilizers in China (GB/T 23349-2009).

2.2 Experimental design and sampling

The experiment included three treatments with four replicates as below, without amendment (CK), alkaline silicon-based amendment (A, 0.5% w/w), and alkaline silicon-based amendment (0.5% w/w) combined with biochar (1.5% w/w) (AB). Total of 1.5 kg soil was packed into each pot (14 cm diameter and 16.5 cm height). The amendment and biochar were thoroughly mixed with soil, respectively. The soil was also supplied with 0.2 g N kg⁻¹ soil as NH₄NO₃ (AR), 0.15 g P kg⁻¹ soil as Ca(H₂PO₄)₂ (AR), and 0.15 g K kg⁻¹ soil as K₂SO₄ (AR) as the base fertilizer. The fertilizer was added to the soil in solution. Nutrient solutions were mixed thoroughly with the

soil amended by A or AB before incubation. Prior to incubation, the pots were remoisten up 70% of soil water-holding capacity via the addition of deionized water. All pots were arranged randomly and incubated for 12 months under natural sunlight conditions (temperature 20–30 °C and relative humidity 30–50%). The soil moisture was controlled at 70% of soil water-holding capacity via the addition of deionized water based on the weight of the pots every 2 days and covered with plastic cover, which had a small hole for gas exchange and minimizing moisture loss. Total 50 g soil was sampled from each pot for determining citric acid-extractable Si, and DTPA-extractable Cu, As, Cd, Cr, and Pb in soils 6 months after incubation. Subsequently, three seedlings of vetiver (shoots 20 cm and roots 5 cm in length) were transplanted into each pot and planted for 6 months. The soil moisture was controlled at 70% of soil water-holding capacity via the addition of deionized water based on the weight of the pots every 2 days. Three months after transplanting of vetiver, soil pore water samples were extracted from each pot using Rhizon soil moisture samples (SMS, model MOM, Rhizosphere Research Products, Netherlands) with a nominal porosity of 0.15 μm; plant height was recorded, the shoots of vetiver were cut above 20 cm of soil surface, washed with deionized water, dried and then weighed. Total of 50 g soil sample was collected from each pot for analysis. Six months after transplanting of vetiver, soil pore water samples were extracted from each pot using Rhizon soil moisture samples; plant height was recorded. And when the vetiver was harvested, the vetiver was divided in two parts (shoots and roots), and then rinsed with deionized water. The plant samples were heated at 105 °C for 30 min, oven dried (48 h at 70 °C) and weighed until constant weight, and then the dry weight (DW) of the shoots and roots was recorded, respectively. The dried plant samples were grounded and sieved with a 0.5 mm sieve for further analysis. The part of soil sample in each pot was air dried, grounded until thoroughly homogenized, and sieved with 2 mm and 0.15 mm sieves for further analysis.

2.3 Chemical analysis

The alkaline silicon-based amendment (A) and biochar (B) were digested with HNO₃-HF according to method 3052 (USEPA 1996) in a microwave digester (Mars6, CEM Corporation, USA). Blanks and certified references of mine samples (GBW (E) 070074, National Institute of Metrology, China) were included to ensure reproducibility and accuracy. The recovery of metals was in the range of 95–110%. The concentrations of P and heavy metals in the digestion were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, ELAN DRC-e, USA). The pH was measured using an HI98185A type pH meter (Hanna, Italy). Available Si was extracted using 0.25 M citric acid and analyzed by silicon molybdenum blue spectrophotometry (Lu

1999). Total C in biochar was measured by an isotopic mass spectrometer (Thermo Finnigan, MAT253, USA). Nondestructive chemical analyses of the amendment were performed via an X-ray fluorescence spectrometer (XRF-1800, Shimadzu, Japan), and the samples were prepared using the squash technique with boric acid. The surface area, total pore volume, and average pore size of samples were measured with a Quadrasorb SI-MP surface area analyzer (Quantachrome, USA). The morphology of amendment and biochar were measured by a Hitachi S-4300 scanning electron microscope (SEM). The X-ray diffraction pattern (XRD, D8 Focus, Bruker, USA) of amendment and soil were performed in a continuous scan mode between 10° and 70° 2θ with a Cu- $K\alpha$ radiation source. Chemical information about functional groups in amendment and biochar was obtained using Fourier-transform infrared spectroscopy (FTIR). FTIR spectra was recorded between 4000 and 400 cm^{-1} using an Excalibur 3100 (Varian, USA) spectrometer for all the samples. The discs were prepared by first mixing 1 mg of dried sample with 500 mg of KBr in an agate mortar and then pressing the resulting mixture at 10 tons cm^{-2} for 15 min under vacuum.

Plant samples were microwave digested with $\text{HNO}_3\text{-H}_2\text{O}_2$ in a microwave digester according to the method 3050B (USEPA 1996). The concentrations of As, Cd, and Pb in the digestion were determined by ICP-MS. The concentrations of P and Cr were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 5300DV, USA). The concentration of Si in plants was determined by ICP-OES after microwave digestion with a mixture of HNO_3 , HCl and HF (Novozamsky et al. 2008). Blank and standard reference materials of plants (GBW-07605, tea leaves, China National Centre for Standard Materials) for analytical procedure were used for quality control. The recovery rates for P, Si, and heavy metals were within $90 \pm 10\%$.

After collection of the soil pore water samples, the pH, dissoluble organic carbon (DOC), P, Si, and heavy metal were immediately determined. The pH was measured using an HI98185A type pH meter (Hanna, Italy). The DOC was measured using a Multi N/C 3100 (Analytik Jena, Germany). The P and Si in solution were determined by ICP-OES. The heavy metal in solution was determined by ICP-MS.

The soil samples were analyzed according to the recommended analytical methods of the soil and agricultural chemistry (Lu 1999). Soil pH (soil:water, 1:2.5) was measured using an HI98185A type pH meter (Hanna, Italy). The soil organic matter was measured using the oil bath heating method ($\text{K}_2\text{CrO}_4\text{-H}_2\text{SO}_4$). The CEC was determined using the 1.0 M $\text{CH}_3\text{COONH}_4$ leaching method at a pH of 7.0. The sand, silt, and clay content was measured using the standard pipette method. The total N was determined by micro-Kjeldahl digestion followed by steam distillation. The available P was extracted using 0.5 M NaHCO_3 and determined by

the molybdenum blue method. The available K was extracted using 1.0 M $\text{CH}_3\text{COONH}_4$ and determined by ICP-OES. The available Si was extracted using 0.25 M citric acid and analyzed by silicon molybdenum blue spectrophotometry. Total SiO_2 in soil was determined by XRF analysis. The available concentrations of Cu, As, Cd, Cr, and Pb in soils were extracted by DTPA and analyzed by ICP-MS. The total concentrations of As, Cd, Cr, and Pb in soils were measured by ICP-MS after digestion in a microwave system with $\text{HNO}_3\text{-HF}$ according to the USEPA method 3052 (USEPA 1996). Standard reference materials of soil (GBW-07405, China National Centre for Standard Materials) in the analytical procedure were used for quality control. The recovery rates for heavy metals were within $90 \pm 10\%$.

2.4 Data analysis

The phytoremediation ability of vetiver was assessed by the translocation factor (TF) and bioaccumulation factor (BCF) (Galal and Shehata 2015). The TF is defined as the ratio of the metal concentration in the shoots to that in the roots (Christou et al. 2017). The BCF is defined as the ratio of heavy metal concentration in the shoot or root of a plant to that in the soil (Mohamed et al. 2015).

All data were expressed as the observed mean, followed by its standard error (\pm SE). Statistical analyses were performed using SPSS Version 19.0 software (SPSS Inc., Chicago, USA). Means were compared using the least significant difference (LSD) determined by a one-way analysis of variance. Differences were considered statistically significant at $p < 0.05$. Pearson correlations were also performed using SPSS, and values were considered significant at $p < 0.05$ and $p < 0.01$. Graphical analysis was carried out using OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA).

3 Results

3.1 Effect of amendment and incorporated with biochar on the availability of heavy metals, and citric acid-extractable Si in the multi-metal-contaminated soils under incubation for 6 months

The Cu was the heavy metal with the highest concentration in amendment. The application of amendments enhanced DTPA-extractable Cu from 3.64 to 4.21 mg kg^{-1} (Table 1), implying that application of amendments increased available Cu in soils. However, application of silicon-based amendment (0.5% w/w, 564.8 mg Cu kg^{-1}) estimated to increase soil Cu from 28.54 mg kg^{-1} (tested soil background) to 31.36 mg kg^{-1} , which was lower than the Class I (35 mg kg^{-1}) of

Table 1 Influence of amendment and incorporated with biochar on concentrations (mg kg^{-1}) of citric acid-extractable Si and DTPA-extractable As, Cd, Cr, and Pb in the multi-metal-contaminated soils under incubation for 6 months

		Si	Cu	As	Cr	Cd	Pb
Before incubation		868.2	3.82	0.19	0.06	0.60	34.37
After incubation	CK	991.73 \pm 19.56 b	3.64 \pm 0.09 b	0.26 \pm 0.01 c	0.07 \pm 0.02 a	0.61 \pm 0.02 a	39.26 \pm 1.29 a
	Amendment	1040.53 \pm 18.86 a	4.21 \pm 0.18 a	0.30 \pm 0.01 a	0.07 \pm 0.01 a	0.58 \pm 0.02 b	35.09 \pm 1.88 b
	Amendment+Biochar	999.74 \pm 10.47 b	3.85 \pm 0.17 b	0.28 \pm 0.01 b	0.07 \pm 0.01 a	0.55 \pm 0.01 c	34.34 \pm 0.67 b

Means \pm standard error followed by different letters within columns are significantly different ($p < 0.05$)

Environmental Quality Standards for Soils of China (GB15618-1995).

Application of amendment (A) in the multi-metal-contaminated soils significantly decreased DTPA-extractable Cd and Pb in soils, combined application of amendment (A) and biochar (B) was more effective than individual application of A (Table 1). On the contrast, application of amendment in the multi-metal-contaminated soils significantly increased the DTPA-extractable As in soils, individual application of A was more effective than combined application of A and B. In addition, application of amendment in the multi-metal-contaminated soils significantly increased the citric acid-extractable Si in soils, individual application of A was more effective than combined application of A and B (Table 1).

3.2 Effect of amendment and incorporated with biochar on the properties of pore water of the multi-metal-contaminated soils grown vetiver

Application of amendment in the multi-metal-contaminated soils resulted in an increase in pH by 0.30–0.32 units in the pore water collected in the 3 months after planting vetiver; with no significant differences were observed between individual application of A and combined application of A and B. No obvious differences in pH of the pore water collected in the 6 months after planting vetiver were observed among treatments (Table 2).

Application of amendment in the multi-metal-contaminated soils increased concentration of P in the pore water collected 3 months after planting vetiver, with individual application of A was more effective than combined application of A and B (Table 2). No obvious differences in concentration of P in the pore water collected 6 months after planting vetiver were observed among treatments (Table 2).

Application of amendment in the multi-metal-contaminated soils significantly decreased concentration of Si in the pore water collected 3 months and concentration of Cu in the pore water collected 6 months after planting vetiver. However, no obvious differences in concentration of Si and DOC in the pore water collected 6 months after planting vetiver were observed among treatments (Table 2).

3.3 Effect of amendment and incorporated with biochar on the concentrations of heavy metals in pore water of the multi-metal-contaminated soils grown vetiver

Application of amendment in the multi-metal-contaminated soils decreased concentration of Cd, As, and Pb in the pore water collected 6 months after planting vetiver. Combined application of A and B was more effective than individual application of A, except for Pb (Table 3). Similar effects were observed in the pore water collected 3 months after planting vetiver; however, the effect was more pronounced in the pore water collected 6 months after planting vetiver than collected 3 months after planting vetiver (Table 3). Application of amendment in the multi-metal-contaminated soils always increased concentration of Cr in the pore water collected 3 months and 6 months after planting vetiver (Table 3).

3.4 Effect of amendment and incorporated with biochar on the pH, DTPA-extractable heavy metals, and citric acid-extractable Si in the multi-metal-contaminated soils grown vetiver

Application of amendment in the multi-metal-contaminated soils decreased DTPA-extractable Cd in soils collected 3 months and 6 months after planting vetiver, respectively; no obvious differences were observed between individual application of A and combined application of A and B (Table 4). Individual application of A tended to increase DTPA-extractable As in soils collected at 3 months and 6 months after planting vetiver, especially for the 3 months, but combined application of A and B tended to decreased DTPA-extractable As in soils (Table 4). These effects were not observed for Cr and Pb (Table 4).

Application of amendment decreased the citric acid-extractable Si in soils collected 3 months after planting vetiver, although it increased the citric acid-extractable Si in soils collected at 6 months after planting vetiver (Table 4). Individual application of A in the multi-metal-contaminated soils significantly enhanced soil pH and DTPA-extractable Cu in soils (Table 4).

Table 2 Influence of amendment and incorporated with biochar on the pH and concentrations (mg L^{-1}) of DOC, P, and Si in the pore water of multi-metal-contaminated soils planted with vetiver for 3 months and 6 months

Treatments	pH	DOC	P	Si	Cu
3 months					
CK	7.86 ± 0.14 b	86.28 ± 18.64 a	0.45 ± 0.09 c	15.34 ± 3.54 a	6.56 ± 1.31 a
Amendment	8.16 ± 0.11 a	72.60 ± 12.94 a	1.29 ± 0.2 a	9.39 ± 1.98 b	5.34 ± 2.87 a
Amendment+ biochar	8.18 ± 0.04 a	64.48 ± 11.37 a	1.03 ± 0.02 b	11.28 ± 3.14 ab	4.75 ± 0.96 a
6 months					
CK	7.90 ± 0.41 a	68.10 ± 10.17 a	1.88 ± 0.47 a	2.35 ± 1.15 a	3.67 ± 1.12 a
Amendment	7.92 ± 0.16 a	78.33 ± 28.86 a	1.97 ± 0.21 a	3.11 ± 2.03 a	2.31 ± 0.94 ab
Amendment+ biochar	7.81 ± 0.30 a	63.83 ± 6.46 a	1.80 ± 0.27 a	3.64 ± 1.16 a	1.94 ± 0.59 b

Means ± standard error followed by different letters within columns are significantly different ($p < 0.05$)

3.5 Effect of amendment and incorporated with biochar on the height and biomass of vetiver grown in the multi-metal-contaminated soils

Application of amendment tended to increase biomass of vetiver, especially the first 3 months of vetiver growth, but no obvious differences were observed between individual application of A and combined application of A and B (Table 5). Vetiver looked taller grown in soils amended by A after planting for 6 months, by AB for 3 months, and which appeared a little short, but strong grown in soils amended by AB during 3–6 months (Table 5).

3.6 Effect of amendment and incorporated with biochar on the concentrations of heavy metals, P, and Si in vetiver grown in the multi-metal-contaminated soils

Combined application of A and B significantly decreased concentrations of Cd and Pb in shoots of vetiver harvested 3 months of planting, but which significantly increased concentration of As in shoots of vetiver harvested 3 months of planting (Fig. 1). Combined application of A and B significantly increased concentration of Pb in shoots of vetiver harvested 6 months of planting (Fig. 1). Application of

amendment tended to decrease concentrations of Cd, Pb, and Cr in roots of vetiver 6 months after planting, especially with combined application of A and B (Fig. 1). Individual application of A and combined application of A and B decreased the concentrations of Si and P in shoots of vetiver harvested 6 months of planting (Table 6). Individual application of A and combined application of A and B had no influence on concentrations of Cu in shoots and roots of vetiver (Table 6).

3.7 Effect of amendment and incorporated with biochar on the translocation and bioaccumulation of heavy metals in vetiver grown in the multi-metal-contaminated soils

Application of amendment tended to increase TF values for Pb, Cd, and Cr in vetiver harvested 6 months after planting, especially for Pb in vetiver grown in soils with combined application of A and B (Fig. 2). Application of amendment significantly decreased BCF-root values for Cr, Cd, and Pb; no effects were observed for BCF-shoot values for Cr, Cd, and Pb among treatments (Fig. 2). Application of amendment had no influence on TF, BCF-shoot, and BCF-root values for As (Fig. 2).

Table 3 Influence of amendment and incorporated with biochar on the concentrations (mg L^{-1}) of As, Cr, Cd, and Pb in the pore water of multi-metal-contaminated soils planted with vetiver for 3 months and 6 months

Treatments	As	Cr	Cd	Pb
3 months				
CK	4.98 ± 1.85 a	0.75 ± 0.13 b	0.18 ± 0.08 a	0.16 ± 0.06 a
Amendment	4.66 ± 0.94 a	1.95 ± 0.84 a	0.16 ± 0.08 a	0.08 ± 0.02 b
Amendment+ biochar	4.46 ± 1.78 a	2.09 ± 0.88 a	0.08 ± 0.02 b	0.09 ± 0.02 b
6 months				
CK	5.04 ± 1.43 a	1.60 ± 0.8 b	0.72 ± 0.23 a	0.09 ± 0.04 a
Amendment	3.95 ± 1.65 ab	3.47 ± 0.39 a	0.45 ± 0.17 ab	0.08 ± 0.03 a
Amendment+ Biochar	2.22 ± 0.66 b	3.59 ± 0.75 a	0.27 ± 0.08 b	0.08 ± 0.04 a

Means ± standard error followed by different letters within columns are significantly different ($p < 0.05$)

Table 4 Influence of amendment and incorporated with biochar on the pH, concentrations (mg kg⁻¹) of citric acid-extractable Si and DTPA-extractable As, Cd, Cr, and Pb in the multi-metal-contaminated soils planted with vetiver for 3 months and 6 months

Treatments	pH	Si	Cu	As	Cr	Cd	Pb
3 months							
CK	7.54 ± 0.05 b	833.62 ± 26.1 a	4.33 ± 0.55 b	0.32 ± 0.04 ab	0.07 ± 0.01 a	0.57 ± 0.08 a	42.04 ± 3.49 a
Amendment	7.65 ± 0.07 a	796.33 ± 36.55 a	5.51 ± 0.9 a	0.38 ± 0.09 a	0.07 ± 0.00 a	0.40 ± 0.07 b	42.87 ± 3.60 a
Amendment+ biochar	7.49 ± 0.07 b	748.65 ± 11.29 b	4.84 ± 0.38 ab	0.26 ± 0.02 b	0.07 ± 0.01 a	0.43 ± 0.09 b	37.84 ± 4.95 a
6 months							
CK	7.25 ± 0.06 ab	732.98 ± 28.45 b	4.31 ± 0.43 b	0.42 ± 0.05 a	0.09 ± 0.01 a	0.65 ± 0.04 a	42.93 ± 2.33 a
Amendment	7.32 ± 0.06 a	817.63 ± 75.9 a	5.91 ± 1.08 a	0.54 ± 0.21 a	0.08 ± 0.01 a	0.55 ± 0.07 b	40.24 ± 4.18 a
Amendment+ biochar	7.19 ± 0.03 b	807.86 ± 26.15 ab	6.11 ± 0.78 a	0.52 ± 0.12 a	0.08 ± 0.01 a	0.56 ± 0.01 b	41.29 ± 3.73 a

Means ± standard error followed by different letters within columns are significantly different (*p* < 0.05)

4 Discussion

4.1 Influence of amendment on the availability of heavy metals in the multi-metal-contaminated soils

The amendment-induced decrease of the availability of Cd in the multi-metal-contaminated soils irrespective of individual application of A and combined application of A and B because individual application of A and combined application of A and B were observed to effectively reduce DTPA-extractable Cd in soils under incubation for 6 months (Table 1), and in soils planted vetiver for 6 months (Table 4). Three principal mechanisms could explain the results above. Firstly, the precipitation of Cd in soils and in pore water due to the amendment/biochar-induced increase of pH in soil and pore water (Tables 2 and 4). Lim et al. (2013) observed that application of a lime-based amendment increased soil pH and induced immobilization of Cd in contaminated soils. Secondly, the co-precipitation of Cd with silicic acid, which originated from the amendment, and from activated Si in soils due to amendment-induced pH increase. Gu et al. (2011) reported that application of silicon-rich amendments resulted in a decrease of the available Cd in soils. Thirdly, the absorption of amendment and biochar on Cd in soil and

pore water, because the present amendment contained 19.8% Fe₂O₃. The Fe-rich amendments were used to act as stabilizers of heavy metals (Lee et al. 2014). The results indicated that combined application of A and B was more effective than individual application of A on decreasing availability of Cd in soils. Because DTPA-extractable Cd in soils was lower in combined application of A and B than in individual application of A (Table 1), and concentrations of Cd in pore water of soils after planting vetiver were lower in combined application of A and B than in individual application of A (Table 3). This result could be ascribed to biochar-induced immobilization of Cd, because the oxygen-containing functional groups (carboxyl, carbonyl and ester) were determined in the biochar (Fig. S5, ESM). Biochar was reported to immobilize Cd by complexation of oxygen-containing functional groups, and to absorb Cd on the surfaces of biochar via ion exchange of cation (Li et al. 2017). Application of biochar induced increase of Si in the pore water due to biochar-induced increase of pH in pore water (Zheng et al. 2012).

The amendment-induced decrease of the availability of Pb in the multi-metal-contaminated soils irrespective of individual application of A and combined application of A and B because individual application of A and combined application of A and B were observed to effectively reduce DTPA-extractable Pb in soils under incubation for 6 months (Table 1), and to

Table 5 Influence of amendment and incorporated with biochar on plant height (cm) and biomass (g dry weight pot⁻¹) of vetiver grown in multi-metal-contaminated soils for 3 months and 6 months

Treatments	Plant height	Shoot dry weight	Root dry weight
3 months			
CK	122.00 ± 6.42 a	7.20 ± 2.05 b	–
Amendment	129.38 ± 10.86 a	11.63 ± 2.38 a	–
Amendment+ biochar	133.75 ± 8.33 a	10.98 ± 2.24 a	–
6 months			
CK	76.93 ± 9.98 ab	11.70 ± 2.33 a	7.82 ± 2.04 a
Amendment	84.25 ± 4.68 a	13.97 ± 4.27 a	8.86 ± 2.67 a
Amendment+ biochar	72.68 ± 4.22 b	13.97 ± 1.33 a	9.34 ± 1.25 a

Means ± standard error followed by different letters within columns are significantly different (*p* < 0.05)

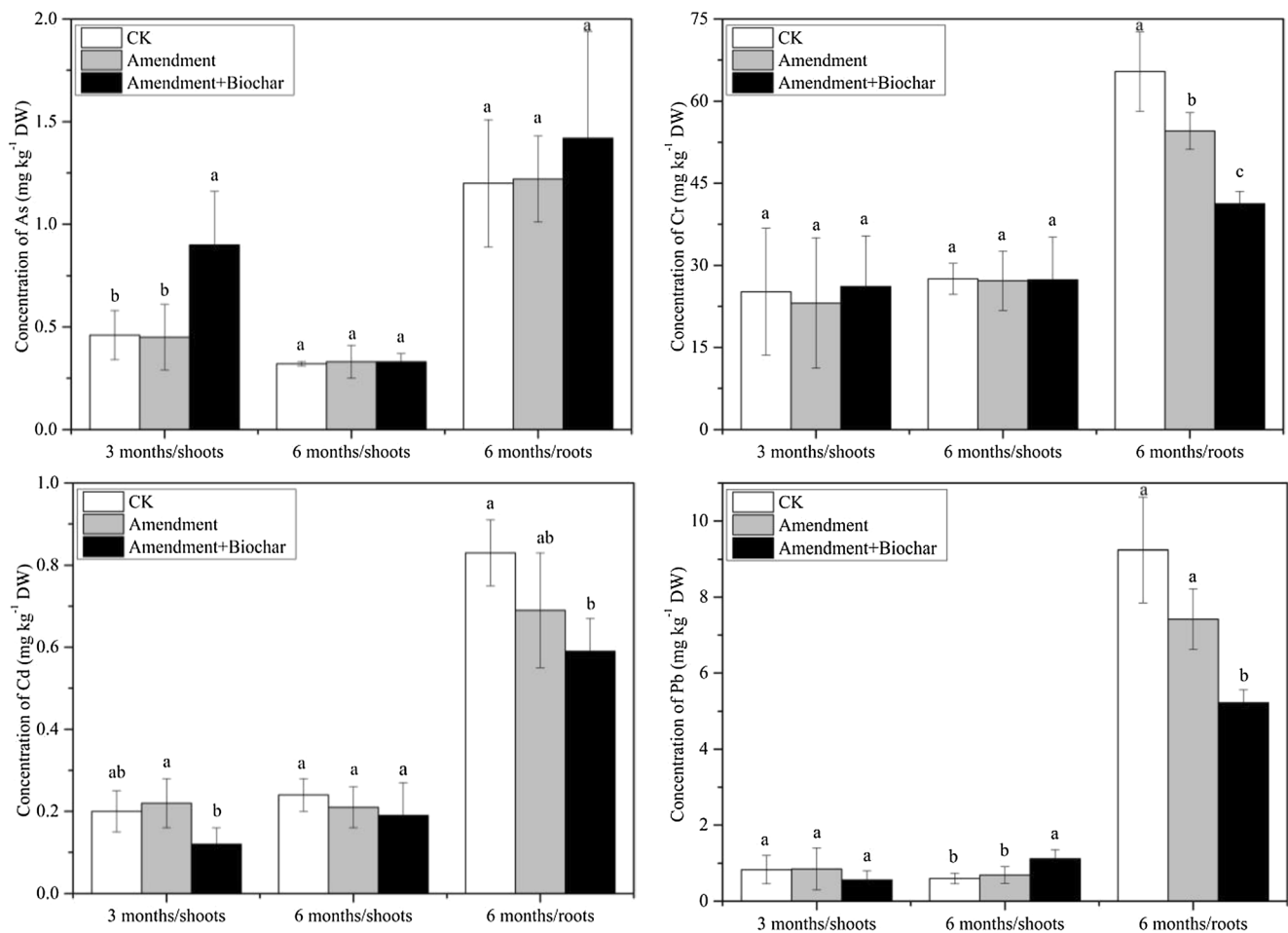


Fig. 1 Influence of amendment and incorporated with biochar on the concentrations (mg kg^{-1} DW) of As, Cr, Cd, and Pb in the shoots and roots of vetiver grown in multi-metal-contaminated soils for 3 months and

6 months. Error bars represent \pm sd ($n=4$). Different letter in the same group indicates a significant difference at 5% level according to LSD test

significantly decrease concentration of Pb in pore water after planting vetiver (Table 3). The primary mechanism could be pH-induced precipitation, Si-induced co-precipitation, and absorption of Pb due to amendment as mentioned above (Chang et al. 2013; Lim et al. 2013). Furthermore, PbPO_4 precipitation

could also contribute the decrease of available Pb, because a negative correlation was observed between the pore water Pb concentration and P concentration ($r = -0.725$, $p < 0.05$). PbPO_4 was not detected on the surface of amended soils due to the detection limit of XRD (Fig. S6, ESM). Phosphate in

Table 6 Influence of amendment and incorporated with biochar on the concentrations (mg kg^{-1} DW) of Si and P in the shoots and roots of vetiver grown in multi-metal-contaminated soils for 3 months and 6 months

Treatments		Si	P	Cu
3 months				
Shoot	CK	1657.32 \pm 676.5 a	991.53 \pm 176.36 a	2.75 \pm 0.81 a
	Amendment	1839.94 \pm 99.61 a	986.54 \pm 165.49 a	3.11 \pm 0.81 a
	Amendment+ Biochar	1632.37 \pm 310.41 a	1004.0 \pm 278.4 a	2.21 \pm 0.69 a
6 months				
Shoot	CK	1739.48 \pm 111.75 a	1126.24 \pm 142.2 a	2.26 \pm 0.37 a
	Amendment	1221.06 \pm 151.94 b	1021.78 \pm 155.21 ab	1.77 \pm 0.43 a
	Amendment+ Biochar	1313.50 \pm 142.38 b	887.23 \pm 78.4 b	2.42 \pm 0.71 a
Root	CK	1285.48 \pm 152.2 a	1081.75 \pm 105.94 a	13.53 \pm 4.18 a
	Amendment	1342.44 \pm 147.54 a	1119.03 \pm 125.57 a	13.32 \pm 4.01 a
	Amendment+ Biochar	1438.48 \pm 194.64 a	974.88 \pm 86.8 a	11.45 \pm 3.92 a

Means \pm standard error followed by different letters within columns are significantly different ($p < 0.05$)

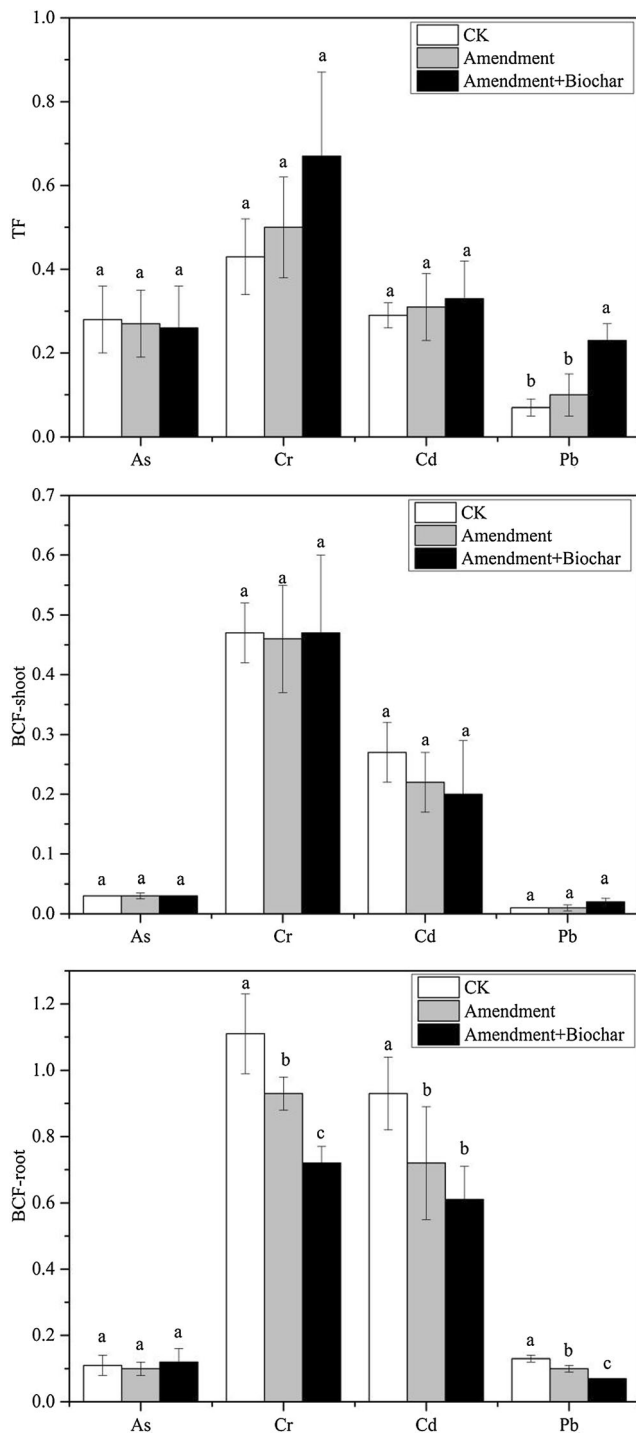


Fig. 2 Influence of amendment and incorporated with biochar on the translocation and bioaccumulation of heavy metals in vetiver grass grown in multi-metal-contaminated soils for 6 months. TF (translocation factor): the ratio of metal concentration in the shoots to that in the roots. BCF-shoot/root: the ratio of the metal concentration in the plant shoot/root to the metal concentration in the soil at harvest. Error bars represent \pm sd ($n=4$). Different letter in the same group indicates a significant difference at 5% level according to LSD test

biochar was reported to immobilize Pb in soils by the formation of Pb-phosphate species (Ahmad et al. 2014).

The amendment-induced increase of the As availability in the multi-metal-contaminated soils amended by A was documented because individual application of A was observed to increase DTPA-extractable As in soils under incubation for 6 months, and in soils planted vetiver for 6 months (Tables 1, 2, 3, and 4). This increase could also be attributable to the As dissolution due to amendment induced increase of soil pH (Tables 2 and 4). The enhancement of soil pH was observed to result in negative charge increase on soil surface, subsequently to decrease the sorption of As in soils (Beesley et al. 2014; Yao et al. 2017). Therefore, individual application of amendment could not be suitable for remediation of contaminated soils with high As. However, the combined application of A and B was observed to decrease the As availability in the contaminated soils because combined application of A and B reduced DTPA-extractable As in the soils under incubation for 6 months and in soils planted vetiver for 6 months (Tables 1 and 4), and to decrease concentration of As in pore water of soils planting vetiver (Table 3). These results could be explained by the biochar-induced sorption of As in soils. The major mechanisms for As sorption by biochar were attributed to the complexation and electrostatic interactions, functional groups (Li et al. 2017).

Individual application of A was observed no influence on the DTPA-extractable Cr in soils under incubation for 6 months and in soils planted vetiver for 6 months (Tables 1 and 4), because the Cr mostly exists in the form of Cr(III), which is stable in soils with pH 7.9–8.2. However, the combined application of A and B was observed to increase the concentration of Cr in pore water of soils planting vetiver (Table 3), which could be attributed to the biochar-induced increase of pH in pore water and soil pH (Table 2). Significant positive correlations were determined between the concentrations of Cr and pH in the pore water ($r=0.771$, $p<0.01$). Other studies found that application of biochar increased soil pH, thereby resulting in an increase of the availability of Cr in soils (Mitchell et al. 2018).

In summary, individual application of A reduced the availability of Cd and Pb, but increased the availability of As in the multi-metal-contaminated soils, which had no significant effect on the availability of Cr in soils. Combined application of A and B could further reduce Cd and Pb availability in the multi-metal-contaminated soils, and also reduced availability of As in soils.

4.2 Influence of amendment on the translocation and accumulation of heavy metals in vetiver grown in the multi-metal-contaminated soils

Both the BCF and TF are used to assess the phytoremediation potential of heavy metals by plants (Abaga et al. 2014). If the TF and BCF for a certain metal in a specific plant > 1 , then the plant is considered suitable for the phytoextraction of that

specific metal, whereas plant with $TF < 1$ is considered suitable for phytostabilization (Galal and Shehata 2015).

The TF is the phytoavailability of metal from the plant roots to shoots (Christou et al. 2017). The results of the present study indicated that the accumulation of As, Cr, Cd, and Pb by vetiver was mainly retained in the roots, as the TF values for As, Cr, Cd, and Pb were < 1 when vetiver grown in multi-metal-contaminated soils without amendment (Fig. 2). Previous studies by Banerjee et al. (2016) reported that TF value for Cr was 0.83 when vetiver grown in mine soil. Meeinkuirt et al. (2013) found that TF value for Pb < 1 in vetiver grown in Pb mine tailings. Ghosh et al. (2015) showed that TF values for As and Cd below detectable range in vetiver grown in fly ash contaminated soils. Gautam and Agrawal (2017) considered that the mechanism underlying heavy metals (Pb, Cd, Cr) tolerance in vetiver was the restricted translocation of heavy metals from roots to shoots.

The BCF is the translocation of available metals from soil to plant (Abaga et al. 2014). In this study, the BCF values for As, Cr, Cd, and Pb were obviously greater in roots than in shoots of vetiver grown in the present multi-metal-contaminated soils (Fig. 2). Findings of present study are consistent with previous observations. Gautam and Agrawal (2017) reported that the BCF value for Pb was 0.60 in roots, 0.06 in shoot; the BCF value for Cd was 0.78 in roots, 0.22 in shoot. Banerjee et al. (2016) reported that the BCF value for Cr was 0.95 in roots, 0.79 in shoot when vetiver grown in mine soil. Chiu et al. (2005) also observed higher accumulation of As in the roots than in shoots of vetiver grown in metal-contaminated soils.

Therefore, vetiver should be the plant for phytostabilization, which immobilize heavy metals through absorption and accumulation by the roots, adsorption onto the roots, and precipitation into the rhizosphere of vetiver. These processes reduce heavy metals mobility and the bioavailability of heavy metals for entry into the food chain. The use of vetiver for stabilizing the multi-metal-contaminated soils could provide conditions for revegetation.

Obvious differences in the phytoremediation potential in vetiver were dependent on heavy metal species. Because the TF values were ordered as follows: $Cr > As/Cd > Pb$, the BCF values followed the decreasing order: $Cr > Cd > As/Pb$ (Fig. 2). The findings of the present study are consistent with the work of Meeinkuirt et al. (2013), who found that TF value for Pb was 0.07–0.45 in vetiver grown in amended soil. The TF value for Cr was 0.83 when vetiver grown in mine-soil (Banerjee et al. 2016), which was 0.11 when vetiver grown in sludge amended soil, but the TF value for Cr was 0.43 in the present study. This phenomenon could be related to ecotypic difference of vetiver used in the experiments. Banerjee et al. (2016) reported that the translocation of heavy metals from roots to shoots was related to the growth period and ecotype of the vetiver. This implies that the vetiver is relatively effective

in translocating Cr from roots to shoots, whereas ineffective in the translocation of Pb from roots to shoots of the vetiver grown in the present tested soils (Fig. 2). It reveals that vetiver has a relatively high potential for the phytoremediation of Cr compared with other heavy metals (As, Pb, Cr).

The results show that, except the BCF value for Cr in roots, both TF and BCF values for As, Cr, Cd, and Pb in vetiver were < 1 (Fig. 2). Similar results were obtained when vetiver grown in soil amended with sewage sludge (Gautam and Agrawal 2017). Implying that the phytoextraction of the As, Cr, Cd, and Pb for vetiver was lower compared with hyperaccumulator, such as *Eleocharis acicularis* accumulates Cd and As, *Medicago sativa* accumulates Pb, and *Pteris vittata* accumulates Cr (Mahar et al. 2016). The vetiver was not the best plant for the phytoextraction of As, Cr, Cd, and Pb in the contaminated soils.

The present study showed that, except Pb in vetiver, the individual application of A, and combined application of A and B showed no influence on the translocation and accumulation of Cr, Cd and As from roots/soil to shoots. Because no differences of TF values for Cr, Cd, and As, and BCF values for Pb, Cr, Cd, and As in shoots of vetiver were observed among CK, individual application of A and combined application of A and B (Fig. 2). Individual application of A and combined application of A and B obviously inhibited the accumulation of Pb, Cr, and Cd in roots, because individual application of A and combined application of A and B significantly decreased BCF values for Pb, Cr, and Cd in roots of vetiver (Fig. 2). This finding could be explained by the immobilization of Pb, Cr, and Cd in amended soils, thereby inhibiting translocation of the metals from soils to roots, which was consistent with the findings from the present study; the application of pig manure and sewage sludge decreased Pb accumulation in vetiver (Chiu et al. 2006; Rotkittikhun et al. 2007); the application of red mud reduced Cr and Cd accumulation in vetiver (Gautam and Agrawal 2017).

Individual application of A and combined application of A and B showed to obviously promote the translocation of Pb from roots to shoots, because individual application of A and combined application of A and B resulted in increase of the TF values for Pb (Fig. 2). There was a significant positive correlation between the concentration of Si in roots and TF value for Pb ($r = 0.739$, $p < 0.01$), between the decreased Si concentration in pore water and concentrations of Pb in the roots ($r = 0.599$, $p < 0.05$). This finding could be ascribed to anti-lead toxicity by binding Si and Pb to the cell wall of plants (Gu et al. 2011). Implying that individual application of A and combined application of A and B had no influence on the phytoextraction of the As, Cr, and Cd for vetiver, excepting for Pb.

In summary, the vetiver should be the plant for the phytostabilization of As, Cr, Cd, and Pb, but not the best plant for the phytoremediation of As, Cr, Cd, and Pb in the tested multi-metal-contaminated soils. The individual application of

amendment, and combined application of A and B could increase phytostabilization of Pb, Cr, and Cd, but had no effect on the phytoextraction of As, Cr, and Cd in vetiver grown in the tested multi-metal-contaminated soils.

4.3 Influence of amendment on the growth of vetiver grown in the multi-metal-contaminated soils

The vetiver could grow well and had no perceived phytotoxic symptoms grown in multi-metal-contaminated soils, indicating that vetiver could tolerate the high Cd, Pb, As, and Cr in soils. The vetiver could be the plant for phytostabilization of As, Cr, Cd, and Pb, because the As, Cr, Cd, and Pb accumulated by the vetiver were largely retained in the roots, as the TF values for As, Cr, Cd, and Pb were < 1 ; the BCF values for As, Cr, Cd, and Pb were obviously greater in roots than in shoots when vetiver grown in multi-metal-contaminated soil without amendment (Fig. 2). These were in agreement with previous reports that vetiver could successfully grow in Cd and Pb contaminated soils (Chen et al. 2004), in fly ash contaminated soils (Ghosh et al. 2015). Therefore, vetiver is a suitable plant to revegetate multi-metals (As, Cr, Cd, Pb) contaminated soils.

Individual application of A markedly improved vetiver growth, especially at seedling period (Table 5), which could be explained by the alleviation of heavy metal toxicity in vetiver through amendment-induced increase in soil pH and the co-precipitation of Si-metal complexes. Significant positive correlations were observed between pH in pore water and plant height ($r = 0.634$, $p < 0.05$) and between pH in pore water and shoot dry weight ($r = 0.581$, $p < 0.05$). Similarly, Yao et al. (2017) reported that the biomass of *B. chinensis* L. was positively correlated with soil pH when the vegetable grown in polluted agricultural soils. In addition, another reason is that amendment-induced decrease of Cr, Cd, and Pb uptake in roots of vetiver because Individual application of A tended to decrease concentration of Cr, Cd, and Pb in roots of vetiver (Fig. 1). A significant negative correlation was observed between shoot dry weight and Pb concentration in the pore water of soils ($r = -0.849$, $p < 0.01$). This phenomenon might indicate that Pb could be a growth-limiting factor for vetiver grown in multi-metal-contaminated soils. This effect could be contributed to that individual application of A markedly improved translocation of Pb from roots to shoots because individual application of A increased the TF value for Pb in vetiver (Fig. 2). An excess supply of Pb was reported to inhibit plant growth by changes to membrane structure and permeability and perturbations to photosynthesis, mineral nutrition, water balance, and enzyme activities (Zhou et al. 2012).

Combined application of A and B was effective in the increase of shoot/root dry weight though it resulted in negative effects on plant height after 6 months planting (Table 5),

which may have been related to the high As accumulation in vetiver inhibited the vetiver growth. Similar results were reported by Mirza et al. (2017), who had shown that As contaminations resulted in pronounce inhibition in the plant height of vetiver. Therefore, compared with individual application of amendment, combined application of A and B should be a better suitable measures for revegetation based on planting vetiver on the multi-metal-contaminated soil because vetiver looked strong.

In summary, vetiver was useful for revegetation of multi-metal-contaminated soils. Individual application of A and/or combined application of A and B could increase vetiver growth by the alleviation of heavy metal toxicity in plants. However, Pb could be a growth-limiting factor for vetiver grown in multi-metal-contaminated soils. The tested amendments showed effective at seeding growth of vetiver grown in multi-metal-contaminated soils, which could be more important for revegetation of multi-metal-contaminated soils by vetiver as remediation plant.

5 Conclusions

The present study confirmed that vetiver could grow in soils contaminated with multi-metals (Cd, Pb, As, Cr) due to the restriction of metal translocation from roots to shoots. The mechanism underlying metal tolerance in vetiver was that the As, Cr, Cd, and Pb accumulated in the vetiver were largely retained in the roots, as the TF values for As, Cr, Cd, and Pb were < 1 ; the BCF values for As, Cr, Cd, and Pb were obviously greater in roots than in shoots.

Individual application of alkaline silicon-based amendment and combined application of amendment and biochar markedly improved vetiver growth, which was more effective for seeding growth because application of A and AB induced accumulation of Cd, Pb, and Cr in the roots, and then enhanced the phytostabilization of Cr, Cd, and Pb in roots based on the BCF values, and the immobilization of Cd and Pb in soils based on increase of soil pH and Si-induced accumulation of Cd, Pb, and Cr in the roots. It could be concluded that vetiver could be a candidate for phytostabilization of As, Cr, Cd, and Pb, but not the best plant for phytoremediation of the As, Cr, Cd, and Pb in the tested multi-metal-contaminated soil. Further investigations are required to determine the feasibility of vetiver as a long-term tool for revegetation of multi-metal-contaminated soils at the field scale.

Funding information This research was supported by the National Natural Science Fund Projects of China (41571318) and Sino-Danish Joint Doctoral Promotion Programme (Chinese Academy of Sciences).

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