Environ Geochem Health
DOI 10.1007/s10653-016-9842-0

ORIGINAL PAPER

Efficacy of woody biomass and biochar for alleviating heavy metal bioavailability in serpentine soil

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Received: 15 February 2016 / Accepted: 10 June 2016

Abstract Crops grown in metal-rich serpentine soils are vulnerable to phytotoxicity. In this study, *Gliricidia sepium* (Jacq.) biomass and woody biochar were examined as amendments on heavy metal immobilization in a serpentine soil. Woody biochar was produced by slow pyrolysis of *Gliricidia sepium* (Jacq.) biomass at 300 and 500 °C. A pot experiment was conducted for 6 weeks with tomato (*Lycopersicon esculentum* L.) at biochar application rates of 0, 22, 55 and 110 t ha^{-1}. The $CaCl_2$ and sequential extractions were adopted to assess metal bioavailability and fractionation. Six weeks after germination, plants cultivated on the control could not survive, while all the plants were grown normally on the soils amended with biochars. The most effective treatment for metal immobilization was BC500-110 as indicated by the immobilization efficiencies for Ni, Mn and Cr that were 68, 92 and 42 %, respectively, compared to the control. Biochar produced at 500 °C and at high application rates immobilized heavy metals significantly. Improvements in plant growth in biochar-amended soil were related to decreasing in metal toxicity as a consequence of metal immobilization through strong sorption due to high surface area and functional groups.

Keywords Soil amendment · Ca/Mg ratio · Chemical stabilization · Black carbon · Charcoal

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Introduction

Serpentine soil is characterized by a high natural abundance of heavy metals such as Ni, Mn, Co and Cr (Hsiao et al. 2009; Oze et al. 2004; Rajapaksha et al. 2012, 2013). Weathering and dissolution may transport these toxic metals into surrounding environment. The presence of excessive metal concentration in the serpentine surroundings may reduce the agricultural productivity and generate the phytotoxicity and bioaccumulation in crops (Houben et al. 2013b). The serpentine-associated soils subjected to agriculture have shown high concentrations of metals in soil and plants, in Northwestern Spain (Fernandez et al. 1999), Canada (Baugé et al. 2013), Greece (Antibachi et al. 2012), Philippines (Susaya et al. 2010) and Japan (Kayama et al. 2002).

Published online: 17 June 2016

Conventional soil remediation techniques such as excavation, soil washing, land filling, etc. are still being used to remediate contaminated soils. However, the techniques are mostly recognized as inappropriate because of commotion in the environment, soil quality and economical unfeasibility (Houben et al. 2013b). Using soil amendments such as biosolid, animal manure, compost and biochar has recently been recognized as an alternative option, in order to reduce the mobility of toxic metals in metal-polluted soils (Hussain et al. 2016b; Zhang and Ok 2014).

Biochar is being considered as the latest promising innovation due to its universal applications in metal remediation, carbon sequestration, climate change mitigation and many other functions (Mohan et al. 2014; Ok et al. 2015; Vithanage et al. 2014b). Further, biochar has been evidenced to act as an efficient sorbent of various organic and inorganic contaminants, because of its high surface area and unique structural properties (Ahmad et al. 2014b; Tang et al. 2013). The addition of biochar as a soil amendment may reduce the bioavailability and the mobility of metal ions (Rajapaksha et al. 2016). Brennan et al. (2014) reported that biomass of maize grown in metal-contaminated soil was significantly increased by adding biochar due to a reduction in the phytotoxicity of Cu and As.

Heavy metals can be immobilized by biochar, and the properties and structure of biochar play an important role in this process. The characteristics of biochar are highly influenced by pyrolysis conditions and the feedstock type (Ahmad et al. 2014b; Wu et al. 2016). The pyrolysis temperature may affect several physicochemical properties of the biochars, such as the O/C ratio, surface area, cation exchange capacity, zeta potential and surface acidic groups, so that biochars possess different sorption abilities for the immobilization of heavy metals (Ding et al. 2014, 2016; Rajapaksha et al. 2016).

Biochar is being extensively used to remediate contaminated soils and waters (Hussain et al. 2016a; Rizwan et al. 2016). For example, Vithanage et al. (2014b) successfully immobilized sulfamethazine from an agricultural soil using invasive plant (*Sicyos angulatus* L.)-derived biochar. Moreover, biochars are also capable of increasing the activities of soil microorganisms such as mycorrhizal fungi, which can improve soil aggregation as reported in some recent studies (Ahmad et al. 2016b, c; Awad et al. 2012; Lehmann and Joseph 2009). The porous structure of biochar is a highly suitable habitat for microbes to colonize, grow and reproduce (Lehmann and Joseph 2009).

Gliricidia sepium (Jacq.) (Family Fabaceae, Leguminous tree with higher biomass production) plant has found wide use as a green mulch, animal fodder and cover crop planted as a plantation in Sri Lanka. Therefore, it has good potential to produce biochar, due to high abundance. Hence, the present study was mainly focused on the characterization of biochar produced by *Gliricidia sepium* (Jacq.) biomass at different temperature and evaluation of the effect of biochar on heavy metals immobilization and phytotoxicity reduction in the heavy metal-rich serpentine soil.

Materials and methods

Soil sample collection and biochar preparation

Pre-characterized serpentine soil (Table 1) obtained from Wasgamuwa (latitude 7°71′67″N and longitude 80°93′33″E), Sri Lanka, was used for this study (Vithanage et al. 2014a). Soil samples were air-dried and mechanically sieved to <2 mm of particle size prior to the experiments. To produce biochar, *Gliricidia sepium* (Jacq.) biomass was ground into small pieces (2 cm) and air-dried for 2 weeks. The ground biomass was placed in the muffle furnace (P300, Nabertherm, Germany) to produce biochar at two different temperatures viz 300 and 500 °C, respectively, under limited oxygen environment. To achieve slow pyrolysis, biomass was heated to the desired temperature at the rate of 7 °C min^{-1}. Holding temperature was 3 h for each biochar. The produced

Table 1 Basic soil properties of serpentine soil

Parameter	Value
pH	6.68 ± 1.02
EC (dS/m)	0.26 ± 0.05
TOC (%)	1.92 ± 0.90
Total metal concentration (mg kg^{-1})	
Ni	6567 ± 232.10
Cr	14,880 ± 356.87
Mn	2609 ± 267.18

biochars were named as BC300 and BC500, respectively. The biochars produced at different temperatures were ground and sieved through 1 mm aperture to achieve homogeneous particle size.

Biochar characterization

Determination of pH and electrical conductivity (EC) of biochars was done in 1:20 suspensions of biochar to distilled water using digital pH (702SM Titrino, Metrohm, Swiss) and EC (Orion 5 star meter, Thermo Scientific) meters, respectively. Cation exchange capacity (CEC) was measured following the ammonium acetate extraction procedure described by Anderson and Ingram (1989). The elemental composition (C,H,N,S and O) was determined by an elemental analyzer (Vario MAX CN, elementar, Hanau, Germany) (Rajapaksha et al. 2015). Surface areas of biochars were measured from N_2 isotherms at 77 K using a gas sorption analyzer (NOVA-1200; Quantachrome Corp, Boynton Beach, FL, USA). The Barrett–Joyner–Halenda (BJH) method was used to determine pore volume and pore diameter from the N_2 adsorption data (Rajapaksha et al. 2015). Proximate analysis, including moisture, mobile matter, ash and residual matter contents, was determined according to the modified thermal analysis methods of McCarl et al. (2009). Briefly, proximate analysis, moisture, was determined by calculating the weight loss after heating the biochar at 105 °C for 24 h to a constant weight. Mobile matter, reflecting the non-carbonized portion in biochar, was determined as the weight loss after heating in a covered crucible at 450 °C for 30 min. Ash content was also measured as the residue remained after heating at 700 °C in an open-top crucible. The portion of the biochar that is not ash is called as resident matter and was calculated by the difference in moisture, ash and mobile matter. The transmission spectra were obtained between 4000 and 400 cm^{-1}, with 1 cm^{-1} resolution and 128 scans using Fourier transform infrared spectroscopy (FTIR) (Nicolet 6700, USA) (Herath et al. 2015b).

Pot experiment

The pot experiment was conducted in a greenhouse during January 29, 2014–March 12, 2014. Local tomato (*Lycopersicon esculentum* L.) variety was selected as the trial crop because it has received numerous considerations due to crop growing in areas close to serpentine outcrops worldwide including Sri Lanka (Herath et al. 2015b; Kanellopoulos et al. 2015). Untreated soil (control) and soil amended with three amended rates of biochar were used for this experiment. The biochar was thoroughly mixed in 250 g of soil with four application rates, i.e., 0, 22, 55 and 110 t ha^{-1} (0, 1.0, 2.5 and 5.0 % by mass, respectively). All amended soils were thoroughly homogenized in large plastic containers and individually prepared prior to use. Plastic pots (11.5 cm in diameter and 10.5 cm in height) were filled with ~250 g of biochar-amended soil. After that, the pots were placed in a dark room for the soil mixtures to equilibrate over 2 weeks with 70 % of water-holding capacity. Five seeds of tomato (*Lycopersicon esculentum* L.) were sown in each pot, and plants were grown for 6 weeks in the greenhouse. Each amendment was performed in triplicate. The soil was irrigated with an equal amount of tap water (30 ml) three times per week to maintain soil moisture at 70 % of the water-holding capacity. During the experiment no fertilizer was added to soil amendments to enhance the growth of plants. The treatments consisted of a control soil without any amendment (S), biomass amended rate of 22 t ha^{-1} (BM-22), biomass amended rate of 55 t ha^{-1} (BM-55), biomass amended rate of 110 t ha^{-1} (BM-110), 300 °C biochar amended rate of 22 t ha^{-1} (BC300-22), 300 °C biochar amended rate of 55 t ha^{-1} (BC300-55), 300 °C biochar amended rate of 110 t ha^{-1} (BC300-110), 500 °C biochar amended rate of 22 t ha^{-1} (BC500-22), 500 °C biochar amended rate of 55 t ha^{-1} (BC500-55) and 500 °C biochar amended rate of 110 t ha^{-1} (BC500-110).

Meteorological parameters were collected daily by thermometer, wet and dry bulb hydrometer and lux meter installed in the greenhouse. During the experiment, the minimum, maximum and the mean air temperature was 22, 30 and 28 °C, respectively. The mean relative humidity and light intensity was observed 75 % and 400 lux respectively.

$CaCl_2$ extraction

The $CaCl_2$ extraction methods provide a proxy for evaluating plant bioavailability of Ni, Mn and Cr in serpentine soils (Rajapaksha et al. 2012). Therefore, the bioavailability of metals in biochars-treated and biochars-untreated soils remained in pots after harvesting of plants was quantified by the $CaCl_2$ extraction method. It is considered to be a simple, cheap and environment friendly method compared to the other methods to evaluate the bioavailable fraction of metals in soil (Ok et al. 2011; Rajapaksha et al. 2012). Hence, 1 g of air-dried soil was extracted with 10 ml of 0.01 M $CaCl_2$. The solid solution was stirred for 2 h, centrifuged and filtered through membrane filtration (0.45 μm pore size). The supernatant was analyzed via atomic absorption spectrometer.

Fractionation of heavy metals

Sequential extraction of heavy metals was performed using the method used by Rajapaksha et al. (2012). Initial extraction was carried out using 1 g of soil. A total of 3 replicate sequential extraction analyzes were completed on the four soil amendment. Filtrate followed by each extraction was analyzed for the concentrations of Ni, Cr and Mn using atomic absorption spectrophotometer (AAS). Consecutive extractions were involved in the sequential extraction; (i) exchangeable: soil was reacted at room temperature for 1 h with 20 cm^3 of magnesium chloride solution (1 M $MgCl_2$, pH 7.0) with continuous agitation, (ii) bound to carbonates: residue from (i) was leached at room temperature for 2 h with 20 cm^3 of 1 M sodium acetate (NaOAc) adjusted to pH 5.0 with acetic acid (HOAc) and with continuous agitation.

In the case of bound to Fe–Mn oxide (iii): Residue from step (ii) was treated with 20 cm^3 of 0.04 M hydroxylamine hydrochloride (NH_2OH-HCl) in 25 % (v/v) HOAc heated at 90 °C with slow continuous agitation for 2 h. Bound to organic matter (iv) was extracted from the residue of step (iii), treated with 3 ml of 0.02 M HNO_3 and 5 cm^3 of 30 % H_2O_2 adjusted to pH 2 with HNO_3 and heated to 85 °C for 2 h with occasional agitation. A 3 cm^3 aliquot of 30 % H_2O_2 (pH 2 with HNO_3) was added, and the sample was heated again to 85 °C for 3 h with intermittent agitation. After cooling, 5 cm^3 of 3.2 M NH_4OAc was added to 20 % (v/v) HNO_3, and the sample was diluted to 20 cm^3 and agitated continuously. For Residual (v): Residue from step (iv) was treated with a mixture of 10 cm^3 concentrated HF and 2 cm^3 concentrated $HClO_4$ and heated to near dryness; then, 1 cm^3 of $HClO_4^+$ and 10 cm^3 of HF were added and heated again to near dryness; 1 cm^3 $HClO_4$ was added, heated until the appearance of white fumes, and finally dissolved with 12 N HC1 and diluted to 25 cm^3 with deionized water. Between each consecutive extraction listed above [(ii) to (v)], the sample was centrifuged at 3500 rpm for 15 min. Additionally, the supernatant was filtered using 0.45-μm filter paper prior to AAS analysis.

Statistical analysis

All results were expressed as the mean values. The differences between non-amended and biochar-amended soils were analyzed by using a one-way analysis of variance (ANOVA). The mean separation was done using Duncan's multiple range test (at $P = 0.05$). All statistical analyzes were carried out using statistical software package (SAS 9.1).

Results and discussion

Biochar characterization

Biomass showed an acidic pH value (6.05), whereas biochars exhibited pH values ranging from weakly acidic to alkaline, depending on pyrolysis temperature (Table 2). The lowest pH value (6.71) was recorded for BC300, which is produced at 300 °C. However, the pH sharply increased and reached to 9.27 for BC500. The increase in pH with increasing pyrolysis temperature is mainly due to concentration of alkali salts and the loss of acidic functional groups at high pyrolysis temperatures (Al-Wabel et al. 2013; Vithanage et al. 2014b). It is speculated that biochars-induced pH will greatly influence the mobility of metals (Ahmad et al. 2013, 2016a).

The EC values of biochars produced at 300 and 500 °C were 0.21 and 0.54 dS m^{-1}, respectively, suggesting that biochars produced at high pyrolysis temperatures increase in soil EC due to the accretion of ashes containing soluble salts (Usman et al. 2016). Compared to the biomass, CEC values of biochars showed an increase with the increase in pyrolysis temperature mainly due to the concentration of cationic elements. Additionally, these elements might not be lost by volatilization (Al-Wabel et al. 2013).

The yield of biochar was reduced with increasing pyrolysis temperature, i.e., from 39.58 to 26.24 % at 300 and 500 °C, respectively. This decrease in the

Table 2 Proximate and ultimate analyses of BM, BC300 and BC500

Parameters	BM	BC300	BC500
pH	6.05	6.71	9.27
EC(dS/m)	0.09	0.21	0.54
CEC(cmol$^+$/kg)	4.59	4.39	4.98
Proximate analysis			
Yield (%)	–	39.58	26.24
Moisture (%)	9.68	3.57	2.66
Mobile matter (%)	76.46	28.58	11.79
Ash (%)	2.37	6.03	14.70
Resident matter (%)	11.47	61.80	70.85
Ultimate analysis			
C (%)	52.10	75.46	92.75
H (%)	7.03	4.76	3.55
O (%)	40.08	19.00	2.80
N (%)	0.72	0.72	0.84
S (%)	0.05	0.04	0.04
Molar H/C	0.13	0.06	0.04
Molar O/C	0.77	0.25	0.03
Surface area (m^2 g^{-1})	–	1.02	76.30
Pore volume (cm^3 g^{-1})	–	0.001	0.01
Pore diameter (nm)	–	38.40	70.30

BM—*Gliricidia sepium* biomass

BC300—Biochar produced at 300 °C

BC500—Biochar produced at 500 °C

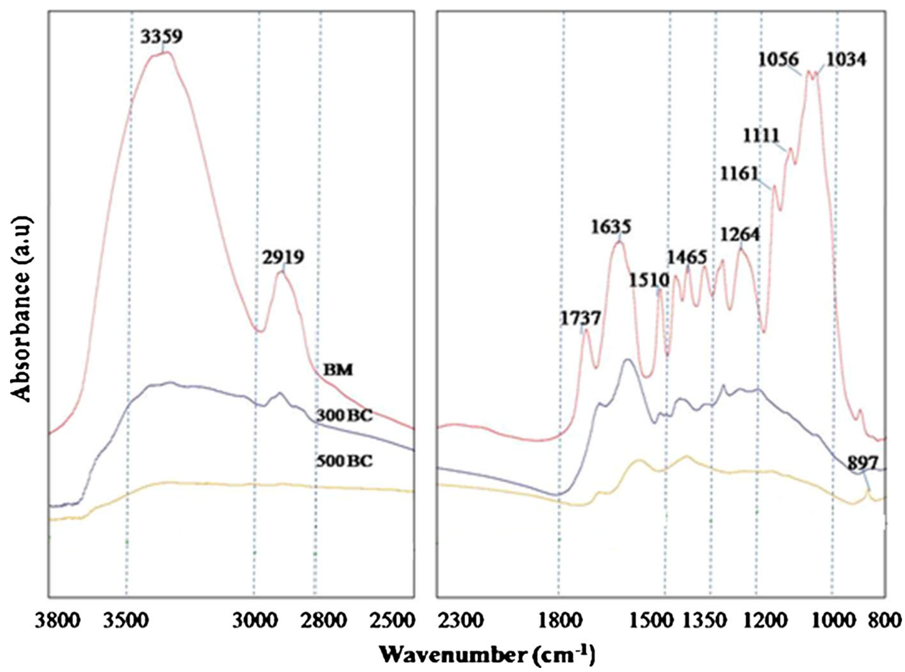
yield at high pyrolysis temperature could be due to a greater loss of volatile matter at the higher temperature (Ahmad et al. 2014a). Moisture and mobile matter percentages were reduced with increasing pyrolysis temperature. The high mobile matter is indicative of high susceptibility toward biodegradation (Ahmad et al. 2014a).

In contrast to mobile matter, the resident matter indicating the fixed or non-biodegradable matter increased with pyrolyzing temperature. The increase was determined to account for 70.85 % of BC500 compared with 61.80 % of BC300 and 11.47 % of biomass. This suggested that biochars have higher fixed C contents compared to its biomass, making them more stable and possibly more useful in sequestering C (Ahmad et al. 2014a; Rajapaksha et al. 2016). The percentage of ash content was increased from 2.37 % for biomass to 6.03 and 14.68 in biochars pyrolyzed at 300 and 500 °C, respectively. These are mainly due to the concentration of alkaline minerals and organic matter residues with the removal of organics. These results agreed with research findings of Ahmad et al. (2014a) and Al-Wabel et al. (2013).

The results showed that the C, N and S contents were increased in biochars as compared to the original biomass, whereas O and H content decreased. The H/C and O/C atomic ratios of the *Gliricidia sepium* (Jacq.) biomass are 0.13 and 0.77, and the respective values for biochars were reduced with increasing pyrolysis temperature. This is due to the progressive dehydration and decarboxylation reactions, indicative of the formation of condensed carbons such as the aromatic rings (Ahmad et al. 2014a; Masto et al. 2013). Molar O/C ratios, related to the degree of maturity, were found to be 0.77, 0.25 and 0.03 for biomass, BC300 and BC500, respectively, suggesting a greater stability of biochars produced at high pyrolysis temperatures. Another manner of molar O/C ratio is its relationship with polarity. Low O/C values betokened a lower degree of polarity, which indicated that BC500 is less polar or more hydrophobic than BC300 and biomass.

The broad peak at 3359 cm^{-1} in the spectrum of biomass indicates the presence of –OH stretching due to strong hydrogen bonding (Fig. 1) (Al-Wabel et al. 2013). Wood cellulose is a polymer rich in hydroxide groups, possibly water, that remains in the BC300 (Chia et al. 2012). However, the intensity of this peak decreased with increasing pyrolysis temperature (BC500), suggesting an ignition loss of –OH at high temperature (Al-Wabel et al. 2013). The band at 2919 cm^{-1} of biomass can be attributed to aliphatic –CH_2 stretching, and the presence of this band in BC300 indicates that the cellulose has not been entirely carbonized during pyrolysis (Chia et al. 2012). This band had disappeared completely in BC500, suggesting the removal of polar functional groups from biochars (Ahmad et al. 2014a). A peak at 1737 cm^{-1} is due to C=O stretching. This peak comprises a variety of C=O containing functional groups, including ketones, carboxylic acids esters and anhydrides (Chia et al. 2012). The intensity of this peak reduced with increasing temperature resulting in a slight stretching frequency in BC500 due to loss of volatile oxygenated groups with increasing pyrolysis temperature. Biomass is characterized by the small aromatic band at 1510 cm^{-1} originating from lignin and lignocelluloses (Smidt and Meissl 2007). The band at 1264 cm^{-1} indicates the aromatic CO- and

Fig. 1 FTIR spectra of biomass, BC300 and BC500



phenolic –OH stretching, and it is completely eliminated in biochar (Chen et al. 2008). The bands due to aliphatic C–O–C and alcohol-OH (1161–1034 cm^{-1}) in biomass indicate oxygenated functional groups (Chen et al. 2008). The appearance of the peak at 897 cm^{-1} in BC300 and BC500 was assigned to the aromatic –CH out-of-plane bending, predicting the condensation of smaller aromatic units into larger sheets (Ahmad et al. 2014a).

Effects of biochar on uptake of heavy metals and growth of tomato plants

Serpentine soil is rich in Cr, Ni and Mn (Rajapaksha et al. 2012, 2013) and low in plant nutrients (N, P and K), and Ca/Mg quotients are $\ll 1$, thereby limiting the growth of plants (Vithanage et al. 2014a). Tomato plants grown in control soil displayed low biomass production compared to the plants that were grown in biochar-amended soil. The phytotoxicity of heavy metals on the growth of plants was also observed. Two weeks after germination, signs of metal toxicity and nutrients deficiency (leaf chlorosis, necrosis, leaf epinasty and growth retardation) appeared in the above-ground parts of tomato plants grown in control soil and hence these plants were not able to survive 6 weeks after the germination of tomato seeds.

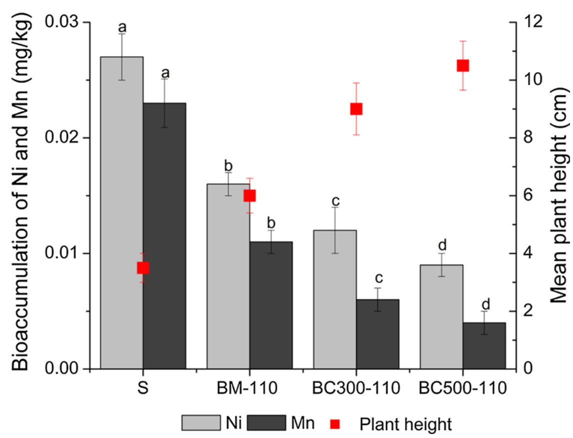


Fig. 2 Relationship between mean plant height and uptake of heavy metals. *Error bars* represent the standard deviation of the mean ($n = 3$). *Different letters* mean statistically significant differences ($P < 0.05$) between *bars* in each treatment

Figure 2 depicts the relationship between mean plant height and uptake of heavy metals in control and 110 t ha^{-1} biochar-amended treatments. With increasing pyrolytic temperature of the biochar, plant uptake of Ni and Mn were significantly reduced. In the presence of BC500-110, the reduction reached 66 % and 82 % for Ni and Mn, respectively. The bioaccumulation of Cr was not detected. The Cr consists of two stable oxidation states: trivalent Cr(III) and hexavalent Cr(VI), and Cr(III) is considered as a more stable ion in many soil systems (Herath et al. 2015a). Therefore, Cr translocation is very low due to high stable status in the soil system. Plants grown in biochar-amended soils showed no toxicity symptoms due to immobilization of toxic Cr, Ni and Mn and also facilitated the uptake of essential nutrients (N, K, Na) (Herath et al. 2015b). Moreover, due to the reduction in bioavailability of toxic heavy metals the plant height increased threefold in BC500-110 compared to the control. Plant-available Mg is abundant in serpentine soils and has been suggested as a major factor for suppressing plant growth due to prohibiting Ca uptake (Oze et al. 2008). It is clearly observed that with increasing pyrolytic temperature and application rates of biochar, soil Ca/Mg ratio significantly increased, and the highest ratio was observed in BC500-110-amended soil compared to the control (Fig. 3). According to the biochar characterization, with increasing pyrolysis temperature cationic ions concentration significantly increased. Other than soil heavy metals reduction, biochar increased Ca/Mg ratio in the serpentine soils. The heavy metals exchange with Ca^{2+}, Mg^{2+} and other cations associated with biochar attributing to co-precipitation and inner sphere complexation with mineral oxides of biochar (Zhang et al. 2013).

The growth of plants on biochar-amended soils is mainly attributed due to the reduction in heavy metals

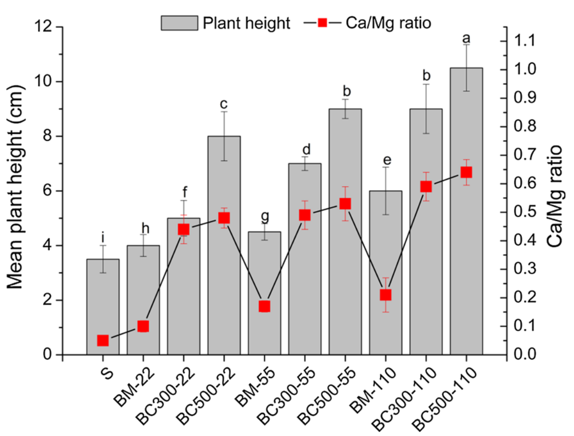


Fig. 3 Relationship between Ca/Mg ratio and growth of tomato plants in different treatments. *Error bars* represent the standard deviation of the mean ($n = 3$). *Different letters* mean statistically significant differences ($P < 0.05$) between *bars* in each treatment

bioavailability, while increasing the soil fertility status. Constructive effects on the biomass production of plants after biochar application have been described by several authors (Fellet et al. 2014; Graber et al. 2010; Saxena et al. 2013). Moreover, biochar may have a powerful ability to remediate heavy metals (Fellet et al. 2014) and provide a favorable environment for microbial growth (Rutigliano et al. 2014).

$CaCl_2$-extractable heavy metals concentrations

The $CaCl_2$ extractability of Ni, Mn and Cr significantly decreased after the incorporation of biochar. The reduction in metal bioavailability significantly increased with the biochar preparation temperatures and their rates of application. The most effective amendment for metal removal was BC500-110 as indicated by the removal efficiencies for Ni, Mn and Cr that were 68, 92 and 42 %, respectively, compared to the control. With increasing application rate of biochar, bioavailable concentrations of Ni had reduced by 17–68 %, for Mn; it was 40–92 % and for Cr; it was 16–42 % in BC500 amendment compared to the control.

Reduction in heavy metal concentrations mainly attributes to the BET surface area and surface functional groups of the biochar. The BET surface area of BC300 and BC500 was 1.02 and 76.30 $m^2\ g^{-1}$, respectively. These results implied that the bioavailability of these metals is decreased significantly with the concentration of biochar amendment and their pyrolyzing temperatures. Furthermore, Ni showed the highest $CaCl_2$ extractability, and hence, the bioavailability of Ni, Mn and Cr in both biochar-amended and biochar-unamended serpentine soil was in the order of Ni > Mn > Cr. Heavy metal immobilization ability of biochar increased with the increase in pyrolyzing temperature and application rate (Herath et al. 2015b), and it is mainly due to the increasing surface area and the pore volume under higher production temperature.

Environmental risks inherent to the presence of heavy metals in soils are mainly dependent on their bioavailable concentrations (Houben et al. 2013a). The application of biochar could change soil physiochemical properties by increasing the pH, surface area, and cation exchange capacity (CEC) of soil. Both soil pH and CEC increased with pyrolysis temperature and rate of amendments (Table 3). The interactions of cationic metals with biochar generally depend on its

Table 3 Effects of biomass and BC amendments on pH, CEC and TOC in serpentine soil

Treatments	pH	CEC (cmol+/kg)	TOC (%)
S	5.75 ± 0.26	3.74 ± 0.35	1.6 ± 0.15
1 % BM	5.80 ± 0.43	4.21 ± 0.53	2.42 ± 0.21
1 % BC300	5.87 ± 0.44	4.34 ± 0.56	2.10 ± 0.25
1 % BC500	5.90 ± 0.51	5.21 ± 0.68	2.43 ± 0.19
2.5 % BM	5.94 ± 0.12	4.32 ± 0.34	3.63 ± 0.23
2.5 % BC300	5.99 ± 0.34	5.62 ± 0.38	2.71 ± 0.18
2.5 % BC500	6.15 ± 0.51	7.13 ± 0.42	2.42 ± 0.15
5 % BM	6.14 ± 0.53	6.02 ± 0.71	4.29 ± 0.35
5 % BC300	6.20 ± 0.42	7.88 ± 0.22	3.44 ± 0.27
5 % BC500	6.50 ± 0.16	9.75 ± 0.25	2.48 ± 0.22

CEC which tends to be increased with raising pH of the system. Hence, the bioavailable concentrations of Ni, Mn and Cr implied that the bioavailability of these metals decreased significantly with the biochar application rates and their pyrolyzing temperatures. However, considering the upper limits of Ni, Mn and Cr (1, 800 and 30 mg kg^{-1}, respectively) in non-polluted soil environment (Christofaki 2011), the highest amount of exchangeable Ni above the upper limit still indicated the threat of Ni phytotoxicity to tomato plants, which can be overcome by using a higher application rate of BC500. The data of $CaCl_2$-extracted metals implied that the reduction in exchangeable fractions of all these metals is mainly due to the immobilization of Ni, Mn and Cr depending on biochar application rates and their pyrolyzing temperatures, thereby reducing the uptake of these metals from serpentine soil to tomato plants.

Heavy metals in different soil fractions

Figure 4 illustrates the effects of different biochar amendments on the distribution of Ni, Mn, and Cr in sequential extractions. Characteristically metals of anthropogenic inputs tend to exist in the first four fractions (Ratuzny et al. 2009), and the residual fraction is associated with silicates as well as with other primary oxides and natural occurrence in the parent rock (Rajapaksha et al. 2012).

The concentration of Ni, Mn and Cr in the exchangeable fraction of serpentine soil decreased significantly with the application rates as well as the pyrolyzing temperatures of biochar compared to the

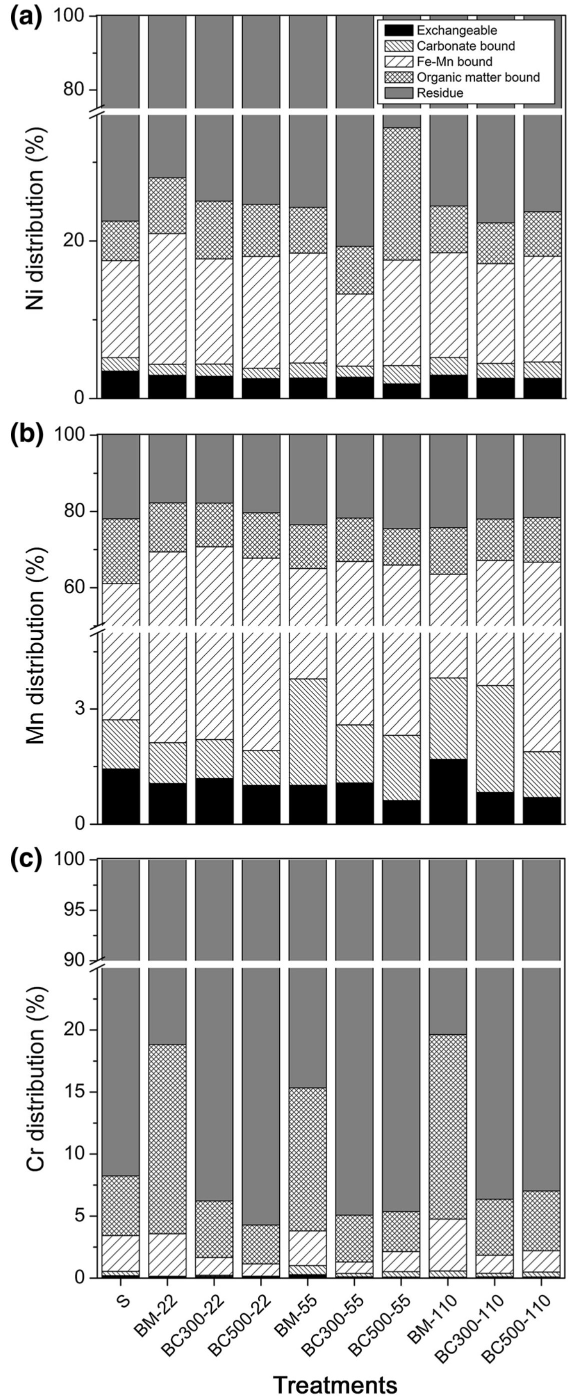


Fig. 4 Fractionations of soils, incubation with different treatments **a** Ni, **b** Mn, **c** Cr

control soil. With increasing application rates of biochars, exchangeable Ni, Mn and Cr concentrations were reduced by 19–30, 58–60 and 1–50 %, respectively, in BC500 treatment compared to the control. Application of biochar alters the metals in different fraction, and carbonate-bound Mn and Cr were 42 and 76, while Ni decreased 26 % in BC500-110 amendment compared to the control. Fe–Mn oxide fraction is a potential major source of Mn in serpentine soil, and addition of BC500-110 to serpentine soil resulted in 5, 21 and 35 % decrease in Fe–Mn oxide fractions of Ni, Mn and Cr, respectively, compared to the control soil. The organic matter-bound Ni, Mn and Cr was increased 13, 15 and 13 % compared to that in control soil. With regard to BC500-110-amended serpentine soil, the order of individual geochemical fractions of Ni, Mn and Cr, greatest to least are residual > Fe–Mn oxide bound > organic matter bound > carbonates bound > exchangeable; Fe–Mn oxide bound > residual > organic matter bound > carbonates bound > exchangeable; residual > organic matter bound > Fe–Mn oxide bound > carbonates bound > exchangeable, respectively.

Overall, the present study revealed that both BC300 and BC500 can effectively immobilize the bioavailable Ni, Mn and Cr in the exchangeable phase of serpentine soil. Immobilization of Ni, Mn and Ni in serpentine soil by biochar is likely to be due to several sorption mechanisms, including (i) organometallic interactions, (ii) sorption via π electron donor–acceptor interaction and (iii) pore diffusion (Ahmad et al. 2014b; Tang et al. 2013). The Ni, Mn and Cr are considered as transition metals possessing an excellent coordination affinity to bond with deferent functional groups of biochar surfaces such as OH, C=O, –COOH and C=N. The O and N atoms of these groups can act as ligands which tend to be donating their lone pairs of electrons to electron-deficient metal centers forming strong organometallic interactions. The presence of oxygenated functional groups on the surfaces of both BC300 and BC500 is evident with the FTIR data. On the other hand, aromatic structure of the biochar surface, particularly in BC500, promote the sorption of metals via π electron donor–acceptor interactions, because aromatic carbons containing double and triple bonds are a pool of π electrons which can readily donate electrons to the metal ions, thereby creating π electron donor–acceptor interactions (Herath et al. 2015a); (Ahmad et al. 2016b, c; Awad et al. 2012; Lehmann and Joseph 2009). The diffusion of elemental metals preliminary takes place through the pores of biochar surface which is encouraged by physical characteristics such as surface area, pore volume and pore size. Compared to the BC300, BC500 showed high surface area and pore size; it facilitates higher diffusion and sorption of heavy metals onto biochar (Table 2). However, the sorption may also be limited due to organic carbon present in the soil and many organic carbon (i.e., humic substances, water-soluble carbon) (Karabcova et al. 2015) can readily be attached to the BC surface, causing inner pores unavailable for metal diffusion (Herath et al. 2015a). Hence, a molecular level understanding within microscopic evidences for these sorption mechanisms is urgently needed to be addressed by future researches.

Conclusions

The present study was conducted to investigate the effect of woody biochar on soil quality by evaluating the bioavailability and phytotoxicity of heavy metals in serpentine soil. Pyrolysis temperature greatly influenced the physicochemical properties of biochar. Application of biochar to serpentine soil increased tomato plants growth associated with increased plants biomass mainly due to the immobilization of heavy metals and increasing Ca/Mg ratio. Immobilization of Ni, Mn and Cr was observed in biochar-amended serpentine soil. The decrease in exchangeable metals concentrations primarily causes a reduction in their bioavailability, thus alleviating the phytotoxicity. Furthermore, the present study shows that the application of *Gliricidia sepium* (Jacq.) biochar to heavy metal-rich serpentine soil may immobilize heavy metals in soil, reduce a bioavailable heavy metal fraction, and thereby reducing translocation and accumulation in plants.

Acknowledgments The biochar characterizations were partly performed at the Central Laboratory of Kangwon National University, Korea Basic Science Institute (KBSI) and the National Center for Inter-University Research Facilities of Seoul National University in Korea.

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