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BIOCHAR FOR A SUSTAINABLE ENVIRONMENT

Bioenergy-derived waste biochar for reducing mobility, bioavailability, and phytotoxicity of chromium in anthropized tannery soil

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Abstract
Purpose This study was aimed to investigate the potential of biochar (BC), a waste byproduct of a bioenegy industry, Sri Lanka, as a soil amendment to immobilize and reduce the phytotoxicity of Cr in tannery waste-polluted soil (TWS).
Materials and methods The TWS and bioenergy waste BC were characterized for physio-chemical parameters. A pot experiment was conducted by adding three BC application rates, 1, 2.5, and 5 % (*w*/*w*) to investigate the immobilizing capacity and bioaccumulation of chromium (Cr) in tomato plants (*Lycopersicon esculentum L.*). Soils and plants were digested via microwave digestion and analyzed for total Cr. Further, sequential extraction was conducted to assess the fractionation of Cr before and after the application of bioenergy waste BC on TWS.
Results and discussion The total Cr concentration in TWS was 12,285 mg/kg. The biomass of tomato plants grown in the 5 % BC amendment doubled compared to the biomass in BC-unamended soil. Bioaccumulation of Cr in plants grown in 5 % BC-amended TWS showed a decrease by 97 % compared to that of the BC-unamended soil. The $CaCl_2$ extractability of Cr indicated that the bioavailability of Cr in the 5 % BC amendment has decreased by 68 % compared to the control. Sequentially extracted Cr in the exchangeable fraction decreased by 98 % in the 5 % BC amendment.
Conclusions Pore diffusion, and adsorption via π-π electron donor-acceptor interactions were the primary mechanisms to be involved in the Cr retention in BC. Results suggested that the addition of BC to TWS reduces the mobility, bioavailability, and phytotoxicity of Cr in tomato plants.

Keywords Adsorption · Bioenergy · Immobilization · Phytotoxicity · Sequential extraction

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1 Introduction

Presence of excessive levels of heavy metals in soils is a widespread environmental problem due to its serious consequences for agricultural crop productivity and human health. Of the heavy metals of greatest environmental and toxicological concern, Cr is generally considered to be one of the most severe and high-risk pollutants originating from anthropogenic activities. Many industrial operations such as dye production, leather tanning, anodizing of aluminum, cooling towers, electroplating, and steel production have been seriously contributed to the release of Cr into the environment at excessive levels to adjacent water systems and to soil (Almaroai et al. 2014). On the other hand, Cr may directly release into the environment from Ultramafic bodies such as serpentine soil

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and sediments (Rajapaksha et al. 2012). Tanning industries are being recognized as one of the major sources of pollution in soils, since it disposes large quantities of effluents and sludge carrying a variety of contaminants including organic and inorganic nitrogen compounds, chromium, sulfides, suspended solids, and dissolved solids (Khan 2001; Durai and Rajasimman 2011).

Generally, Cr consists of two stable oxidation states: trivalent Cr (III) and hexavalent Cr (VI). The form of Cr (III) is considered to be an essential trace element in mammalian metabolism of glucose, cholesterol, and fat plant nutrient, whereas the Cr(VI) state is of particular concern since it is capable of easily penetrating the cell wall and exert its noxious influence in the cell itself, which may later lead to various cancer diseases (Costa and Klein 2006; Gil et al. 2006). Moreover, Cr(VI) compounds are generally more soluble, mobile, and bioavailable in the environment compared to Cr(III) compounds. Therefore, risks encountered with Cr to environmental contamination depend on its oxidation state. Tannery sludge disposal over about 20 years has resulted in high total Cr levels in soil ranging between 7 and 10 % (Cabeza et al. 1998). It is found that much of Cr in tannery waste-polluted soil is in the form of Cr(III) under sufficient reducing conditions and it occurs in top more layers of the soil with low solubility, whereas Cr(VI) is present in relatively low levels with high potential release into the deeper soil layers (Sakthivel and Vivekanandan 2009).

The growth of some plants in tannery waste-polluted soil (TWS) may accumulate high concentrations of Cr in their edible parts (Khan 2001). The prolonged consumption of metal accumulated plants such as *Zea mays* L. (Almaroai et al. 2013). *Allium sativum* L. (Jiang et al. 2001). and *Brassica napus* L. (Houben et al. 2013) may pose serious health risks when their consumption leads to concentrations above the toxicity threshold, even for micronutrient metals such as Cu, Mn, and Zn. The permissible limit values of Cr in plants recommended by WHO is 1.3 mg/kg, and hence, tomato plants tend to show phenotypic symptoms under high concentrations of Cr(VI) (Goupil et al. 2009). In a laboratory study, the maximum accumulation of Cr in the leaves of *Vallisneria spiralis*, *Azadirachta indica*, and *Alternanthera sessilis*, was found to be 1378, 458, and 201 mg/kg, respectively, after 9 day of Cr exposure (Sinha et al. 2002). Another study investigated the relationship between Cr biomagnification, accumulation and mycorrhizal fungal infection in *Dalbergia sissoo*, *Acacia arabica*, and *Populus euroamericana* growing in tannery effluent polluted soil (Khan 2001). Hence, the cultivation of crop plants in areas within and adjacent to tannery waste polluted lands and other heavy metal-enriched sites may be of particular concern, due to both phytotoxicity and metal accumulation. Therefore, the restoration of such heavy metal-rich soils using novel and economically feasible technologies is an urgent necessity before they are used in agriculture. Soil washing/flushing (Davis and Olsen 1995) chelate-enhanced phytoremediation/phytoextraction (Mahimaraja et al. 2011) and application of soil amendments are some of the more common soil treatment processes applied for the remediation of Cr polluted soils (Choppala et al. 2012). Chromium remediation in soils involves extraction from the soil matrix by washing with a chelating agent, and the major risk of applying this remediation approach is the increase in the oxidation potential of soil enhancing the conversion of trivalent Cr into the soluble and more toxic hexavalent form of Cr (Di Palma et al. 2012). On the other hand, the use of conventional soil remediation technologies such as soil replacement, solidification, electro-kinetic extraction, and washing strategies has become limited due to their high cost and environmental pollution (Vane and Zang 1997).

Bioremediation of contaminated soils is a widely used technology in which native or introduced microorganisms and/or biological wastes such as compost, animal manures, and plant residues are used to detoxify or transform toxic forms of metals to less toxic forms (Baker et al. 1994; Sadowsky 2000; Almaroai et al. 2013). Several studies have revealed that the Cr(VI) in soils can be effectively reduced to Cr(III) by application of biosolids compost and farmyard or poultry manures (Tokunaga et al. 2003; Choppala et al. 2015). Organic carbon in these amendments stimulates the release of dissolved organic carbon (DOC) by microbial communities, which acts as an electron source for the reduction of Cr(VI) (Bolan and Duraisamy 2003). Recently, a considerable interest has been expressed in the use of organic carbon-rich amendments like biochars (BCs) to remediate heavy metal-contaminated soils (Cao and Harris 2010; Beesley et al. 2011; Park et al. 2011). Generally, BC is a carbon-rich product that is produced by pyrolysing bio-waste materials. It is capable of improving physical, chemical, and biological properties in soils due to its high organic carbon content. The application of BC as a soil amendment leads to increase soil fertility and enhance plant growth by supplying and retaining essential nutrients while improving soil physical and biological properties (Houben et al. 2013). The positive effects of BC on the immobilization of bioavailable heavy metals in contaminated soils have been investigated under greenhouse conditions (Park et al. 2011; Houben et al. 2013; Zhang et al. 2013). Green waste compost and BC amendments were successfully applied to reduce the mobility and uptake of Pb and Cu to ryegrass plants during successive harvests (Karami et al. 2011). In a similar study, as mobility was investigated in three different brownfield soils amended with green waste compost and BC planted with *Miscanthus* (Hartley et al. 2009). However, no many studies have been conducted regarding the effects of BC application on the changes in properties of Cr-polluted soils and immobilization of Cr in such soils. BC derived from maize stalk has applied to heavily Cr-polluted soil, and results obtained in this study showed that BC addition increases soil fertility, enhances nutrient uptake, and ameliorates Cr-polluted soils (Nigussie et al. 2012). The addition of bioamendments including black carbon, chicken manure BC, and cow manure found to increase the reduction rate of Cr (VI) to Cr (III) in contaminated soil thereby reducing the bioavailability.

Due to the fact that the presence of excessive metal levels in soils surrounding, TWS may have serious consequences for groundwater and agricultural productivity, the remediation of sites adjacent to such metal-enriched settings can provide much-needed land for the cultivation of plants to use as food or animal feed. This is the first time reporting of using a waste byproduct of a bioenergy industry (Dendro) on the immobilization of Cr contaminated in TWS, along with postulated mechanisms. Hence, this study was aimed to investigate the potential of waste woody BC from a bioenergy industry as a soil amendment to immobilize bioavailable Cr and reduce its phytotoxicity in tomato plants (*Lycopersicon esculentum L*; *Solanaceae*). Subsequently, the outcomes of this study are believed to be a good option as a green strategy in order to manage wastes generated from the Dendro bioenergy industry in Sri Lanka.

2 Materials and methods

2.1 Characterization of TWS and amendments

Soil used in this study was collected from two areas close to tannery industries, which were located about 10 and 20 km North of Colombo, Sri Lanka. The BC was collected as a waste byproduct from a bioenergy industry (Dendro) in Sri Lanka. In the bioenergy industry, this BC was produced by pyrolysing the woody biomass of *Gliricidia sepium* (Jacq.) Steud. (Fabaceae) in a closed reactor. The end temperature of this process was recorded as 900 °C at which *Gliricidia* wood is gasified for the generation of electricity. The BC obtained from this power plant was air-dried and ground in a blender and sieved to <1 mm of particle size prior to use in the experiments.

The soil and BC samples were air-dried and sieved to pass through a 2- and 1-mm sieve, respectively. The pH and electrical conductivity (EC) of soil and BC were measured in a 1:5 soil-to-water ratio suspension using a digital pH meter and EC meter, respectively. Exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) were determined using the 1-M ammonium acetate method (Ross 1995). Soil organic matter was determined following the Walkley-Black method (Walky and Black 1934). In order to determine the total Cr concentration, an aliquot of soil samples was digested in *aqua regia*, and digested samples were analyzed by using atomic absorption spectroscopy (GBC AAS 933A). The surface area of BC was determined following the BET method (Peterson et al. 2012). The Fourier transform infrared spectroscopy (FTIR) spectra of vacuum-dried sample pellets, prepared with fused-KBr, were obtained with a resolution of 1 cm^{-1} between 4000 and 400 cm^{-1}. The spectra were analyzed using OMNIC version 8.0 software.

2.2 Pot experiment

Untreated soil (control) and soil treated with three rates of BC applications were used in the experiment. Soil was amended by mixing dry BC with a mass fraction of 1, 2.5, and 5 % (*w*/*w*). Amended soils were thoroughly homogenized in large plastic containers prior to use. Plastic pots (12.0 cm diameter, 9.0 cm height) were filled with ~250 g of BC-amended and BC-unamended soil. The pots were then placed in a dark room for the soil mixtures to equilibrate over 2 weeks. Each treatment was performed in triplicate. After the equilibration period, the pots were transferred to an outdoor greenhouse and arranged in a completely randomized design (CRD). In each pot, 25 seeds of tomato were sown and plants were grown for 9 weeks. All pots were irrigated with 30 cm^3 of tap water three times per week.

2.3 Plant tissue analysis

From each treatment, three whole plants per pot were harvested 9 weeks after sawing tomato seeds. Whole plants were used as it was difficult to obtain enough biomass for metal analysis by separating shoots and roots. Harvested plants were thoroughly washed with tap water following a diluted EDTA solution and finally with distilled water to remove any metal ions trapped on the root surface. After air-drying in a forced draft oven at 60 °C for 48 h and subsequent cooling in a desiccator, the dry weight of each plant was measured. The total amount of Cr in plant tissues was analyzed by AAS after digestion with 10 cm^3 of concentrated HNO_3 acid in a closed vessel temperature controlled microwave digester system (Milestone ETHOS PLUS labstation with HRP-1000/10S high pressure segmented rotor).

2.4 $CaCl_2$ extraction

The $CaCl_2$ extraction procedure was conducted as a proxy for evaluating plant available Cr in BC-amended and BC-unamended soil. From each treatment, 1 g of soil was extracted with 10 cm^3 of 0.01 M $CaCl_2$ by stirring the solution for 2 h, centrifuging, and filtering through a membrane filtration (0.45-μm pore size). The supernatant was analyzed by AAS.

2.5 Sequential extraction

Sequential extractions involved the selective extraction of trace metals from soil solid fractions, providing detailed information on the availability of heavy metals among the different geochemical phases in soil (Rajapaksha et al. 2012). Ideally, the extractants are chosen to selectively target a specific soil compartment with minimal dissolution of non-targeted phases. The sequential extraction procedure was carried out on both control and post-harvest soils following standard methods (Peijnenburg et al. 2007). A mass of 1 g soil (dry weight) was used for the initial extraction. A total of five replicate sequential extraction analyses were completed on the four soil treatments. The concentrations of Cr were measured in the effluent after each extraction using AAS. The following is a list of the extraction procedures performed on the soil amendments.

(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.
(iii) Bound to Fe-Mn oxide: Residue from (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.
(iv) Bound to organic matter: Residue from (iii) was treated with 3 cm^3 of 0.02 M HNO_3 and 5 cm^3 of 30 % H_2O_2 adjusted to pH 2 with HNO_3, heated to 85 °C for 2 h with occasional agitation. A 3.0-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 in 20 % (*v*/*v*) HNO_3 was added and the sample was diluted to 20 cm^3 and agitated continuously.
(v) Residual: Residue from (iv) was treated with a mixture of 10 cm^3 concentrated HF and 2 cm^3 concentrated $HClO_4$ and heated to near dryness. It was then treated with 1 cm^3 of $HClO_4^+$, 10 cm^3 of HF, 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 successive 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.

2.6 Microbial analysis

Post-harvest soil in each pot was subjected to a microbial analysis to evaluate the effects of BC on the total microbial count in BC-amended and BC-unamended soil. A dilution series was prepared from 10^{-1} to 10^{-5} and each dilution was plated in nutrient agar (NA) and potato dextrose zgar (PDA) media to determine the number of bacteria and fungi in soil, respectively. The NA and PDA plates were incubated at 37 °C in 24 and 72 h, respectively. Finally, the number of colony forming units (CFU) was counted (Odeyemi et al. 2011).

2.7 Statistical analyses

Statistical analyses were carried out to compare the average results of different BC application rates on the growth of plants and the accumulated concentrations of Cr in plant tissues 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 contrast were used after performing the ANOVA for complete randomized design (CRD). All statistical analyses were carried out using statistical software package (SAS 9.1).

3 Results and discussion

3.1 Physico-chemical characteristics of TWS and BC amendments

The basic physico-chemical parameters of TWS and BC amendments are presented in Table 1. The average concentration of total Cr in the soil was 12,285 mg/kg. The maximum concentration of Cr(VI) determined was 210 mg/kg that was approximately 58-fold lesser than the concentration of Cr(III). The Cr(III) form may complex with humic matter which could potentially reduce the available Cr(III) to be oxidized to the most toxic form of Cr(VI) (Bartlett 1991). Also, the pH of the soil is one master variable that controls the redox reaction between Cr(III) and Cr(VI). In this case, the pH of soil samples was high, which generally supports the reduction of Cr(VI) by organic matter, Fe(II) and sulfides (James et al. 1995). Alternatively, much Cr(VI) in the soil may have undergone microbial reduction by indigenous Cr-resistant bacteria (Chen and Hao 1998). Nevertheless, the concentration of Cr(VI) was extremely high and dissolution of this Cr(VI) into the environment can be catastrophic. The presence of Cr(VI) soil may also be due to the presence of Mn minerals in the soil (Fendorf 1995; Oze et al. 2007).

The influence of BC treatments on changes in several physico-chemical parameters including pH, EC, and total organic carbon (TOC) in TWS were investigated (Table 1). The increase in soil pH after the addition of BC is mainly due to the alkali nature of the BC. The dissolution of oxides, hydroxides, and carbonates of alkaline substances, decarboxylation of organic anions, and the ammonification of the soil provide nutrients for plant growth as well as increase in soil pH which may minimize the bioavailability of heavy metals in soil (Houben et al. 2013). The FTIR data obtained for *Gliricidia* BM and its BC suggested the dehydration of cellulosic and ligneous contents in the BM and an increase in the

Table 1 Physico-chemical characteristics of tannery waste soil and biochar (n=3)

	pH	EC	CEC	TOC	Metal concentrations/mg kg^{-1}					
		µS cm^{-1}	cmol kg^{-1}	mg g^{-1}	Ca	Mg	K	Na	Cr	Cr(VI)
TWS	4.5±0.05	127.0±0.02	2.7±0.6	177.5±5.8	2488±133	76.2±9.5	1205.4±160.4	36.3±3.5	12285±237	210±8.1
BC	10.1±0.04	1132.0±0.06	5.3±0.8	648.0±12.7	2103±140	562±18	9229±240	427±21	ND	ND
1 % BC	4.9±0.02	74.4±0.08	2.8±0.5	213.4±16.1	2977.5±277	99.5±6.4	42.6±3.7	52.6±9.6	–	–
2.5 % BC	5.6±0.06	83.6±0.01	2.9±0.6	226.6±8.4	2916.4±164.8	76.7±12.3	733.3±54.0	32.6±3.7	–	–
5 % BC	6.8±0.01	90.0±0.05	3.8±0.2	274.8±10.2	4188.5±193.7	95.8±6.5	1638.7±155.6	62.1±6.8	–	–

TWS BC-unamended soil (control), *BC* biochar, *1 % BC* 1 % BC-amended soil, *2.5 % BC* 2.5 % BC-amended soil, *5 % BC* 5 % BC-amended soil, *ND* not detected

condensation of aromatic units in the BC produced at a high temperature (Fig. 1a). The SEM micrographs of the biomass of *Gliricidia* and its BC derived at 900 °C are shown in Fig. 1b. The structures of both materials clearly showed that the dendro BC consists of a macro-porous surface texture and the surface of the BC is smoother than that of the biomass, suggesting that the production of BC at 700 °C could create more pores and lead a considerable removal of volatile matter present in the biomass.

3.2 Effects of BC on the growth of plants

Figure 2a depicts the variation of the biomass production of tomato plants grown in BC-amended and BC-unamended TWS. The addition of BC to the soil increased significantly the dry matter of tomato. The least biomass production was observed in tomato plants grown in BC-unamended TWS compared to the plants that had received 1, 2.5, and 5 % BC applications. Six weeks after sowing of tomato seeds, signs of Cr toxicity in the aboveground parts of tomato plants were apparent in the BC-unamended TWS (Fig. 3). The common diagnostic symptoms of Cr phytotoxicity such as leaf chlorosis, necrosis, and growth retardation were displayed in plants that grew in the BC-unamended soil. Such symptoms are usual for tomato plants submitted to heavy metal stress and their morphology has been addressed by several studies (Moral et al. 1995).

There was a significant increase in the biomass of tomato plants with increasing BC application rates (Table 2). The biomass of plants grown in BC-unamended TWS was reduced by 67 % compared to the plants that had received 5 % BC application. This biomass reduction could be due to the bioaccumulation of Cr in the tomato plants and thereby resulting phytotoxicity inhibiting their growth. Overall, 5 % BC amendment in TWS resulted in increasing the biomass of tomato plants by 2-fold compared to the BC-unamended soil. Such an increase in biomass production of tomato plants after

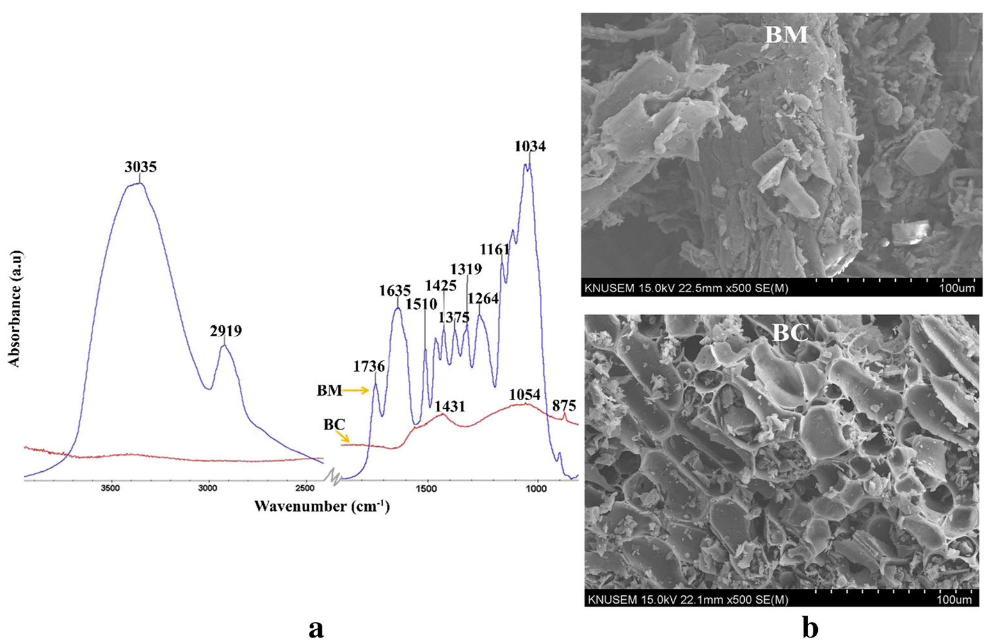


Fig. 1 **a** FTIR spectra of the biomass of Gliricidia (*BM*) and its biochar (*BC*) derived at 900 °C. **b** SEM images of Gliricidia BM and its BC derived at 900 °C

addition of BC to TWS could be due to both its fertilizing effect and the immobilization of Cr in TWS. However, the analysis of tomato plant tissues showed that the addition of BC to TWS had mostly contributed to the promotion of plant growth while enhancing the fertilizing effects. Moreover, these findings agree with those of recent studies (Houben et al. 2013) confirming higher plant productivity when BC is applied, likely resulting from the immobilization of Cr in TWS.

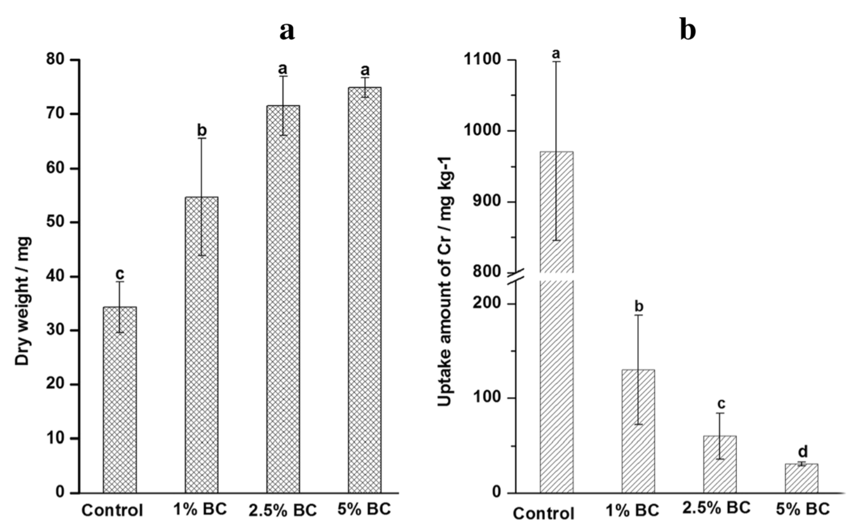
3.3 Effect of biochar on uptake of Cr in tomato plants

Accumulated concentrations of Cr in tomato plants grown in BC-amended and BC-unamended TWS 9 weeks after sowing of tomato seeds is depicted in Fig. 2b. All BC applications significantly reduced the uptake and bioaccumulation of Cr in tomato plant tissues, and maximum accumulated concentration of Cr was found in tomato plants grown in BC-unamended soil (Table 2). Compared to the control, 1, 2.5, and 5 % BC application rates reduced the accumulation of Cr by 87, 94, and 97 % respectively. Overall, the bioaccumulation of Cr in tomato plants grown in BC-unamended soil was 31-fold higher than that of 5 % BC-amended soil. Because of such a high bioaccumulation of Cr, tomato plants grown in BC-unamended TWS were not able to survive longer due to metal-induced phytotoxicity. The permissible limit value of Cr in plants recommended by WHO is 1.3 mg/kg. Accumulation of excessive amounts of Cr is believed to be interfered with Fe uptake and metabolism causing chlorosis and necrosis in plants (Ghani 2011). In this study, since Cr accumulation in tomato plants was far higher the threshold concentration, it is noted that these plants are not capable of surviving at a high level of Cr contamination in TWS. Therefore, this high accumulation of Cr in plants grown in BC-unamended soil in decreasing biomass as well as the occurrence of some visible signs of metal toxicity in the above ground parts of tomato plants. Hence, the reduction of toxic levels of Cr in the presence of BC could be attributed to improving the patterns of essential nutrient uptake by tomato plants, resulting in high biomass production. Hence, plant tissue analysis data suggested that the higher biomass production in the presence of 5 % BC application was accompanied by the enhancement of soil fertility as well as the reduction in plant uptake of metal ions.

3.4 Effects of BC on microbial growth in TWS

Effects of BC on changes in microbial quantity in TWS are also of particular concern. The maximum number of CFU was found in 2.5 % BC application whereas 5 % BC showed significant reduction in CFU (Fig. 4). This positive effect may be attributed to a general improvement in physical and chemical characteristics of the 2.5 % BC application. The pores in BC may provide a suitable habitat for microorganisms by protecting them from predation and drying while providing nutrients (Thies and Rillig 2009). Several recent studies have shown that BC is capable of stimulating the activity of agriculturally important soil microorganisms (Pietikainen et al. 2000). Even though the increment of BC application increases the quality of soil physicochemical properties (Thies and Rillig 2009). Infinite increase in BC application would not be beneficial for the biological activities in soil. Hence, a reduction in the CFU was observed with 5 % BC application. This could be due to the changes in soil structural properties and the adsorption of extracellular enzymes and inorganic nutrients to the BC surface (Thies and Rillig 2009). Moreover, high cation exchange capacity (CEC) in the 5 % BC amendment may enhance the binding capacity of important macro nutrients such as nitrogen and phosphorus, negatively impacting microbial growth (Gomez et al. 2014).

Fig. 2 Effects of BC on **a** the growth of tomato plants and **b** the accumulated concentration of Cr in tomato plants grown in BC-amended/unamended TWS, 9 weeks after sowing of seeds. *Control* BC-unamended soil, *1 % BC* 1 % BC-amended soil, *2.5 % BC* 2.5 % BC-amended soil, *5 % BC* 5 % BC-amended soil. *Error bars* represent the standard deviation of three replicates. The same letters above each bar indicate no difference at a 0.05 significance level



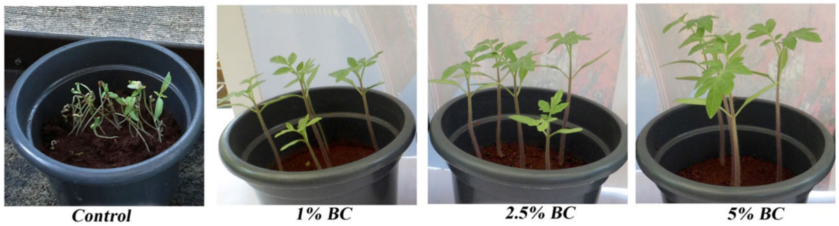


Fig. 3 Representative image showing differences in tomato plant growth 9 weeks after sowing. From *left* to *right*: BC-unamended soil, 1 % BC-amended soil, 2.5 % BC-amended soil, 5 % BC-amended soil. Only one of the three replicates is shown for each treatment

3.5 Bioavailability of Cr and their partitioning in the TWS

Environmental risks associated with the presence of heavy metals in soils are mainly dependent on the bioavailability of metals. The $CaCl_2$ extraction data are generally a good indication of plant bioavailability of metals present in soils and partly in easily exchangeable forms. The $CaCl_2$ extractable concentrations of Cr in BC-amended and BC-unamended TWS are shown in Fig. 5a. Bioavailable concentrations of Cr significantly decreased after the addition of BC to TWS at different rates. Reduction of Cr availability in different BC amendments showed a significant decrease with increasing BC application rates. Compared to the control soil, 1, 2.5, and 5 % BC application rates decreased the $CaCl_2$ extractable Cr concentrations by 28, 52, and 68 %, respectively. Hence, the $CaCl_2$ extractable concentrations revealed that the bioavailability of Cr decreases significantly with increasing BC application rates.

Sequential chemical extraction was used to determine Cr partitioning among different discrete phases of TWS and to elucidate their release and immobilization in the presence of BC at different rates. The amounts of sequentially extracted Cr from different phases, including exchangeable, carbonate, Fe-Mn oxide, and organic matter of BC-amended and BC-unamended TWS, are shown in Fig. 5b. Sixty-three days after the addition of BC amendments to the TWS, a significant reduction of the exchangeable fraction of Cr was observed in all application rates of BC, compared to the control soil. The 1, 2.5, and 5 % rates of BC applications attributed to decrease in exchangeable concentrations of Cr by 93–98 % compared to the control soil. Because of the fact that the exchangeable fraction of Cr is highly responsible for the bioavailability, reduction in the exchangeable concentrations by the BC amendments may result a minimum bioaccumulation of Cr in tomato plants.

The highest amount of Cr in both BC-amended and BC-unamended TWS was found to be in the phase of Fe and Mn oxide, compared to other phases (Fig. 5). This is supposed to be quite environmentally friendly, since naturally occurring redox processes associated with the Fe and Mn oxide phase tend to control the mobility of Cr in the soil by transforming highly mobile and toxic Cr(VI) species into less mobile forms such as Cr(III). In the Fe-Mn oxide phase, Cr(VI) species gets reduced to Cr(III) by organic matter and Fe(II), on the other hand MnO_2 can act as an oxidizing agent of Cr(III) to produce highly mobile forms of Cr including Cr(VI). Hence, the concentration of such mobile forms of Cr in this phase was significantly decreased in all BC amendments (Fig. 5). Interestingly, with the increase of BC application rates from 1 to 5 %, the concentration of Cr in the Fe-Mn oxide phase

Table 2 Comparison of the biomass of tomato plants and accumulated concentrations of Cr in plant tissues due to the influence of different biochar application rates

BC Treatment	Biomass per plant /mg	Total Cr concentration /mg kg^{-1}
Control	34.3±4.7c	971.8±125.5a
1 % BC	54.6±10.8b	130.8±58.3b
2.5 % BC	71.5±5.5a	60.5±23.8b
5 % BC	74.8±1.8a	31.5±2.3b

For each harvest, column means (n=3) with the same letter do not differ significantly at the 5 % level according to the Fisher's multiple comparison test

Control BC-unamended soil, *1 % BC* 1 % BC-amended soil, *2.5 % BC* 2.5 % BC-amended soil, *5 % BC* 5 % BC-amended soil

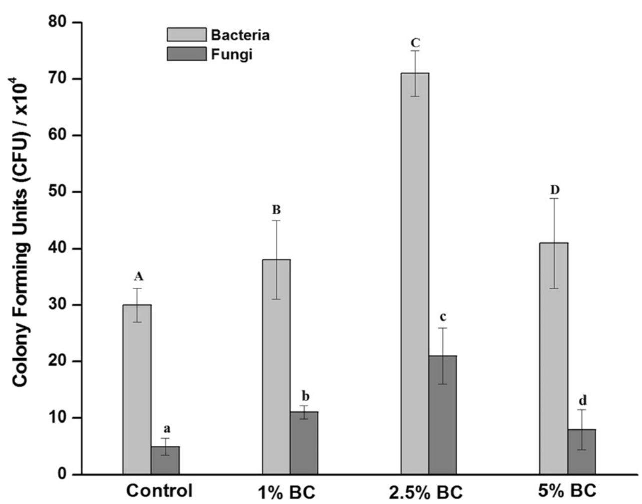


Fig. 4 Total number of bacteria and fungi per gram of soil in different BC treatments. *1 % BC* 1 % biochar-amended soil, *2.5 % BC* 2.5 % biochar-amended soil, *5 % BC* 5 % biochar-amended soil. *Error bars* represent the standard deviation of three replicates. The same letters above each bar indicate no difference at a 0.05 significance level

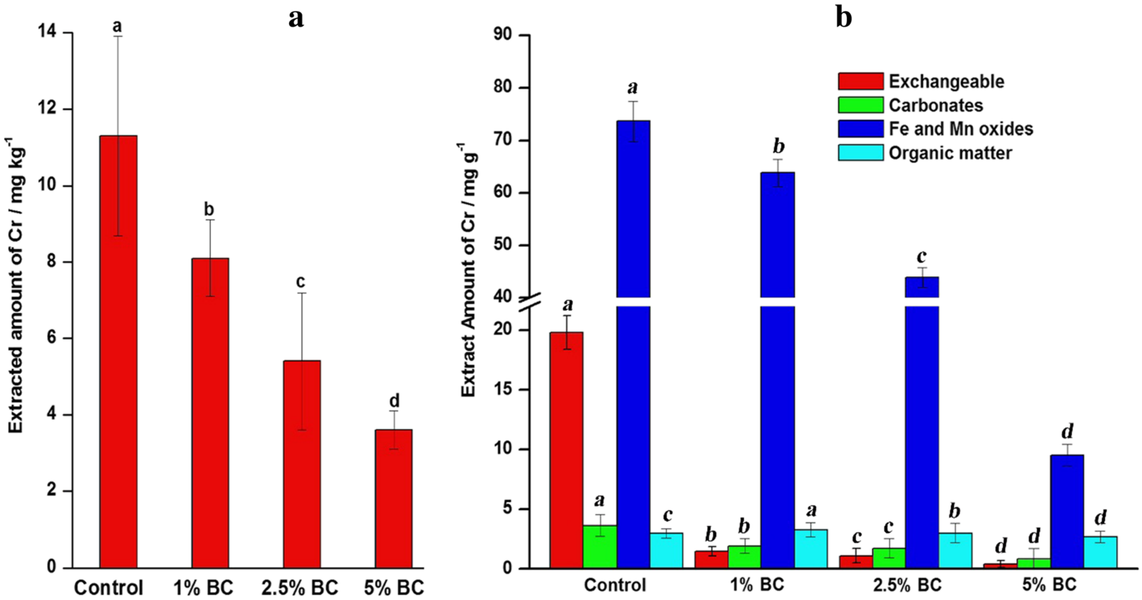


Fig. 5 **a** $CaCl_2$ extractability of Cr in BC-amended and BC-unamended TWS. *1 % BC* 1 % biochar-amended soil, *2.5 % BC* 2.5 % biochar-amended soil, *5 % BC* 5 % biochar-amended soil. **b** Sequentially extracted concentrations of Cr in BC-amended and BC-unamended TWS. *1 % BC* 1 % biochar-amended soil, *2.5 % BC* 2.5 % biochar-amended soil, *5 % BC* 5 % biochar-amended soil. *Error bars* represent the standard deviation of three replicates. The same letters above each bar indicate no difference at a 0.05 significance level

significantly decreased and the highest application rate, 5 % BC treatment reduced the concentration of Cr by over 87 % compared to the control soil. Such a noticeable immobilization of mobile Cr in the BC-amended TWS is likely to be due to the adsorption of Cr on the surface of BC via π*-π electron donor acceptor interactions (Fig. 6).

Moreover, the 5 % BC amendment decreased the concentrations of Cr in the phases of organic and carbonate by 77 and 43 %, respectively. The rest of Cr levels may have been adsorbed onto silicates which was not extractable from the TWS. With regard to the 5 % BC amended of TWS, the order of individual geochemical fractions of Cr bound from greatest to least is Fe-Mn oxide bound>organic matter bound>carbonates bound>exchangeable. Hence, it is clear that the BC application rates resulted in a decrease of Cr concentrations in all phases of TWS, compared to that of the control, which may be attributed to the high immobilization ability of the BC surface. Hence, the sequential extraction data suggested that the reduction of exchangeable fraction of metals is mainly due to the immobilization of Cr contaminated in the TWS, depending on the application rates of BC amendment, thereby reducing the bioavailability of Cr from the TWS to tomato plants. Therefore, the findings clearly corroborate with those of the pot experiment.

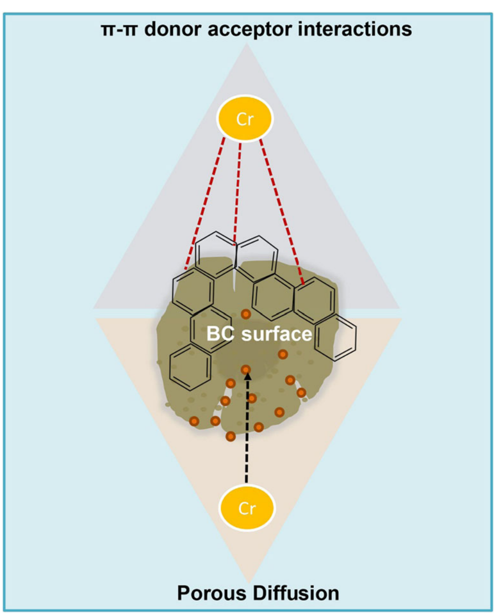


Fig. 6 A graphical representation of potential mechanisms for the immobilization of Cr in BC-amended TWS

3.6 Possible mechanisms for immobility of Cr

As single and sequential data demonstrated, the reduction of bioavailable Cr in TWS in the presence of BC at different rates of application could be due to pore diffusion and adsorption via π electron donor-acceptor interaction (Fig. 6). The porous diffusion and adsorption processes of Cr onto the BC surface could be in part explained by its high surface area (714 m^2/g) and pore size (40.8 Å). The diffusion rate of the metal is governed by the BC pore size and mesopores which highly facilitate the mechanism of diffusion and adsorption of Cr onto the surface of BC. The higher attenuation of Cr(VI) in the presence of BC amendments may be due to its smaller ionic radius (58 pm) which favored its diffusion compared to that of Cr (III) (75 pm). However, the adsorption may also be limited since organic substances such as humic acids present in the soil can readily be attached to the BC surface, rending inner pores unavailable for metal diffusion and further adsorption (Peijnenburg et al. 2007).

The formation of back bonding (back donation) with the π-electron rich graphene surface of the BC could be the main mechanism for the immobilization of Cr in BC-amended TWS. Early transition metals such as Cr are relatively electropositive, and hence, they are found in the highest possible oxidation state due to the oxidation of Cr (d^6) to Cr (VI) (d^0). Donation of electron pairs from π-electron-rich ligands such as benzene and phenolic derivatives present in the carbonized part of the BC makes the metal center electron rich and high in energy (σ bond); as a result, it is less stable. During the process, the electron density of the metal increases, some of π acceptor ligands having π*-orbitals such as PhC=CPh present in the BC surface have the ability to accept dπ-electrons from the metal centre, thereby forming the back bonding (π bond). When the ligands have low-lying orbitals with an appropriate symmetry, the transfer of electron density takes place. Therefore, this back donation introduces the π-character into the bond of the metal and the ligand (M-L) and makes the M-L bond stronger. With the increase of BC application rates, available binding sites also increased in order to form strong M-L bonds, thereby reducing the amounts of bioavailable Cr in TWS. It is clear that the application of BC as a soil amendment has significantly immobilized bioavailable Cr in the TWS. Hence, our study proposed that this bioenergy-derived BC is highly effective in immobilizing Cr found in TWS, thereby providing a distinct advantage in the remediation of such heavy metal contaminated soils.

4 Conclusions

The present study was conducted to investigate the potential of BC, a waste byproduct of a bioenergy industry, as a soil amendment to immobilize bioavailable Cr and reduce the phytotoxicity of TWS. Tomato plants grown in 5 % BC-amended TWS increased the dry biomass by 2-fold compared to the BC-unamended soil. However, the highest bacterial and fungal counts were found in the 2.5 % BC-amended soil. Bioaccumulation of Cr in tomato plants grown in 5 % BC-amended soil also decreased by 97 % compared to the BC-unamended soil. The 5 % BC amendment decreased the $CaCl_2$ extractable Cr by 68 % and sequentially extracted Cr of the exchangeable by 98 % compared to the control soil, suggesting that the reduction of bioavailable Cr is primarily due to the immobilization of Cr in TWS. Hence, the present study contributes further evidence that the release of Cr from TWS and its bioavailability and phytotoxicity were reduced with the increase in the BC application rates. Furthermore, the present study shows that the application of BC to heavy metal-rich lands, including areas that were formerly used for metal extraction, may immobilize metal translocation and accumulation in plants, including agricultural crops. Hence, BC as a byproduct from the bioenergy industry may potentially be used in the restoration of heavy metal-contaminated soil.

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