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ORIGINAL PAPER

Biochar effects on metal bioaccumulation and arsenic speciation in alfalfa (*Medicago sativa L.*) grown in contaminated soil

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Abstract Mining and geogenic activities can lead to elevated concentrations of potentially toxic elements in soil. Biochar amendment to soil is a cost-effective technology and environmentally friendly approach to control soil pollution, improve phytoremediation and mitigate health risks due to agricultural products. Greenhouse pot experiments were conducted to investigate the effects of rice husk biochar on alfalfa biomass, metal bioaccumulation and arsenic speciation. Results indicated that rice husk biochar amendments to contaminated soil increased plants biomass by improving soil fertility and available nutrients. Biochar also increased soil cation change capacity, dissolved organic carbon, while decreased available concentrations of potentially toxic elements (except for arsenic). The accumulation of nickel, lead, cadmium and zinc (except for chromium and arsenic) significantly ($P \leq 0.05$) decreased as compared with unamended control plants. In addition, increases were observed for inorganic arsenite and arsenate. Current findings demonstrate that rice husk biochar can be used as a beneficial amendment for contaminated soil. However, further field experiments are needed to validate its long-term effectiveness where environmental factors are diverse and complex.

Keywords Alfalfa · Arsenic speciation · Biochar · Metals bioaccumulation

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Introduction

Soil contamination with toxic metal (loid)s is one of the main environmental concerns that lead to food quality issues. With the development of the economy and industry, anthropogenic and geogenic processes are the major sources of potentially toxic elements (PTEs), especially zinc (Zn), cadmium (Cd), arsenic (As), cobalt (Co), lead (Pb) and copper (Cu) (Pratas et al. 2013). Soil pollution also occurs due to massive application of chemical fertilizers and pesticides in arable lands (Petrikova et al. 1995). China is a rapid-industrializing and developing country, and anthropogenic and geogenic activities frequently occur, mainly in urban and suburban areas where people also need to grow different cereal and fodder crops for their food and cattle. As a result, these activities (along with other industrial processes) have increased the PTE levels in the agricultural fields. Forage and cereal crops when grown in contaminated soil enable PTEs to enter the food chain and then transfer to human's directly or indirectly via the consumption of crops or animals. This results in severe health risks and chronic diseases. Due to food safety and public health perspectives, PTE accumulation in arable soil and their constant migration into the food chain through various cereal and fodder cropping systems are of great concern (McLaughlin et al. 1999).

Excessive Cd and Pb exposure causes kidney damage, abdominal pain, and stomach and lung cancers (Jarup et al. 1998). Inorganic As (sum of arsenate and arsenite, iAs) is a highly toxic carcinogen that finds its way into humans, and therefore can cause various health risks including infertility, cardiovascular diseases and neurological disorders (Smoke and Smoking 2004).

In order to remediate the PTE-contaminated soil and reduce public health risks, biochar (carbon (C)-rich material) amendments have been widely used in the last decade (Komkiene and Baltrenaite 2016; Ghosh et al. 2015). The benefits of biochar amendments to soil have been reported to decrease the soil bulk density, increase water dynamics, improve ecosystem function and increase soil cation exchange capacity (Méndez et al. 2013). Growing rice at an alarming rate, global climatic communities have raised many questions about the management of the fibrous residues of rice plants. Turning rice husk into biochar and then using this as a soil amendment will be a useful and cost-effective strategy for sustainable environmental management and remediation. As a perennial plant belonging to family fabaceae and "Queen of forage" in the world, alfalfa (*Medicago sativa* L.) is highly beneficial to soil health and cropping system and has more nutritional quality for animal feed. However, excessive metal bioaccumulation in alfalfa can result in a deterioration and contamination of food chain. Therefore, it was selected to investigate how rice husk biochar (RHB) affects PTE uptake and accumulation into this forage plant grown in metal-contaminated soil. As far as it is concerned, no such research work has been conducted in the past. Therefore, greenhouse pot experiments were conducted at Institute of Urban Environment, Chinese Academy of Science, Xiamen, China, from early March, 2015 to late May, 2015. The aims and objectives of the present research were to characterize rice husk biochar (RHB) and examine its effects on: (1) PTE availability in soil, (2) alfalfa biomass, (3) PTE uptake into and their accumulation in alfalfa and (4) arsenic speciation in alfalfa.

Materials and methods

Soil sampling

Soil samples (0–10 cm) were collected in triplicate near Luoyuan County, Fuzhou, Fujian Province China. The surrounding area is known to be heavily contaminated with PTEs especially As. Soil samples were air-dried and sieved (2 mm mesh) after transportation to the laboratory. Before the experiment, the properties of soil and biochar like pH, electric conductivity (EC), total carbon (C), total nitrogen (N), total sulfur (S), dissolved organic carbon (DOC), total organic carbon (TOC), particle size, porosity and surface area, total and available PTEs and macro nutrients were measured (Rayment and Higginson 1992). The results are presented in Table 1. Procedural detail is given in supporting information (SI).

Biochar characterization

Functional groups and elemental composition of RHB were characterized through Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM–EDS) and X-ray diffractograms (XRD) techniques. Procedural explanation is given in SI.

Table 1 Basic physicochemical characteristics of soil and RHB ($n = 4$) and their comparison with permissible limits set for soil (pH > 6.5) by the State Environmental Protection Administration (SEPA, 1995)

Parameters	Soil	RHB	Parameters	Soil	RHB	Background soil	
pH ($CaCl_2$)	6.68	8.45	Concentration	Total	Total	SEPA	Fujian Province
EC (μS/cm)	290	823	Cr (mg/kg)	46.7	55.3	ND	
BET surface area ($m^2\ g^{-1}$)	12.4	23.8	Ni (mg/kg)	20.1	15.6	ND	
Pore volume ($cm^3\ g^{-1}$)	0.0001	0.0063	As (mg/kg)	768	13.1	30	5.88
Pore size (nm)	10.7	3.57	Cd (mg/kg)	8.16	11.1	0.3	0.05
Soil particle size			Pb (mg/kg)	1265	547	300	35.6
Clay (%)	5.58	ND	Zn (mg/kg)	946	63.8	250	79.5
Silt (%)	49.9	ND	Co (mg/kg)	8.64	0.24	ND	
Sand (%)	43.9	ND	K (mg/kg)	1124	6365	8.77	
N (%)	0.17	0.82	P (mg/kg)	459	1876	0.50	
C (%)	1.29	48.0	Si (mg/kg)	124	108	0.31	
S (%)	0.18	0.42	Na (mg/kg)	1340	762	1.06	

RHB represents rice husk biochar. ND: No Data. Available PTE extracted with EDTA-Na_2 (0.05 M), triethanol amine (TEA) (0.1 M) and $Cacl_2$ (0.01 M). Soil background value taken from literature method (Chen et al. 1992) for Fujian Province, China

Experimental design

The experimental design was completely randomized block design with four replicates. Biochar was produced from rice husk through pyrolysis at 500 °C for 5 h under a continuous flow of N_2 using a high-performance automatic controlled furnace (GWL-1200, Henan, China)(See SI for more detail). Greenhouse pot experiments were conducted at Institute of Urban Environment, Chinese Academy of Science, Xiamen, China, from early March, 2015 to late May, 2015. Biochar was homogenously mixed on a dry weight (d.w) basis with multi-metal-contaminated soil at application rates of 1, 3 and 5 % (*w*/*w*), referred to as RHB1 %, RHB3 % and RHB5 %, respectively. Soil without biochar amendment (CK) was also included. Each treatment was repeated for four times ($n = 4$) in a polyvinyl chloride pots (24 cm height and 15 cm diameter) having 4 kg soil per pot. Before cultivation, these pots were irrigated with deionized water and kept for 1 week at 60 % of maximum water-holding capacity. After 1 week, about 5 g soils was taken from each replicate using a small soil corer and used for analysis of changes occurred in the biochar amended and unamended CK. Alfalfa seeds were surface sterilized with 30 % H_2O_2 for 10 min and thoroughly washed with deionized water. These experiments were conducted for 13 weeks in a greenhouse under natural conditions with 12/12 h day/night light, keeping daytime temperature of 12 ± 2 °C and night temperature of 8 ± 3 °C, and relative humidity of 8 ± 5 %. Pots were completely randomized in order to compensate temperature and light differences.

Soil analyses

Prior to the cultivation, dissolved organic carbon (DOC) of amended soil was extracted according to the method by Feng et al. (2012). Briefly, 40 mL of 0.5 M K_2SO_4 solution was added to 4.0 g moist soil samples, shaken for 1 h at 200 rcf and then centrifuged. The supernatant was filtered through 0.22-µm filter membrane and used for DOC measurement with a total carbon analyzer (TOC-V_{CPH} Shimadzu, Japan). Available P (Colwell P) was determined following the method by Rayment and Higginson (1992). The EDTA-extractable fraction of soil was also analyzed for the available concentrations of metal (loid)s in amended and unamended soils. Total concentrations of major and trace metals in biochar-amended and biochar-unamended soil samples were determined by extraction using strong acid (concentrated HNO_3 and $HClO_4$) digestion (Wong and Li 2004).

Plants analyses

Plants were harvested after 13 weeks of growth and washed thoroughly with deionized water. Plants were oven-dried at 70 °C for 72 h, and the biomass was recorded on a dry weight (d.w) basis. Shoots, roots and leaves were powdered prior to chemical analysis. Plants samples (0.2 g) were digested with highly pure nitric acid (HNO_3) (GR, Merk, Germany) and hydrogen peroxide (H_2O_2)(GR, Sinopharm, Shanghai, China)(HNO_3/H_2O_2: 1/1 *v*/*v*) in a microwave-accelerated reaction system (Mars5, CEMCorp, Matthews NC, USA). The concentrations of elements such as sodium (Na), potassium (K), phosphorus (P), silicon (Si) and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin-Elmer Optima 7000 DV, Downers Grove, IL, USA), while As, Cd, Pb, Co, nickel (Ni) and chromium (Cr) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, 7500 CX, Santa, Clara, CA, USA). The As species in alfalfa plants were measured using high-performance liquid chromatography (HPLC)-ICP-MS technology after extracting the samples (200 mg) in polypropylene tubes with 10 ml 1 % HNO_3 in microwave-accelerated reaction system (Jia et al. 2012). Inorganic As (iAs) species such as arsenite (As III) and arsenate (As V) and organic species including methyl arsonic acid (MMA) and dimethyl arsinic acid (DMA) were quantified using anion-exchange column (PRPX-100, Hamilton Company, USA). Mobile phase of the mixture of 10 mM $(NH_4)_2HPO_4$ and 10 mM NH_4NO_3 (pH 6.2) was used. Inorganic arsenic was calculated from the sum of arsenite (As III) and arsenate (As V). The extraction efficiency ranged from 77–100 % (Table 3).

Quality control and data analysis

In order to verify the accuracy and precision of the analysis, sample blanks and certified reference materials (for soil GBW07401-GSS-1 and plants GBW07602-GSV-1) which were obtained from the National Research Centre for Standards in China, these were included during total soil and plant PTE extraction batch. The recovery rate ranged from 78 to 114 % for soil and 89 to 108 % for plant samples. Detailed explanation is given in the supporting information (Table S2). The data were analyzed using statistical software package (Statistix 8.1, USA) with one-way ANOVA and then plotted by Sigma plot 12.0 (Systat Software, Inc, San Jose, CA, USA).

Results and discussion

Properties of soil and rice husk biochar

The total concentrations of PTEs such as Cr, Ni, As, Cd, Pb, Zn and Co in contaminated soil were 46.7, 20.1, 768, 8.16, 1265, 946 and 8.64 mg/kg, respectively, while the total concentrations of macronutrients including Na, K, P and Si were 1340, 1124, 459 and 124 (mg/kg), respectively. Similarly, the total concentrations of PTEs like Cr, Ni, As, Cd, Pb, Zn and Co in RHB were 55.3, 15.6, 13.1, 11.1, 547, 63.8 and 0.24 mg/kg, respectively (Table 1). In soil, the concentrations of As, Cd, Pb and Zn exceeded their maximum permissible limits (MPL) (30, 0.3, 300 and 250 mg/kg, respectively) set for soil (pH > 6.5) by the State Environmental Protection Administration, China (SEPA, 1995).

Comparison of control and biochar-amended soil before alfalfa cultivation

The detailed comparison of the control and RHB-amended soils (RHB1 %, RHB3 % and RHB5 %) is given in Table 2. The amendments of RHB changed almost all soil properties such as pH, EC, cation exchange capacity (CEC), DOC, TN, TC, S and Colwell P. Soil pH increased from 6.68 to 6.81, a range related to the maximum adsorption of metals (Kołodyńska et al. 2012). CEC significantly increased from 1.98 to 2.34 (cmol kg^{-1}) (Table 2). The increase in CEC of biochar-amended soil may be due to the formation of carboxylic-C and aromatic-OH functional groups on biochar surfaces that in turn decreased the Cd-exchange capacity of the soil (Liang et al. 2006). Amendments of RHB decreased available concentrations of PTE like Cr (from 71 to 85 %), Cd (from 19 to 34 %), Pb (from 33 to 43 %) and Zn (from 25 to 54 %) as compared to the control treatment (Table 2). These results are in agreement with previous findings that observed significant decrease in metals availability (Cd, Pb and Zn) in miscanthus straw biochar-amended soil (Houben et al. 2013). Amendments of soil with biochar derived from other feed stocks have also been investigated to reduce the available concentrations of PTEs (Khan et al. 2015; Ahmad et al. 2012). The available concentration of As increased from 28 % to 79 % with amendments of RHB as compared with an un-amended control (Table 2). This increase in available As concentration in amended soil may be attributed to the fact that biochar possesses negatively charged functional groups (Cheng et al. 2006), which limits adsorption of As and hence increases As availability in amended soil. In addition, the increased availability of As in soil solutions may be attributed to the completion for adsorption between arsenate and silicate on soil solids. Competition between arsenate and silicate sorbed on soil particulates had been shown in illite and kaolinite (Sharma and Kappler 2011) and in ferrihydrite and goethite (Luxton et al. 2008). The available concentrations of macronutrients like K (from 12 to 26 %), P (from 21 to 94 %) and Si (from 27 to 55 %) increased significantly in RHB-amended soils as compared to the control (Table 2). Other studies revealed that biochar amendments increased available

Table 2 Changes in the characteristics of soil amended with RHB after 1-week flooding but before alfalfa cultivation. Mean values are shown ± 1 standard deviation ($n = 4$)

Parameters	Control	RHB1 %	RHB3 %	RHB5 %
pH ($CaCl_2$)	6.68 ± 0.0	6.72 ± 0.12	6.76 ± 0.01	6.81 ± 0.01
EC (μS/cm)	1.44 ± 0.01	1.49 ± 0.02	1.51 ± 0.01	1.59 ± 0.03
CEC (cmol kg^{-1})	1.98 ± 0.13	2.25 ± 0.31	2.27 ± 0.1	2.34 ± 0.01
TN (%)	0.17 ± 0.01	0.18 ± 0.02	0.24 ± 0.03	0.32 ± 0.05
TC (%)	1.29 ± 0.01	1.75 ± 0.11	2.54 ± 0.21	3.65 ± 0.01
S (%)	0.18 ± 0.03	0.28 ± 0.01	0.38 ± 0.01	0.50 ± 0.02
DOC (mg kg^{-1})	3.31 ± 0.14	3.59 ± 0.11	3.60 ± 0.11	3.82 ± 0.24
TOC	1.35 ± 0.01	1.36 ± 0.02	1.61 ± 0.11	1.77 ± 0.32
Colwell P	22.9 ± 2.6	30.3 ± 2.5	47.7 ± 2.02	48.5 ± 2.92
Available PTE concentrations (mg/kg)				
Cr	0.21 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	0.03 ± 0.01
Ni	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.08 ± 0.03
Cd	3.70 ± 0.4	3.00 ± 0.01	2.98 ± 0.1	2.43 ± 0.2
As	0.73 ± 0.02	0.83 ± 0.03	1.26 ± 0.04	1.31 ± 0.1
Pb	3.91 ± 0.42	2.61 ± 0.2	2.41 ± 0.1	2.20 ± 0.9
Zn	78.5 ± 0.4	58.52 ± 0.2	42.8 ± 1.3	35.6 ± 1.9
Available macronutrients concentrations (mg/kg)				
K	41.62 ± 2.4	46.3 ± 0.1	50 ± 2.1	52 ± 2.4
P	4.07 ± 0.9	4.93 ± 0.4	5.85 ± 0.4	7.93 ± 0.1
Si	21.42 ± 0.1	27.3 ± 0.1	31.1 ± 0.2	33.3 ± 0.3

Bioavailable PTEs and nutrients were extracted with ethylene diamine tetra acetic acid EDTA-Na_2 (0.05 M), triethanol amine (TEA) (0.1 M) and $CaCl_2$ (0.01 M) concentrations of P, K, Na and As, but reduced available concentrations of Cd, Pb and Zn (Namgay et al. 2010).

Effect of biochar on biomass

In this study, RHB showed beneficial effects on alfalfa plant growth and biomass production (Table S1). The amendment of RHB significantly improved alfalfa biomass as compared with the control. Root and shoot biomass significantly ($P \leq 0.05$) increased with the application rates of RHB1 % and RHB3 %. However, RHB5 % reduced shoot and root biomass. It is clear that amendments of RHB1 % and RHB3 % are ideal in terms of alfalfa plant biomass productivity. The decrease in alfalfa biomass with amendment of 5 % biochar may be caused by increased bioaccumulation of PTEs like Cr, As and their toxicity to alfalfa which might cause chlorosis, necrosis and yellowing of plants during the 9-week period. Yu et al. (2009) also found increased biomass of spring onion grown in soil amended (1 %) with Eucalyptus sapwood biochar.

Bioaccumulation of potentially toxic elements

Bioaccumulation of PTEs like As, Cd, Cr, Ni, Pb and Zn was affected significantly with RHB amendments as compared to an un-amended control (Fig. 1). Cr concentration was nonsignificantly increased in shoots, while Ni, Pb, Cd and Zn concentrations were decreased significantly ($P \leq 0.05$) by 39–74 %, 23–43 %, 26–61 % and 17–37 %, respectively, with the amendments of RHB (Fig. 1). Our results are in agreement with previous findings that Pb, Zn and Cd concentrations were significantly ($P \leq 0.05$) reduced up to 60, 37 and 71 %, but As bioaccumulation increased with amendments of rice husk, bran and straw biochar (Zheng et al. 2013). Similarly, Ni, Cd, Pb and Zn concentrations in leaves were significantly ($P \leq 0.05$) decreased by 33–49 %, 32–74 %, 24–52 % and 17–32 %, respectively, with amendments of RHB. Decreases in PTE uptake rate were observed with the increasing application rate of biochar (Fig. 1). Amendments of chicken manure biochar (15 %) significantly decreased Cd (by 88 %) and Pb (by 96 %) bioaccumulation in the aboveground tissues of mustard (*Brassica juncea*) (Park et al. 2011).

In roots, Ni, Cd, Pb and Zn concentrations were significantly ($P \leq 0.05$) decreased (from 36 to 62 %, 16 to 38 %, 18 to 54 % and 23 to 44 %, respectively) with amendments of RHB (Fig. 1). Current results are in consistent with previous findings (Lu et al. 2014) that amendments of rice straw biochar (1 and 5 %) significantly reduced Cd (49 %), Pb (71 %) and Zn (60 %) accumulation in *Sedum plumbizincicola*. This decrease in PTEs bioaccumulation may be attributed to increase in pH of the amended soil (Zheng et al. 2012) because pH can affect the

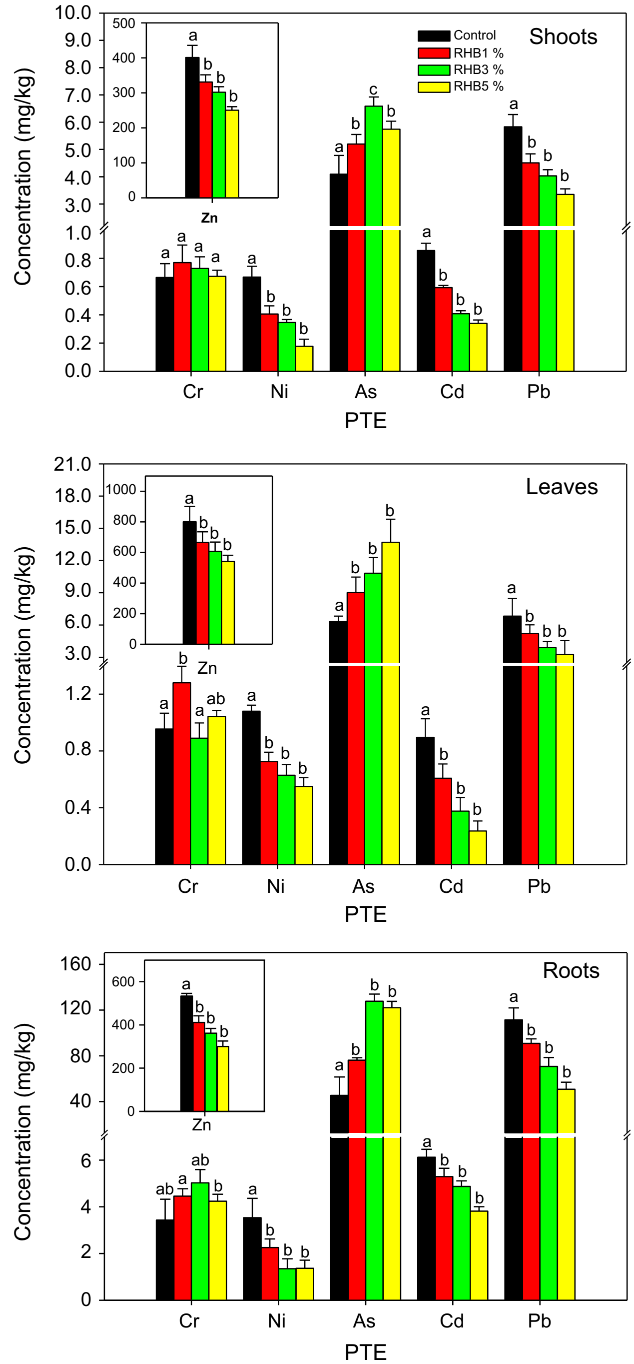

Fig. 1 PTE concentrations in alfalfa shoots, leaves and roots grown in the control and soil amended with RHB1 %, RHB3 % and RHB5 %. *Error bars* represent standard deviations ($n = 4$). *Different letters* indicate significant difference ($P \leq 0.05$) between treatments, while similar letters and parameters without letters indicate non-significant difference

chemistry of adsorbent, surface charges and also change the ionization of PTEs in soil solution and its subsequent bioaccumulation in plants (Kołodyńska et al. 2012). Moreover, PTE has less mobility at higher soil pH (Houben et al. 2013). DOC concentration can be enhanced in amended soil. While DOC acts as chelator and forms stable complexes with PTEs. Therefore, increased DOC will reduce PTE availability in soil solutions and finally decrease their bioaccumulation in alfalfa plants (Zheng et al. 2012). Various other factors like oxygen-functional groups on biochar surface and alteration in microbial activities in amended soil can also affect PTEs availability and its subsequent uptake into alfalfa plants (Steinbeiss et al. 2009; Xu et al. 2013).

Bioaccumulation of macronutrients

Nutrients are required for proper growth and development of plants. Nutrients regulate plant shape, size, color and essential process like photosynthesis, respiration and reproduction. In the current study, accumulation of macronutrients like P, K, Si and Na was significantly ($P \leq 0.05$) increased in shoots (from 94 to 187 %, 42 to 134 %, 58 to 197 % and 150 to 350 %, respectively) with amendments of RHB. Similarly, P, K, Si and Na concentrations in leaves were also statistically increased (from 55 to 146 %, 80 to 184 % and 75 to 118 %, respectively) amended with RHB. P (46–209 %), K (50–130 %), Si (41–63 %) and Na (85–179 %) concentrations in roots with amendments of RHB were significantly increased (Fig. 2). These increases in accumulation of nutrients in alfalfa may be due to the improved soil fertility and nutrients in RHB-amended soil (Major et al. 2012) because biochar increases beneficial nutrients in soil solution and then their accumulation in plants (Martinsen et al. 2014).

Bioaccumulation of arsenic and its species

Arsenic is one of the toxic metalloids in the environment and is discharged to the environment at alarming rates through various geogenic and anthropogenic activities. In this study, amendments of RHB contrastingly affected the accumulation of As and its species in various tissues of alfalfa. Accumulation of total As significantly ($P \leq 0.05$) increased in shoots (89–94 %), leaves (95–106 %), roots (97–124 %) as compared with unamended controls (Fig. 1). Similarly, accumulation of iAs species such as As III was significantly increased in shoots and leaves (Table 3). In roots and shoots, bioaccumulation of As III and As V increased by 29–297 % and 28–57 % with application of RHB. Leaves bioaccumulation As V was significantly increased (73 %) with the amendments of RHB5 %, while roots bioaccumulation was increased (96 %) with RHB1 % amendment. However, the bioaccumulation of organic species including MMA and DMA was not quantified because their concentration was lower than the detection limits (0.1 µg/kg) of the HPLC -ICP-MS (Table 3). The increased accumulation of arsenic species

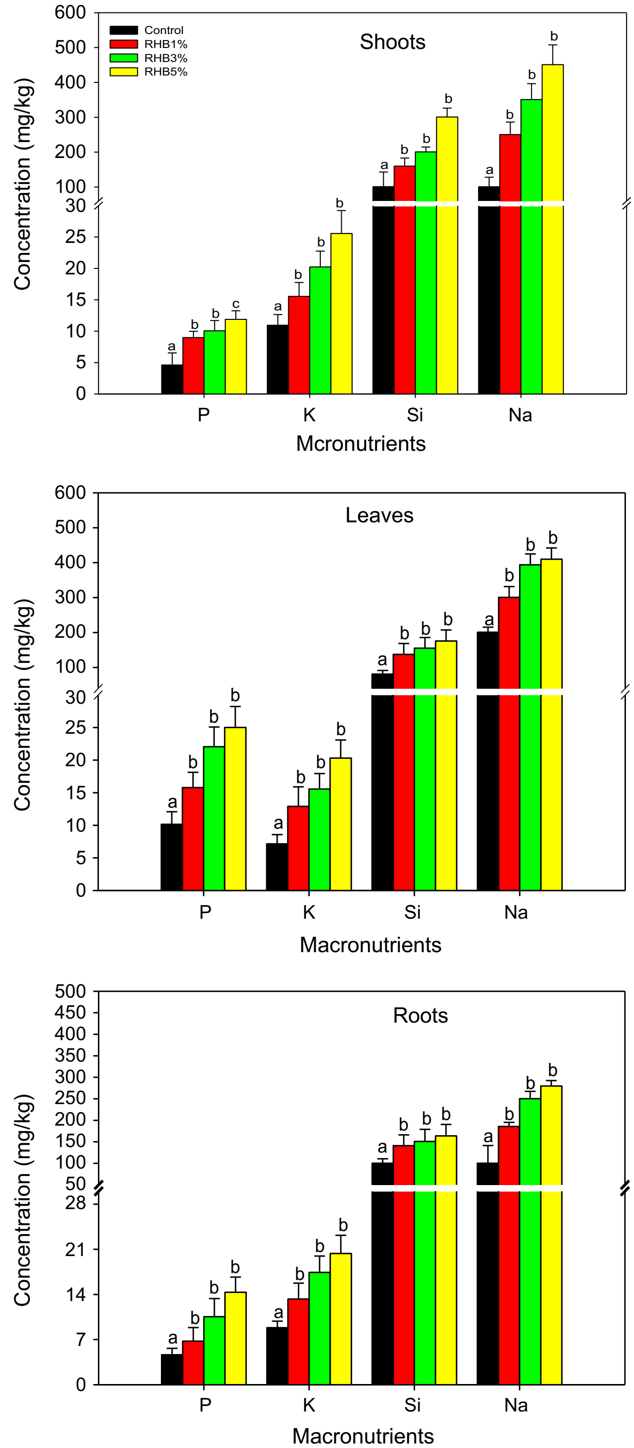

Fig. 2 Macronutrients concentrations in alfalfa shoots, leaves and roots grown in the control and soil amended with rice husk biochar RHB1 %, RHB3 % and RHB5 %. *Error bars* represent standard deviations ($n = 4$). *Different letters* indicate significant difference ($P \leq 0.05$) between treatments, while similar letters and parameters without letters indicate nonsignificant difference

may be due to increased available concentration of Si (as discussed earlier) and P in RHB-amended soil. During As uptake, these two elements strongly interact which may in

Table 3 Concentrations (mg/kg) of As species in alfalfa ($n = 4$) and their extraction efficiency as compared to bioaccumulated total As

Parameters	Control			RHB1 %			RHB3 %			RHB5 %		
	Shoots	Leaves	Roots	Shoots	Leaves	Roots	Shoots	Leaves	Roots	Shoots	Leaves	Roots
As III	0.59 b	0.95 b	5.66 a	0.76 b	2.08ab	7.35 a	1.19 a	3.04 a	11.99a	1.27 a	3.48 a	22.48a
As V	3.85a	5.02b	51.60b	4.95a	7.36ab	101.60a	6.07a	7.98ab	86.38b	5.48a	8.73a	72.61ab
DMA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MMA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
iAS	4.44 b	5.96b	57.26b	5.71ab	9.44ab	108.95a	7.26 a	11.03a	98.36 a	6.75ab	12.21a	95.09 a
TAs	4.97	6.33	59.3	5.98	9.51	109	7.59	11.8	128	6.75	13.7	122
Extraction efficiency (%)	89.3	94.2	96.5	95.4	99.3	99.8	95.68	93.3	77.0	100	89.1	77.9

The value of inorganic arsenic (iAs) is the sum of arsenite (AsIII) and arsenate (As V), and BDL means below detection limits (0.1 g/kg). Extraction efficiency for As species was calculated from the sum of As species (AsIII and As V) divided by total accumulated As extracted with H_2O_2 and concentrated HNO_3 and multiplied by 100

turn increased the As accumulation and speciation in alfalfa. Meharg and Macnair (1992) discovered that As V accumulates in plants from soil via P transporters. An increase in available P means that As is mobilized. From soil to plants the transfer of arsenic is high, which subsequently increases its speciation. This may be due to the extensive root system of alfalfa, its high availability in soil and its high uptake rate. In the current study, iAs was found to be a dominant form, while DMA and MMA were not quantified. The extraction efficiency of iAs as compared to total As was observed for shoots (89.3–96.5 %), leaves (95.4–99.8 %) and roots (77.9–100 %), as given in Table 3. It seems that DMA and MMA were not taken by alfalfa because its uptake rate is very slow compared to inorganic As species and another reason could be the upland condition of soil which may be not led to formation of organic species (Abedin et al. 2002).

Conclusion

Amendments of RHB significantly ($P \leq 0.05$) reduced available concentrations of PTE and their subsequent accumulation in alfalfa. However, arsenic and its species increased significantly as compared with the unamended control. The selected biochar improved alfalfa biomass and agronomic properties of soil by increasing cation-exchange capacity (CEC), dissolved organic carbon (DOC) and beneficial nutrients. This study found that a rice husk biochar (RHB) can be used as a beneficial amendment in the management of contaminated soil by growing alfalfa plants, but not for soil highly contaminated with arsenic. However, the application of current findings needs to be examined under field conditions for further investigations and mitigating health risks arise due to agricultural products.

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