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ORIGINAL PAPER

Effects of carbon nanotube and biochar on bioavailability of Pb, Cu and Sb in multi-metal contaminated soil

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Abstract This study examined the effects of carbon nanotube and biochar on the bioavailability of Pb, Cu and Sb in the shooting range soils for developing low-cost remediation technology. Commercially available multi-walled carbon nanotube (MWCNT) and biochar pyrolyzed from soybean stover at 300 °C (BC) at 0.5, 1 and 2.5% (w w^{-1}) were used to remediate the contaminated soil in an incubation experiment. Both DTPA (bioavailable) and TCLP (leaching) extraction procedures were used to compare the metal/loid availability and leaching by the amendments in soil. The addition of BC was more effective in immobilizing mobile Pb and Cu in the soil than that in MWCNT. The BC reduced the concentrations of Pb and Cu in the soil by 17.6 and 16.2%, respectively. However, both MWCNTs and BC increased Sb bioavailability by 1.4-fold and 1.6-fold, respectively, in DTPA extraction, compared to the control. The toxicity characteristic leaching procedure (TCLP) test showed that the leachability of Pb in the soil amended with 2.5% MWCNT was 1.3-fold higher than that the unamended soil, whereas the BC at 2.5% decreased the TCLP-extractable Pb by 19.2%. Precipitation and adsorption via electrostatic and π–π electron donor–acceptor interactions were postulated to be involved in the interactions of Pb and Cu with surfaces of the BC in the amended soils, whereas ion exchange mechanisms might be involved in the immobilization of Cu in the MWCNT-amended soils. The application of BC derived from soybean stover can be a low-cost technology for simultaneously immobilizing bioavailable Pb and Cu in the shooting range soils; however, neither of amendments was effective in Sb immobilization.

Keywords Multi-walled carbon nanotube · Bioavailability · Black carbon · Soil remediation · Charcoal

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Introduction

Anthropogenic sources such as paints, pesticides, mining, metal piping, burning of coal and military training may release hazardous levels of metal(loid)s into soil environment (Herath et al. 2015; Singh et al. 2011). Soil in military shooting range are reputed to build up high concentrations of metal(loid)s such as Pb, Cu and Sb, which can become a point-source of pollution in local catchments (Rajapaksha et al. 2015). Bullets lodged in local soil can release metal(loid)s in a wide range of species or forms, e.g., hydroxides, carbonates, sulfates and carboxylates (Rajapaksha et al. 2015), and their bioavailability threats soil and water quality of surrounding ecosystems, and associated agricultural crop quality and human health (Herath et al. 2015; Suthar et al. 2008; Vithanage et al. 2015). Bioavailable metal fraction is the most critical phase in the soil, as it is the absolute concentration of particular heavy metal(loid)s that can have impact on a variety of organisms. Bioavailability tends to result in the bioaccumulation attacking biological organisms which leads to the toxicological bioavailability (Jin et al. 2014). Hence, the bioavailability of hazardous heavy metal(loid)s is one of the important indicators for assessing the effectiveness of remediation/immobilization technologies in contaminated soils as they are related to the extent of their toxicity and probability of ill consequences in multi-metal contaminated soils (Jin et al. 2014; Uchimiya et al. 2012).

For example, high level of metal(loid) exposure disturbs metabolic functions of critical organs such as heart, brain, kidney, bone and liver, subsequently displacing the essential nutritional minerals from their original place, thereby deterring their biological functions in human body (Singh et al. 2011). Hence, effective remediation of such metal(loid)s are urgently needed to reduce their detrimental effects in multi-metal contaminated lands on environmental health and quality.

Soil remediation methods such as soil replacement, solidification, electro-kinetic extraction, soil washing, land filling and excavation are costly to be applied in a large spatial scale and may cause secondary soil contaminations (Herath et al. 2015). Because of these concerns, more attention has recently been attributed toward low-cost and environmentally friendly technologies for rehabilitating polluted soils. The applications of biochar and carbon nanotube as soil amendments have been recommended for in situ remediation of metal(loid) contaminated soils. This is because functional C-materials derived from biological wastes are capable of adsorbing and lowering bioavailability for the metal(loid)s contaminated soils (Ahmad et al. 2014a, b; Herath et al. 2015; Houben et al. 2013; Rajapaksha et al. 2015).

Biochar is a product of carbon-rich waste or biomass produced by thermal degradation under anoxic conditions (Beesley et al. 2010). The application of biochar in contaminated soils can not only adsorb metal(loid)s but also increase the retention of essential nutrients, thereby enhancing plant growth through improvement of physical and biological soil properties (Ahmad et al. 2014a, b, 2016; Cao et al. 2011; Herath et al. 2015; Singh et al. 2011; Zheng et al. 2012). Although many investigations have focused on remediation of heavy metals in contaminated soils using biochars, very few on metalloids such as As and Sb (Inyang et al. 2014; Liu et al. 2013).

The biochar benefits in remediating metalloid contaminated soils remain variable in published studies, in terms of plant uptake, which perhaps depend on rhizosphere mechanisms of different species. A recent study investigated the mobility of Cd, Zn, Pb and As in a historically multi-metal contaminated paddy soil amended with biochars produced from rice straw, husk and bran, and found that the accumulation of Cd, Zn and Pb in rice shoot decreased by up to 98, 83 and 72%, respectively, but As level increased by up to 327% (Inyang et al. 2014). The exchangeable Sb in an army firing range soil was significantly increased when it was amended with biochar, but Sb accumulation in maize plants was lowered (Liu et al. 2013).

Carbon nanotubes are cylindrical graphene materials having unique characteristic features such as ultra-low weight, high mechanical strength, and chemical and thermal stability (Pillay et al. 2009). The application of multi-walled carbon nanotubes (MWCNTs) as adsorbents has currently taken much interest because of their hollow- and multi-layered structures with exceptionally high specific surface area and sorption capacities for various contaminants (Pillay et al. 2009; Rajapaksha et al. 2015; Yang et al. 2013). However, studies have been mostly limited to the removal of heavy metals from aqueous solutions using MWCNTs, for example, the removal of Ni^{2+} from water by a MNCNT with an adsorption capacity of 18 mg g^{-1} (Ahmad et al. 2013). Non-functionalized MWCNTs showed an adsorption of Cr(VI) up to 98% of a 100 μg g^{-1} Cr(VI) solution (Almaroai et al. 2014) and removed Cu(II), Ni(II), Pb(II) and Zn(II) from water (Mebius 1960). However, only few studies have focused on the use of synthetic nanoparticles for the remediation of metal(loid)s in contaminated soils (Ahmad et al. 2013; Rajapaksha et al. 2015).

The present study aims to comparatively investigate the effects of MWCNTs and biochar derived from crop residue on remediation of multi-metal(loid)s in contaminated shooting range soils, as low-cost organic carbon-based remediation technology. Particular focuses have been placed on the effects on soluble and exchangeable forms of the metal(loid)s concerned in the contaminated soil.

Materials and methods

Preparation and characterization of soil and materials

Soil tested was collected from an area within or adjacent to a military shooting range located in South Korea, which lodged with many bullets from shooting practice. The soil samples were air-dried and then sieved through a 2-mm stainless steel sieve after removing bullet fragments.

Biochar was produced from soybean stover. Soybean stover was collected and dried under the sun for a week and then was oven-dried at 50 °C for 24 h (Vithanage et al. 2015). For BC production, the dried soybean stover was subjected to a slow pyrolysis at 300 °C for 2 h with a heating rate of 7 °C min^{-1} under limited O_2 in a modified N11/H Nabertherm furnace (Nabertherm GmbH, Lilienthal, Germany). Biochar was then crushed and ground to <1.0 mm particle size. The MWCNTs used were commercially available. (CM-150, Hanwha Nanotech, Incheon, Korea).

Incubation experiment

The soils were treated with BC and MWCNTs at the rates (mass%) of 0 (control), 0.5, 1 and 2.5% in the incubation experiment. The BC and MWCNTs treatments were thoroughly mixed with the dry soil of 200 g in each replicate, which were watered to 70% water holding capacity and incubated in high density polyethylene bottles for a month at 25 °C in an automated incubator (MIR-554, SANYO Electronic, Co., Ltd., Tokyo, Japan). At the end of the incubation, the treated soils were sampled for analyzing soil properties, cations, metal(loid)s (i.e., Pb, Cu and Sb) and anions.

Chemical analysis

Total concentrations of Pb, Cu and Sb from aliquots of soil samples (0.5 g) were determined by means of an inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 7300 DV, Perkin Elmer, USA) after being digested in a microwave-assisted digestion unit with a mixture of concentrated HNO_3, HCl and HF acids (MARS, HP-500 plus, CEM Corp., USA). Soil organic matter content was determined by the Walkley and Black titration method (Anderson and Ingram 1998), and total C and N contents were also quantified by using an elemental analyzer (vario MAX CN, Elementar, Germany).

The pH of the BC and MWCNTs was measured in a suspension of 1:10 BC/de-ionized water (w v^{-1}), using a digital pH meter (Orion, Thermo Electron Corp., USA). Moisture was estimated by calculating the weight loss after heating the BC at 105 °C for 24 h to a constant weight. Mobile matter (analogous to volatile matter), which reflects the non-carbonized portion in BC, was determined as the weight loss after heating in a covered crucible at 450 °C for 30 min (Mebius 1960). Ash content was also measured as the residue remaining after heating at 700 °C in an open-top crucible. The portion of the BC not being ashed, referred to as resident matter (analogous to fixed matter), was calculated by the difference in moisture, ash and mobile matter. Each sample was analyzed in triplicate. Exchangeable Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in the BC were extracted using the ammonium acetate (NH_4OAc) procedure (Peijnenburg et al. 2007) and analyzed by using the ICP-OES. The organic matter content was determined following the Walkley–Black method (Luo et al. 2011). The surface functional groups of both BC and MWCNTs were characterized by Fourier-transform infrared spectroscopy (FTIR) with transmittance mode (Bio-Rad Excalibur 3000MX spectrophotometer, Hercules, CA, USA). The specific surface area, total pore volume and pore diameter were determined using a gas sorption analyzer using N_2 gas

(NOVA-1200; Quantachrome Corp., Boynton Beach, FL, USA).

Soil pH and electrical conductivity (EC) were measured electrometrically at a soil/water ratio of 1:5. Total (i.e., Al, Ca, Mg, K, Na, Fe, Pb, Cu and Sb) and exchangeable cations (i.e., Ca^{2+}, Mg^{2+}, K^+ and Na^+) in the soil were analyzed using an ICP-OES after extraction in 1 M NH_4OAc. Soil cation exchange capacity (CEC) was calculated from the values of exchangeable cations. Changes in the dissolved organic carbon (DOC), in response to the amendments, were determined by using an organic C analyzer (TOC-ASI, Shimadzu, Japan). The water-soluble anions including NO_3^-, PO_4^{2-}, SO_4^{2-}, and Cl^- were extracted by equilibration for 24 h in soil/water suspension at 1:10 ratio and determined by using an ion chromatography (Metrohm Compact IC-861, Switzerland) (Table 1).

Bioavailability and leachability of heavy metals/metalloids

Potentially bioavailable pool of metal(loid)s was estimated by the diethylenetriaminepentaacetic acid (DTPA) single extraction method (Jin et al. 2014; Rajapaksha et al. 2012). Aliquots of 10-g soil samples were extracted by using 20 mL of 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine buffered solution for 2 h. The filtrates were analyzed for Pb, Cu and Sb by using an ICP-OES.

The toxicity characteristic leaching procedure (TCLP) was used to determine the leachability of metal(loid)s in the BC- and MWCNT-amended soils (Jin et al. 2014). The TCLP extraction is a standard soil extraction method that can be used to access the hazardousness of particular contaminant in the environment in terms of its leachability. Aliquots of 20 mL TCLP extracting solution No. 1 (pH 4.93) were added to polypropylene tubes containing soil samples (1 g per each tube) and subsequently mixed for 18 h at 30 rpm, in which supernatants were filtered through Whatman Grade No. 42 filter papers (2.5 μm). The filtrates were analyzed for concentrations of Pb, Cu and Sb by means of ICP-OES.

Statistical analysis

Statistical analyses were carried out by using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p < 0.05$) for multiple comparisons. Mean separation procedure (least significant different test) and group comparison contrasts were used in a randomized complete block design. All statistical analyses were carried out using the Statistical Analysis System (SAS 9.1). The coefficient of determination (R^2) and Pearson's correlation (r) among the variables were also calculated.

Results and discussion

Physicochemical characterization of shooting range soil

The shooting range soil used in this study was a sandy loam composed of 67.1% of sand, 26.9% of slit and 6.0% of clay. Soil pH and EC were 8.0 and 0.05 dS m^{-1}, respectively (Table 1). Total concentrations of Pb, Cu and Sb were 17,468, 1168, and 164 mg kg^{-1}, respectively. The Pb content far exceeded the Korean standards adopted for shooting range soil management (Rajapaksha et al. 2015). The concentrations of Cu and Sb did not exceed the threshold values for a Korean military area, but remained much higher than the permissible levels of Cu and Sb recommended for agricultural purposes in Korea (Rajapaksha et al. 2015). These findings indicated that the shooting range soil sampled was significantly contaminated with Pb, Cu and Sb, triggering the necessity of soil remediation for preventing in situ and off site pollution impacts, particularly in the event that the land is to be transferred back to civil use in the future.

Effects of BC and MWCNTs on soil properties

Effects of BC and MWCNTs on soil pH, CEC, and NH_4OAc-extractable Pb, Cu and Sb are summarized in Table 2. The pH values of BC and MWCNTs were 7.3 and 7.1, respectively. The application of 2.5% MWCNTs slightly increased the CEC that would be expected to increase with time due to gradual oxidation of the MWCNT surface and adsorption of organic acids (Cao et al. 2011). In BC-amended soils, there were significant increases ($p < 0.05$) in available cations such as K^+, Ca^{2+} and Mg^{2+} (Table 2). This was mainly due to the presence of these cations in the ash fraction of the BC itself, which were dissolved and

Table 1 Physicochemical properties of shooting range soil and biochar

Soil			Biochar		
Parameter	Unit	Value	Parameter	Unit	Value
pH	–	8.00 ± 0.06	pH	–	7.3 ± 0.02
EC	dS m^{-1}	0.05 ± 0.00	EC	dS m^{-1}	3.8 ± 0.03
Texture		Sandy loam	Surface area	m^2 g^{-1}	5.6 ± 0.08
Sand	%	67.10	Pore size	nm	2.72 ± 0.08
Silt	%	26.90	Total PO_4^{3-}	mg kg^{-1}	9.96 ± 1.70
Clay	%	6.00			
Exchangeable cations			Proximate analysis		
Na^+	$cmol_{(+)}$ kg^{-1}	0.05 ± 0.00	Yield	%	37.03 ± 1.20
K^+	$cmol_{(+)}$ kg^{-1}	0.21 ± 0.03	Mobile matter	%	46.34 ± 2.17
Ca^{2+}	$cmol_{(+)}$ kg^{-1}	1.19 ± 0.01	Moisture	%	4.50 ± 1.94
Mg^{2+}	$cmol_{(+)}$ kg^{-1}	1.27 ± 0.01	Fixed matter	%	46.34 ± 2.70
Organic matter	g kg^{-1}	5.24 ± 0.10	Ash	%	10.4 ± 1.83
Total C	%	0.47 ± 0.09			
Total N	%	0.02 ± 0.00	Ultimate analysis		
Total metal contents			C	%	68.8 ± 1.5
Pb	mg kg^{-1}	17,467.63 ± 426.38	H	%	4.3 ± 1.8
Cu	mg kg^{-1}	1168.43 ± 90.85	O	%	25.0 ± 2.4
Sb	mg kg^{-1}	164.25 ± 19.32	N	%	1.8 ± 0.3
Water extracted metals			S	%	0.04 ± 0.01
Pb	mg kg^{-1}	47.68 ± 2.46	H/C	–	0.74
Cu	mg kg^{-1}	5.90 ± 0.44	O/C	–	0.27
Sb	mg kg^{-1}	3.56 ± 0.22			
NH_4OAc extracted metals					
Pb	mg kg^{-1}	5595.53 ± 25.97			
Cu	mg kg^{-1}	128.80 ± 4.66			
Sb	mg kg^{-1}	not detectable			

released into soil solution. The application of both MWCNT and BC in the shooting range soil reduced the NH_4OAc-extractable Pb and Cu (Table 2). The extractability of Pb and Cu in the 2.5% MWCNT-amended soil was reduced by 20.0 and 6.0%, respectively, compared to the control. Comparatively, the extractable Pb and Cu in the 2.5% BC-amended soil were reduced by 45.5 and 36.5%, respectively, compared to the control. These findings indicate that the addition of the BC to shooting range soil is more effective in immobilizing exchangeable Pb and Cu than the MWCNT. The NH_4OAc-extractable Sb was not detectable in the control soil, and the MWCNT- and BC-amended soils. It may be resulted from the electrostatic repulsion between negatively charged OAc^- and Sb anion.

The contents of DOC in the soils amended with the MWCNTs were overall lower than that in the control soil, whereas relatively higher DOC concentrations were observed in the BC-amended soils (Fig. 1). The content of DOC in the soil amended with the highest application rate of BC (i.e., 2.5%) was twice as much as that in the control soil. This could be due to the dissolution of organic content in the BC amendments. The dissolved organic carbon is capable of complexing with mobile forms of metals in the soil and subsequently, such DOC-metal complexes tend to be formed at BC surface (Antoniadis and Alloway 2002).

The BC and MWCNTs treatments altered the profile of extractable anions in the soil, such as NO_3^- and SO_4^{2-} but not PO_4^{3-} ions (Fig. 1). The low levels of PO_4^{3-} ions may be due to its precipitation

Table 2 Changes of pH, cation exchangeable capacity (CEC) and ammonium acetate (NH_4OAc)-extractable metal concentrations in the shooting range soil amended with multi-walled carbon nanotubes (MWCNTs) and soybean stover-derived biochar (BC) at different application rates

Soil amendment	pH	CEC/cmol$_{(+)}$ kg^{-1}	NH_4OAc extracted metals/mg kg^{-1}							
			Na	K	Ca	Mg	Pb	Cu	Sb
Control	$8.27^{c} \pm 0.06$	$1.99^{a} \pm 0.51$	$20.0^{b} \pm 0.4$	$70.2^{d} \pm 1.4$	$361.2^{d} \pm 13.7$	$222.1^{d} \pm 9.1$	$5339.2^{a} \pm 172.3$	$191.3^{a} \pm 4.9$	ND†
0.5% MWCNT	$8.20^{d} \pm 0.02$	$1.97^{a} \pm 0.11$	$19.5^{b} \pm 0.5$	$67.5^{e} \pm 2.9$	$346.8^{e} \pm 9.7$	$215.0^{e} \pm 5.6$	$4075.7^{e} \pm 306.2$	$155.3^{e} \pm 37.3$	ND
1% MWCNT	$8.22^{d} \pm 0.03$	$1.96^{a} \pm 0.83$	$20.0^{b} \pm 0.2$	$67.8^{e} \pm 0.4$	$343.5^{f} \pm 8.9$	$210.7^{f} \pm 4.7$	$4307.4^{b} \pm 434.3$	$185.4^{b} \pm 23.6$	ND
2.5% MWCNT	$8.30^{c} \pm 0.06$	$1.96^{a} \pm 0.54$	$21.0^{ab} \pm 0.2$	$67.2^{e} \pm 1.3$	$336.9^{g} \pm 17.3$	$205.8^{g} \pm 10.3$	$4263.4^{c} \pm 168.2$	$179.6^{c} \pm 5.6$	ND
0.5% BC	$8.30^{c} \pm 0.02$	$2.09^{a} \pm 0.32$	$19.6^{b} \pm 0.7$	$157.7^{c} \pm 4.1$	$394.4^{c} \pm 7.9$	$227.5^{c} \pm 4.2$	$3771.7^{f} \pm 165.0$	$163.2^{d} \pm 15.2$	ND
1% BC	$8.41^{b} \pm 0.01$	$2.20^{a} \pm 0.19$	$21.1^{ab} \pm 1.2$	$254.8^{b} \pm 5.3$	$447.0^{b} \pm 1.6$	$243.4^{b} \pm 2.1$	$4208.0^{d} \pm 44.3$	$148.3^{f} \pm 20.6$	ND
2.5% BC	$8.61^{a} \pm 0.01$	$2.53^{a} \pm 0.55$	$22.2^{a} \pm 0.3$	$515.1^{a} \pm 1.10$	$562.7^{a} \pm 15.2$	$275.7^{a} \pm 6.1$	$2908.4^{g} \pm 79.6$	$121.4^{g} \pm 8.0$	ND

The same letter above each value indicates no difference at a 0.05 significance level

† Not detectable

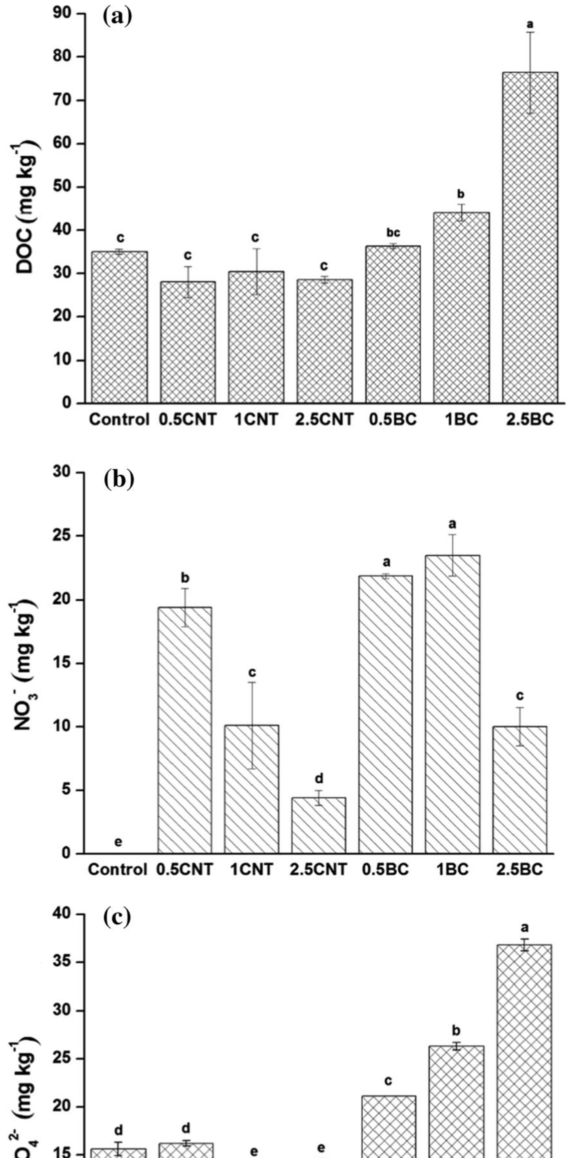

Fig. 1 Effects of multi-walled carbon nanotubes (MWCNTs) and soybean stover-derived biochar (BC) on **a** dissolved organic carbon (DOC), **b** NO_3^-, and **c** SO_4^{2-} in shooting range soils. Error *bars* represent the standard deviation of three replicates. The same letters above each bar indicate no difference at a 0.05 significance level (0.5CNT: 0.5% MWCNT-amended soil; 1CNT: 1% MWCNT-amended soil; 2.5CNT: 2.5% MWCNT-amended soil; 0.5BC: 0.5% BC-amended soil; 1BC: 1% BC-amended soil; 2.5BC: 2.5% BC-amended soil)

with different minerals present in the soil or strong interactions with binding sites of Al and Fe-minerals in the BC- and MWCNT-amended soils (Ahmad et al. 2013). Increasing application rates of the BC and MWCNTs from 0.5 to 2.5%, decreased the level of NO_3^- from 21.9 to 10.0 mg kg^{-1} and 19.2 to 4.4 mg kg^{-1} in the BC- and MWCNT-amended soils, respectively. This may be due to high activity of microorganisms in BC- and MWCNT-amended soils. The levels of SO_4^{2-} in the BC-amended soils increased with increasing application rates and the highest application rate of BC at 2.5% increased the release of SO_4^{2-} by nearly 2.4-folds of the control, but not in the MWCNT-amended soils. Overall, the addition of BC is more effective in altering the chemical properties of soils in comparison with that of the MWCNTs.

Bioavailability of Pb, Cu and Sb

The additions of MWCNTs did not reduce the DTPA-extractable Pb compared to the control (Fig. 2). Nevertheless, the BC treatments (i.e., 0.5, 1 and 2.5%) reduced the DTPA-extractable Pb by 2.3, 27.4 and 7.7%, respectively, compared to the control (Fig. 2a). A considerable reduction was observed in the DTPA-extractable Pb in the BC-amended soils, indicating that the addition of BC is more effective than that of MWCNTs for immobilizing bioavailable pool of Pb in the soil. The concentration of DTPA-extractable Cu was also slightly lower in the soils amended with both BC and MWCNTs, but the reduction was not significant with increasing application rates (Fig. 2b). The application rate at 2.5% of both BC and MWCNTs amendments decreased the DTPA-extractable Cu by 16.2 and 17.4%, respectively, compared to the control. However, in contrast to the extractable Pb and Cu, the DTPA-extractable Sb was obviously high in both BC- and MWCNT-amended soils, relative to the control. The average extractable concentration of Sb in the BC- and MWCNT-amended soils was 1.4-fold to 1.6-fold higher than that in the control soil (Fig. 2c). Overall, the DTPA-extractable metalloids indicated that the BC is relatively more effective to simultaneously reduce the bioavailability of both Pb and Cu, but none of these amendments are suitable for immobilizing Sb in the shooting range soil. These findings would be beneficial in near future to develop metal-cleanup strategies and cost-effective management practices in order to remediate such multi-metal contaminated soils.

Leachability of Pb, Cu and Sb

In this study, TCLP data were used to assess whether the existing concentrations of selected metal(loid)s in the shooting range soil can be accepted into the environment. If TCLP analytical results of a waste are below the TCLP maximum contamination levels, this waste can be accepted into the environment. The TCLP-extractable Pb concentration in the control exceeded the permissible level of 5 mg L^{-1}, suggesting that this shooting range soil may be categorized as a toxic waste. The total Pb (17,468 mg kg^{-1}) and TCLP-extractable Pb concentrations (7246 mg kg^{-1}) implied that this shooting range soil is highly contaminated by Pb. Hence, the remediation and restoration of such soils using suitable management practices is much needed. The addition of MWCNTs to the soil increased the TCLP-extractable Pb with increasing the application rate and the extractable Pb in the 2.5% MWCNT-amended soil was 1.3-fold higher than that in the control (Fig. 2d). This further confirmed that the MWCNTs would be less effective to immobilize Pb in this shooting range soil. Interestingly, there was a considerable reduction of TCLP-extractable Pb in the BC-amended soils, and the application rate of 0.5, 1 and 2.5% BC decreased the extractable Pb by 1.7, 7.0 and 19.2%, respectively, compared to the control. Therefore, it is further evident that the tested BC in this study may be selected as a low-cost remediating material for immobilizing heavy metals in shooting range soils.

The application of MWCNTs reduced on average, the concentration of TCLP-extractable Cu by 10.0% only, compared to the control with little impact on the extractability of Cu in the amended soils (Fig. 2e). However, TCLP-extractable Cu in the BC-amended soils slightly decreased with increasing the application rate and the 2.5% BC amendment reduced the extractable Cu by 29.1%, compared to the control. Both types of amendment are inappropriate for Sb remediation. Overall, the results of the TCLP corroborated well with the findings of DTPA-extractable metal(loid)s in the BC- and MWCNT-amended soils.

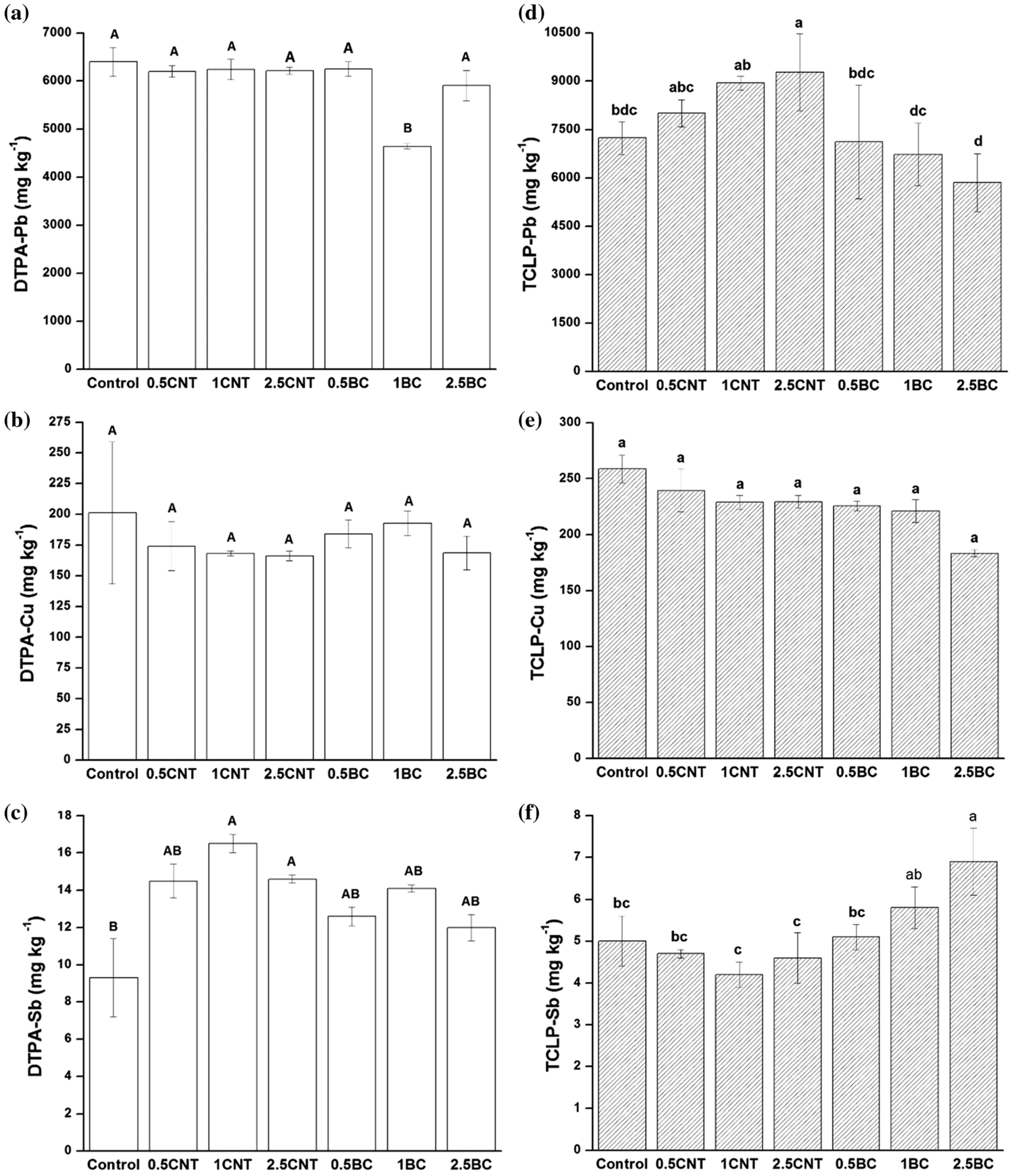

Fig. 2 Exchangeable (DTPA-extractable)- and toxicity characteristic leaching procedure (TCLP-extractable)-**a**, **d** Pb, **b**, **e** Cu, and **c**, **f** Sb in the shooting range soil amended with multi-walled carbon nanotubes (MWCNTs) and soybean stover-derived biochar (BC). *Error bars* represent the standard deviation of three replicates. The same letters above *each bar* indicate no difference at a 0.05 significance level (0.5CNT: 0.5% MWCNT-amended soil; 1CNT: 1% MWCNT-amended soil; 2.5CNT: 2.5% MWCNT-amended soil; 0.5BC: 0.5% BC-amended soil; 1BC: 1% BC-amended soil; 2.5BC: 2.5% BC-amended soil)

This study further demonstrated the correlation of soil quality parameters such as pH, DOC and CEC on the TCLP-extractable heavy metal(loid)s in the BC- and MWCNT-amended soils. In the MWCNT-amended soils, the TCLP-extractable Pb, Cu and Sb were positively correlated with the soil CEC (Fig. 3). The strong correlation of Pb, Cu and Sb mobility with CEC in the MWCNT-amended soils indicated that ion exchange could be the potential mechanism of metal immobilization in the MWCNT-amended soils. For the BC-amended soils (Fig. 4), TCLP-extractable Pb and Cu were negatively correlated with parameters of pH, DOC and CEC, indicating that the immobilization of both Pb and Cu in the BC-amended soils can be governed by ion exchange, precipitation and adsorption via physical and chemical interactions. In contrast to extractable Pb and Cu, the TCLP-extractable Sb was positively correlated with pH, DOC and CEC, suggesting that the Sb mobilization may be encouraged with increasing the application rate of BC.

Mechanisms of BC and MWCNT in regulating the extractability of Pb, Cu and Sb

Biochar derived from soybean stover was tentatively identified as a candidate for remediating soils contaminated with both Pb and Cu, but not Sb. The biochar used in this study are evident with the presence of various surface functional groups such as hydroxyl, carboxylic, phenolic, amine, carbonyl and lactonic (Ahmad et al. 2012; Vithanage et al. 2015). Furthermore, high O/C molar ratios of BC is also mainly due to the O-containing functional groups on the BC surface (Houben et al. 2013). The carboxylic and carbonyl groups present on the surface of BC can form strong organometallic complexes with metal cations, Pb^{2+} and Cu^{2+}, thereby leading to a great retention of these metal ions in the BC. Moreover, soil pH and CEC play a vital role in immobilizing Pb and Cu. Since the BC-amended soil is under alkaline conditions, Pb and Cu tend to easily react with free hydroxyl ions precipitating as metal hydroxide, which may decrease the mobility of these metals in the BC-amended soils. Furthermore, Pb and Cu can be precipitated with phosphates, carbonates and sulfates present in the BC-amended soils. On the other hand, cation exchange mechanisms are capable of governing the metal immobilization process, by which the cations such as Ca^{2+} and Mg^{2+} present on the BC surface can be readily exchanged by metal cations. The point of zero charge (*pzc*) of this BC is pH 7.1 and, hence, under alkaline soil conditions (pH $>$ 8.0), the surface of BC becomes negatively charged, which can govern the electrostatic attraction between the BC surface and positively charged metal ions. Moreover, the aromatic BC surface is a pool of π electrons, which could facilitate π–π* donor–accepter interactions with the electron deficient metal cations. A graphical representation of specific mechanisms for the immobilization of Pb and Cu in the BC-amended shooting range soil is illustrated in Fig. 5.

Dissolved organic carbon has a crucial role in the release of heavy metals in soils due to the formation of soluble organometallic complexes, which would highly facilitate the adsorption of metal ions on particular adsorbents (Antoniadis and Alloway 2002). For the BC-amended soils, the DOC significantly increased with increasing the application rate, thereby increasing the immobilization efficiency of Pb and Cu via the adsorption of soluble DOC-metal

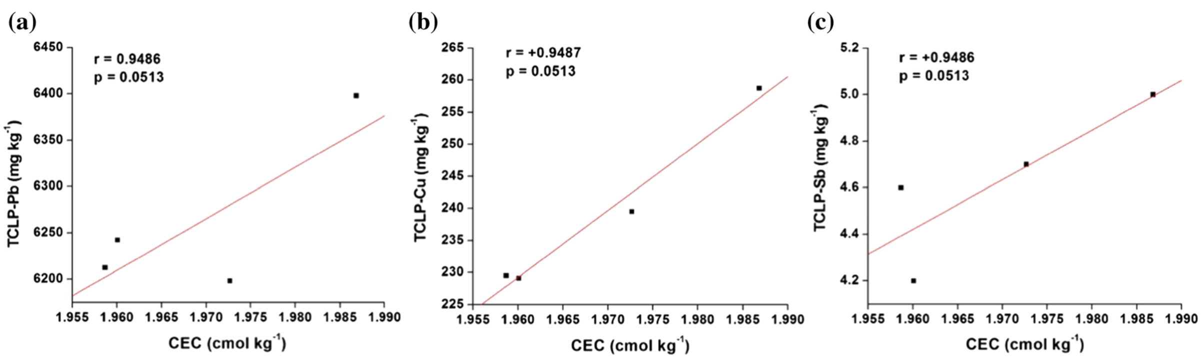

Fig. 3 Correlations between **a** cation exchange capacity (CEC) and toxicity characteristic leaching procedure (TCLP)-Pb, **b** CEC and TCLP-Cu, **c** CEC and TCLP-Sb in the shooting range soil amended with multi-walled carbon nanotubes (MWCNTs)

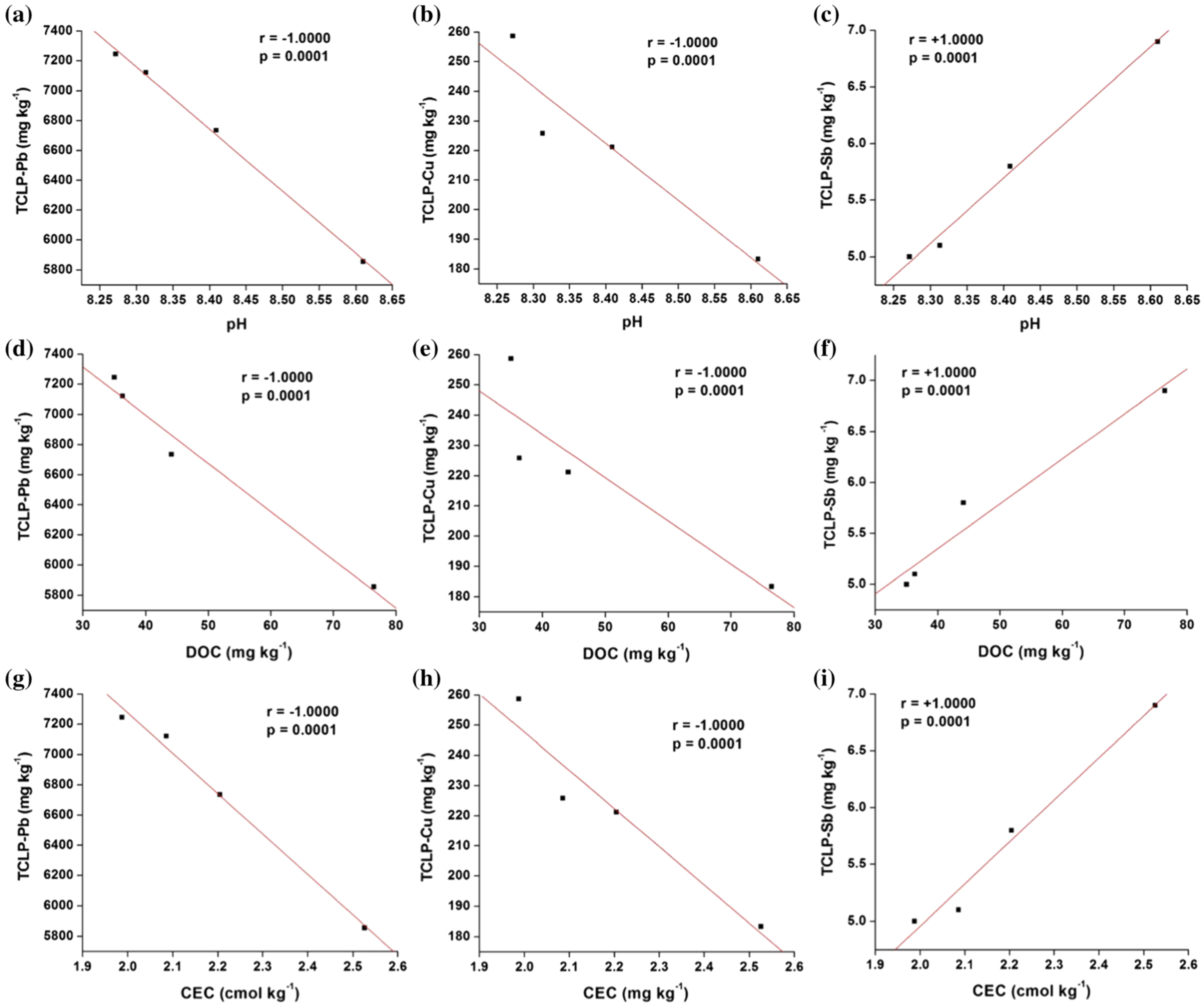

Fig. 4 Correlations between **a** cation exchange capacity (CEC) and toxicity characteristic leaching procedure (TCLP)-Pb, **b** pH and TCLP-Cu, **c** pH and TCLP-Sb, **d** dissolved organic carbon (DOC) and TCLP-Pb, **e** DOC and TCLP-Cu, **f** DOC and TCLP-Sb, **g** CEC and TCLP-Pb, **h** CEC and TCLP-Cu, **i** CEC and TCLP-Sb in the shooting range soil amended with soybean stover-derived biochar (BC)

complexes on the BC surface. However, the addition of MWCNTs did not enhance the release of DOC; hence, the sufficient free metal ions would not be available for the adsorption reactions. This may be a reason for showing the least immobilization of Cu and Pb in the MWCNT-amended soils. On the other hand, the least immobilization of Pb and Cu in the MWCNT-amended soils could be partially explained by the pH*pzc* of the MWCNTs, which was found to be pH 8.5 for the tested MWCNTs. The surface of MWCNTs becomes positively charged because the pH value in the amended soils is below the pH*pzc* ($pH < 8.5$), that may exhibit predominantly electrostatic repulsive forces with positively charged MWCNT surface and positively charged divalent Pb and Cu ions. This may also be the case for relatively less immobilization of Pb and Cu in the MWCNT-amended soils. At the same time, competition of positive other charges such as H^+ may have reduced the divalent cation sorption into the amendment. Although the aqueous medium studies have shown that the MWCNTs are promising for the removal of divalent cations from the system, it is not the case in the soil due to the complex mixture of different ions and functional groups as well as the presence of Dissolve Organic Carbon (DOC). Nevertheless, the addition of MWCNTs can be a

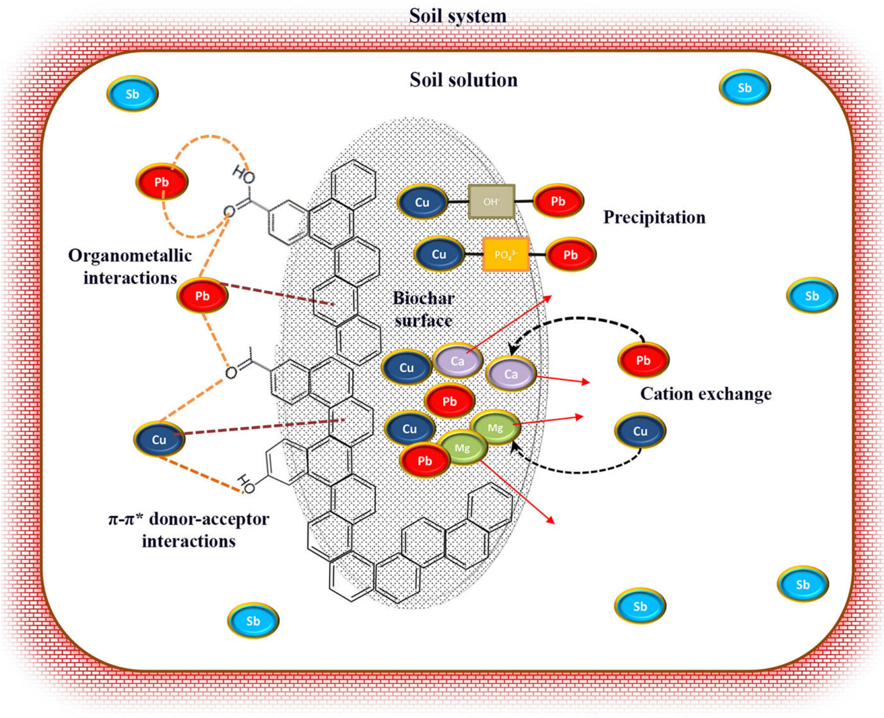

Fig. 5 A graphical representation of specific mechanisms for the immobilization of Pb and Cu in the shooting range soil amended with soybean stover-derived biochar (BC)

considerable tool to immobilize bioavailable Cu (e.g., by 17.4% in this study) in amended soils since it may be precipitated as hydroxides.

Both subjected amendments resulted in a considerable desorption of Sb to the soils compared to the control. This could be explained by analyzing the surface charge of amendments and the speciation of Sb in the soils. In soils, the Sb predominantly exits as oxyanions $Sb(OH)_6^-$ (Okkenhaug et al. 2013). The surface of BC is also negatively charged and shows repulsive electrostatic forces between Sb anions. It may lead to a mobilization of Sb from soil. Since the surface of MWCNTs is positively charged, it is expected to be immobilized Sb due to electrostatic interactions. However, the MWCNTs showed converse effects. This may be due to competitive effects of other anions such as PO_4^{3-} and SO_4^{2-} for the available sorption sites of the MWCNTs as well as due to the presence of DOC as competitors.

Conclusions

Biochar as a soil amendment is relatively effective in reducing bioavailable concentrations of Pb and Cu, but not Sb in the shooting range soils, based on the results of the DTPA-extractable metal(loid)s in this study. The application of BC to the soil also improved soil properties including CEC, DOC and nutrients such as SO_4^{2-}, NO_3^- and PO_4^{3-} in comparison with that of the MWCNTs. Precipitation and adsorption via electrostatic and π–π electron donor–acceptor interactions postulated to be involved in immobilizing Pb and Cu in the BC-amended soils, whereas the ion exchange mechanisms led to immobilize Cu in the MWCNT-amended soils. Therefore, future research is essential to apply a suitable type of BC to maximize the effectiveness of immobilization on metal(loid)s in multi-metal contaminated soils.

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