



Full Length Article

Evaluation of Green Waste and Popular Twigs Biochar Produced at Different Pyrolysis Temperatures for Remediation of Heavy Metals Contaminated Soil

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Abstract

Remediation of heavy metals contaminated soil through biochar addition depends upon the characteristics of biochar. Feedstock and pyrolysis temperature used for biochar production are the main factors that influence the properties of biochar important for metals immobilization in soil. In this study, immobilization of the most prevalent heavy metals [(lead (Pb (II)), cadmium (Cd) and chromium (Cr)] by green waste and popular twigs biochar produced at different pyrolysis temperatures (low 350°C and high 650°C), has been investigated, following the determination of physical and chemical properties of biochar. Soil was spiked with Pb, Cd and Cr and then amended with different biochar types in an incubation experiment. Soil chemical properties and available concentration of metals were measured at three incubation durations (0, 45 and 90 days). Biochar produced from green waste at 350°C pyrolysis temperature had maximum concentration of oxygen-containing functional groups (0.38 meq g⁻¹) and had maximum CEC (43.7 cmol_c kg⁻¹) as compared with all other biochars. However, fixed carbon content (69.8%) and surface area (309.58 m² g⁻¹) was found maximum in the biochar produced at 650°C pyrolysis temperature from popular twigs and green waste, respectively. Available concentrations of all three heavy metals were significantly decreased with the passage of time after biochar addition as compared with control and the minimum available concentrations of metals (Pb 6.33 mg kg⁻¹, Cd 1.42 mg kg⁻¹ and Cr 3.14 mg kg⁻¹) were determined in the treatment where green waste biochar produced at 350°C pyrolysis temperature was added to soil after 90 days of incubation duration. Soil chemical properties was significantly improved after biochar addition, especially soil CEC which was found maximum (21.90 cmol_c kg⁻¹) in the treatment where green waste biochar produced at 350°C pyrolysis temperature was added to soil. We concluded that the addition of green waste biochar produced at low pyrolysis temperature (350°C) to heavy metals contaminated soil showed minimum available concentration of metals due to its better physicochemical characteristics and more oxygen-containing functional groups and by improving the soil chemical properties as compared with other biochars. © 2017 Friends Science Publishers

Keywords: Biochar; Heavy metals; Oxygen containing functional groups; Soil properties

Introduction

Heavy metals pose a risk to human health because of their persistence, toxicity and non-biodegradable nature and wide spread occurrence in natural and human altered environment (Khan *et al.*, 2008). They enter the human body by consumption of heavy metals contaminated food crops, water and dust (Murtaza *et al.*, 2010). It is estimated that 70% of these toxic metals enter into the human body via consumption of food crops, which grow on metal contaminated soil (Murtaza *et al.*, 2010). When these toxic metals enter the human body, they cause severe fatal diseases (e.g. cardiovascular problems, kidney and nervous diseases, immunological defense and growth retardation, constipation and anemia bone diseases) and many other problems in humans (WHO, 1992; Turkdogan *et al.*, 2003).

It has become crucial to prevent the entry of these toxic metals into food chain and thus reduce their risk to human health.

Physical (e.g. membrane filtration, packed-bed filtration), chemical (e.g. precipitation, ion exchange and electro-coagulation) and biological methods (e.g. phytoremediation and bioremediation approaches) have been adopted to treat heavy metals contaminated soil (Malamis *et al.*, 2011). Most of these technologies however, may be associated with high operational cost, sludge disposal problems and short term effect (Sud *et al.*, 2008). These problems have amplified the need to develop alternative, long term and inexpensive technologies for heavy metal contaminants. Therefore, bio-sorbents have been recommended to be a potential candidate to satisfy this need for remediation of toxic metals in

contaminated soil (Demirbas, 2008; 2009). The use of biochar as an economical bio-sorbent to immobilize the metallic contaminants in soil is an emerging and promising remediation approach (Liu and Zhang, 2009; Uchimiya *et al.*, 2010b; Zhang *et al.*, 2016).

Bioavailability of heavy metals in biochar amended soil depends on the biochar characteristics and soil properties. Biochar has a wide range of characteristics that depend upon the type of feedstock used as well as the pyrolysis conditions used during biochar production (Ahmad *et al.*, 2014). Surface area, oxygen containing functional groups, pH, ash and carbon (C) content are the important properties of biochar for decreasing the bioavailability of the heavy metals in the soil (Lima *et al.*, 2014). During pyrolysis, numerous functional groups such as amino, hydroxyl, ester, aldehyde, ketone, nitro and carboxyl are formed. This phenomenon takes place mainly on the outer layer of the well-developed graphene layers and surfaces of macro and micro pores (Zwieten *et al.*, 2010). Some of the functional groups act as electron acceptors, while others as electron donors. These properties describe biochar nature, from low pH to high pH and from hydrophilic to hydrophobic (Amonette and Joseph, 2009). Hydrogen, N, O, P and S are assimilated in the highly aromatic rings and find out the electro negativity of produced biochar and thus modifying the cation exchange capacity. Like other properties, the pH of biochar is highly influenced by feedstock type, production temperature and duration (Yuan *et al.*, 2011a, 2011b). Similarly, CEC and EC values of biochar ranges 40–500 cmole_c kg⁻¹ and 1–5 dS m⁻¹, respectively, depending upon type of feedstock used and pyrolysis conditions (Amonette and Joseph, 2009).

There are various mechanisms by which biochar decrease the bioavailability of heavy metals from soil. These includes sorption (Beesley and Marmiroli, 2011; Lu *et al.*, 2012), precipitation (Park *et al.*, 2013), altering soil pH (Wu *et al.*, 2012) and changing the redox state of the heavy metals (Choppala *et al.*, 2012). Sorption may be due to the complexation of different oxygen-containing functional groups present on the biochar surface with heavy metals (Uchimiya *et al.*, 2011b) and by exchange of metals with different cations, e.g. Ca, potassium (K) and magnesium (Mg), present on the biochar surface (Lu *et al.*, 2012). Different compounds (carbonates (CO₃), phosphates (PO₄) and sulphates (SO₄)) present on the biochar surface precipitate the heavy metals by forming different compounds such as lead phosphate (Cao *et al.*, 2009; Karami *et al.*, 2011; Park *et al.*, 2013). When biochar (high pH) is applied to the soil, it increases the pH of the soil which results in the immobilization of heavy metals (Wu *et al.*, 2012). Biochar additions into the soil can also change the redox state of the heavy metals thus decreasing their mobility and bioavailability (Hussain *et al.*, 2017). An example is when the polycyclic aromatic sheets of biochar donate π electrons to Cr (VI) and reduce it into Cr (III) which is less toxic and less mobile so its bioavailability is

decreased (Choppala *et al.*, 2012). Organomineral layers are formed on the biochar surface on which new reactive surfaces are formed by oxidation during aging. Thus, biochar immobilizes the heavy metals for long periods of time and its capacity of heavy metals adsorption increases with the passage of time (Bian *et al.*, 2014).

Biochar is considered as a promising heavy metals adsorbent, due to its favorable physicochemical properties. However, there are a limited number of studies on the effects of various biochars produced from different feedstocks and pyrolysis temperatures on metals immobilization in soil. This study was therefore, conducted to characterize the biochar produce from different feedstocks and pyrolysis temperatures and evaluate the most efficient biochar for remediation of heavy metals contaminated soil.

Materials and Methods

Production of Biochar

Two feedstocks (i.e. green waste (*Cynodon dactylon*) and popular twigs cuttings) were selected for biochar production. Green waste consisting of Bermuda grass (*Cynodon dactylon*) cuttings, were collected from the lawns of the University of Agriculture, Faisalabad (UAF). Popular twigs were collected from popular trees (having ages of almost 10–15 years) of the field areas of UAF.

Two hundred grams of each crushed feedstock was pyrolyzed in a laboratory setup as described by Sanchez *et al.* (2009). Muffle furnace (Gallonhop, England) was used to carry on the pyrolysis process. The pyrolysis was done in a Pyrex flask of 2 L capacity. For the removal of gases and vapors from the working area a bended outlet composed of a glass rod was used. The junction of the Pyrex flask and glass rod was sealed with high temperature resistant silicon grease to avoid the entry of oxygen into reaction chamber. The increase in furnace temperature per unit time was adjusted at 8–9°C min⁻¹. After attaining the maximum pyrolysis temperature (350 and 650°C temperature for each feedstock) the maximum temperature was maintained for an additional 20 min. After the completion of pyrolysis the furnace was allowed to cool until the temperature of the reaction chamber reached 50°C. After cooling, the Pyrex flask was removed from the furnace chamber and the biochar was collected from the Pyrex flask.

Characterization of Biochar

Surface areas were measured using a NOVA 2000 surface area analyzer in duplicate by nitrogen adsorption isotherms at 77 K (Quantachrome, Boynton Beach, FL). Moisture, volatile matter and ash content were measured by using the method described by Uchimiya *et al.* (2011a). After the determination of moisture, volatile matter and ash fixed C content were calculated by difference (Uchimiya *et al.*, 2011a).

The biochar pH was measured using 1:20 biochar:deionized water ratio and after shaking for 90 min on a mechanical shaker. Modified ammonium acetate compulsory displacement method was used to measure the biochar CEC (Gaskin *et al.*, 2008).

Boehm Titration (Boehm, 1966) was used to determine the surface acidic oxygen containing functional groups (i.e. carboxylic, lactonic and phenolic groups). Macronutrients i.e. P, K, calcium (Ca) and Mg, as well as the micronutrients zinc (Zn), copper (Cu) and iron (Fe), were extracted from biochar samples after digestion in hydrogen peroxide (H₂O₂) and sulfuric acid (H₂SO₄) (Wolf, 1982). UV-visible spectrophotometer (UV-1201, Shimadzu, Tokyo, Japan) was used to determine the P concentration by the vanadate-molybdate method (Chapman and Pratt, 1961). Flame photometer (PFP7, Jenway, Essex, UK) was used to determine K concentration while atomic absorption spectrophotometer (AAAnalyst 100, Perkin-Elmer, Norwalk, USA) was used to determine Ca, Mg, Zn and Fe content in biochar digest.

The C, hydrogen (H), N and S content in different biochars were analyzed on a Vario Micro CHNS-O Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The percentage of oxygen content were determined by difference method:

$$\text{Oxygen (\%)} = 100 - (\text{Ash} + \text{carbon} + \text{hydrogen} + \text{nitrogen})\%$$

The results obtained from CHNS-O analyzer were used to calculate elemental ratios i.e. C:N, C:P and C:S and molar ratios i.e. H:C, O:C and (O+N):C.

A 0.5 g sample of each biochar was digested in a 4:1 ratio of concentrated nitric and perchloric acids on a hot plate. After digestion, the volume was made to 50 mL by using distilled water. The samples were analyzed for a suite of elements including Cd, Pb and Cr by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (ICP-OES optima 5300 DV) having a limit of detection (3 µg kg⁻¹) and a limit of quantification (9 µg kg⁻¹). Water soluble PO₄³⁻ and CO₃²⁻ content were extracted by mixing 5 g biochar with 50 mL deionized H₂O and determined using ion chromatography (Waters 2690 Separations Module, Waters Corporation, USA) (Cao and Harris, 2010).

Incubation Experiment

Basic soil analysis: The soil was air-dried and passed through a 2 mm sieve. A sub sample of the sieved soil was analyzed for various soil physico-chemical properties. The sandy loam soil texture (sand 56.3%; silt 22.5%; clay 21.2%) was determined by the hydrometer method (Gee and Bauder, 1986). The pH of a saturated soil paste was 7.95 and was measured by Calomel glass electrode assembly. Electrical conductivity of the saturated soil paste extract was 1.30 dS m⁻¹. Soil organic carbon, determined by the

Walkley-Black method (Nelson and Sommers, 1982) was 4.3 g kg⁻¹ of the soil. The CEC of soil was estimated by the method of Sumner and Miller (1996) and was 14.3 cmol_c kg⁻¹. Soil total N was determined by the Kjeldhal method (Jackson, 1962) and was 0.062%. Extractable K was determined according to method of Richards (1954) and was 109 mg kg⁻¹. Olsen P was determined by extracting the soil with sodium bicarbonate solution (Olsen and Sommers, 1982) and was 7.31 mg kg⁻¹. Calcium carbonate content were determined according to the method of Leoppert *et al.* (1984) and was 3.43%. Total Pb, Cd and Cr concentrations were 200, 30 and 60 µg kg⁻¹, respectively) and were measured by ICP-AES after digestion with aqua regia (3:1 of HNO₃:HCl). Similarly, available Pb, Cd and Cr concentrations (less than 10 µg kg⁻¹) were measured by extracting the soil with AB-DTPA and then analyzing the filtrate by ICP-AES (Houba *et al.*, 2000).

Experimental Setup

A completely randomized design two factorial, incubation experiment was conducted in a glass-house at the Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF). This experiment resulted in the selection of the best biochar types (from 4 types of biochars as described in the biochar production and characterization) on the basis of effective remediation of artificially HM spiked soil as well as by observing the temporal changes in soil properties by biochar addition. There were two experimental factors: (1) biochar types (4 types of biochar along with control) and (2) sampling times (3 sampling times: 0, 45 and 90 days after treatments application). Each treatment was replicated three times.

Glass cups having capacity of 500 g were used and filled with 400 g of soil. The soil was spiked with 250, 6 and 12 mg kg⁻¹ of Pb, Cd and Cr respectively, according to their permissible limits in soil by using ultra-pure salts of lead nitrate, potassium dichromate and cadmium nitrate. After that, the four different biochars (GWB350°C, GWB650°C, PTB350°C and PTB650°C along with the control) were applied at a rate of 2% (w/w) and the soil was mixed. These cups were placed in the glass house and changes in soil of available concentrations of Pb, Cd and Cr were determined by collecting the soil samples 0, 45 and 90 days after treatment application. Soil pH and EC was also analyzed by using the standard methods as described in basic soil analysis but soil TOC content were analyzed by using TOC-TRL analyzer (TERRALAB equipments, Ankara, Turkey).

Statistical Analysis

Statistical analysis and data computations of biochar characterization and incubation experiment were made on

Microsoft Excel 2013® (Microsoft Corporation, Redmond, WA, USA) and Statistix 8.1® (Analytical Software, Tallahassee, USA). However, significantly different means in case of biochar characterization were compared by using LSD (least significant difference) test at $p \leq 0.05$ (Steel et al., 1997), while in case of incubation experiment, significantly different treatment means were separated by using Tukey's multiple comparison test ($P \leq 0.05$).

Results

Characterization of Biochar

Nutrient concentration and molar ratios in biochar: The maximum concentrations of N, O, H and S were obtained when biochar was produced at low pyrolysis temperature (i.e. 350°C) as compared with biochar produced at high pyrolysis temperature (i.e. 650°C). Carbon (C), Ca, Mg and micronutrients were significantly ($P \leq 0.05$) higher in concentrations in the biochar produced at high pyrolysis temperature. Among feedstocks, GW biochar showed significantly ($P \leq 0.05$) higher concentrations of all macro and micro nutrients as compared with PT biochar (Table 1). However, C content were maximum in biochar produced from PT at both low and high pyrolysis temperatures.

Similarly, there was also a significant ($P \leq 0.05$) effect of feedstock and pyrolysis temperature on elemental and molar ratio of nutrients in biochar (Table 1). Maximum values of O:C, H:C and O+N:C ratio were found for the biochar produced at low pyrolysis temperature.

Among feedstocks, GW biochar showed significantly ($P \leq 0.05$) higher molar ratios of O:C, H:C and O+N:C as compared with PT biochar. The maximum values of the C:N, C:P and C:S ratios were found in the biochar produced at high pyrolysis temperature (650°C) as compared with low pyrolysis temperature (350°C) produced biochars. It was also clearly evident (Table 1) that by increasing the pyrolysis temperature the C:N, C:P and C:S ratios were increased and maximum values were calculated in the biochar produced at 650°C.

Physicochemical Properties of Biochar

There was a significant ($P \leq 0.05$) increase in surface area and pH of biochar produced from both GW and PT feedstocks when pyrolysis temperature was increased from 350°C to 650°C (Table 2). The significantly ($P \leq 0.05$) highest values of surface area and pH i.e. 310 m² g⁻¹ and 10.3, respectively were measured in GW biochar produced at 650°C. The significantly ($P \leq 0.05$) highest value of CEC of 43.7 c mol_e kg⁻¹ was determined in GW biochar produced at 350°C. Among feedstocks, GW biochar showed higher values of pH, surface area and CEC as compared with PT biochar at both high and low pyrolysis temperatures.

Proximate Composition of Biochar

Proximate composition of biochar was significantly ($P \leq 0.05$) affected by feedstock type and pyrolysis temperature used for biochar production (Fig. 1).

Table 1: Effect of feedstock and pyrolysis temperature on concentrations, elemental and molar ratios of nutrients in biochar

Nutrient concentration	Units	Biochar types			
		Green waste biochar		Popular twigs biochar	
		350°C	650°C	350°C	650°C
C	%	39.30 ± 2.4 c	49.40 ± 2.4 b	46.70 ± 3.2 b	66.70 ± 3.2 a
H	%	3.96 ± 0.4 a	1.96 ± 0.4 c	3.16 ± .27 b	1.23 ± .17 d
O	%	19.10 ± 1.8 a	9.07 ± 1.8 c	14.10 ± 2.1 b	5.74 ± .35 d
N	%	1.47 ± 0.1 a	0.77 ± 0.1 b	0.53 ± .16 c	0.30 ± .07 d
P	g kg ⁻¹	4.31 ± 0.5 a	2.97 ± .25 b	1.61 ± .30 c	0.84 ± .22 d
K	g kg ⁻¹	16.20 ± 2.4 a	12.20 ± 1.4 b	13.60 ± 2.6 ab	6.56 ± .86 c
S	g kg ⁻¹	8.65 ± 1.3 a	3.98 ± .27 bc	5.65 ± 1.3 b	2.31 ± .56 c
Ca	g kg ⁻¹	22.40 ± 2.4 a	24.70 ± 3.5 a	15.10 ± 2.9 b	17.70 ± 1.1 b
Mg	g kg ⁻¹	17.00 ± 2.2 ab	19.30 ± 2.5 a	12.30 ± 1.4 c	14.60 ± .89 bc
Zn	mg kg ⁻¹	177 ± 12 a	203 ± 21 a	97.00 ± 8.5 b	107 ± 8.5 b
Fe	mg kg ⁻¹	202 ± 18 b	255 ± 29 a	112 ± 8.7 c	125 ± 14 c
Cd	mg kg ⁻¹	0.03 ± .01 b	0.08 ± .02 a	0.04 ± .01 b	0.09 ± .02 a
Pb	mg kg ⁻¹	0.07 ± .03 a	0.11 ± .05 a	0.06 ± .02 a	0.09 ± .02 a
Cr	mg kg ⁻¹	0.47 ± .17 b	0.83 ± .19 a	0.33 ± .12 b	0.49 ± .16 b
PO ₄ ²⁻	mg kg ⁻¹	53.70 ± 9.5a	16.30 ± 4.5 bc	19.0 ± 4.0 b	05.30 ± 2.5 c
CO ₃ ²⁻	%	1.23 ± .06 b	1.86 ± 0.14a	0.90 ± .19 c	1.39 ± .12 b
Elemental ratio					
C:N ratio		26.80 ± 00.31 c	65.00 ± 10.54 bc	93.14 ± 31.7 b	225.22 ± 37.3 a
C:P ratio		92.30 ± 13.69 c	166.40 ± 7.24 bc	295.50 ± 43.9 b	819.50 ± 163.3 a
C:S ratio		46.40 ± 08.85 c	124.30 ± 10.8 bc	86.62 ± 26.8 b	298.20 ± 64.27 a
Molar ratio					
H:C ratio		01.22 ± 0.20 a	00.48 ± 00.13 c	00.81 ± 0.06 b	00.22 ± 00.03 d
O:C ratio		00.36 ± 00.04 a	00.14 ± 00.03 c	00.23 ± 0.02 b	00.07 ± 0.003 d
O+N:C ratio		00.40 ± 00.04 a	00.15 ± 00.03 c	00.24 ± 0.02 b	00.07 ± 0.003 d

Values are mean of the three replicates ± standard deviation (n=3). Different letters within a row indicate significant difference among treatments at $\alpha=0.05$ according to the LSD test

The maximum amounts of ash and volatile matter were observed in biochar produced from GW at both low and high pyrolysis temperatures, but yield and FC (fixed carbon) content were higher when biochar was produced from PT feedstock at both low and high pyrolysis temperature.

Oxygen-containing Functional Groups in Biochar

The significantly ($P \leq 0.05$) highest concentrations of oxygen-containing functional groups (i.e. carboxylic, lactonic and phenolic groups) was measured in low pyrolysis temperature produced biochar as compared with high temperature produced biochar from both GW and PT feedstocks (Fig. 2). Similarly, among feedstocks GW biochar showed significantly ($P \leq 0.05$) higher concentrations of oxygen-containing functional groups (i.e. carboxylic, lactonic and phenolic groups). The maximum values of carboxylic, lactonic, phenolic and total concentrations of oxygen-containing functional groups were 0.121, 0.130, 0.130 and 0.381 meq g⁻¹, respectively in the GW biochar produced at 350°C.

Temporal Changes in Heavy Metals Spiked Soil

Temporal changes in soil available heavy metals concentration: Addition of low pyrolysis temperature produced biochars to soil significantly ($P \leq 0.05$) decreased the available concentrations of heavy metals i.e. Pb, Cd and Cr as compared with high pyrolysis temperature (Fig. 3a, b and c) produced biochars addition. The maximum decrease in available Pb concentration (up to 80%) as compared with the control was observed for the GW biochar produced at 350°C after 90 days of incubation (Fig. 3a). The maximum decrease in available Cd concentration (up to 65%) as compared with control was observed for the GW biochar produced at 350°C after 90 days of incubation (Fig. 3b). This was followed by the PT biochar produced at 350°C which decrease the available Cd concentration up to 45% as compared with control. Similarly, the maximum decrease in available Cr concentration (up to 55%) as compared with the control was observed after 90 days when GW biochar produced at 350°C was added to soil (Fig. 3c).

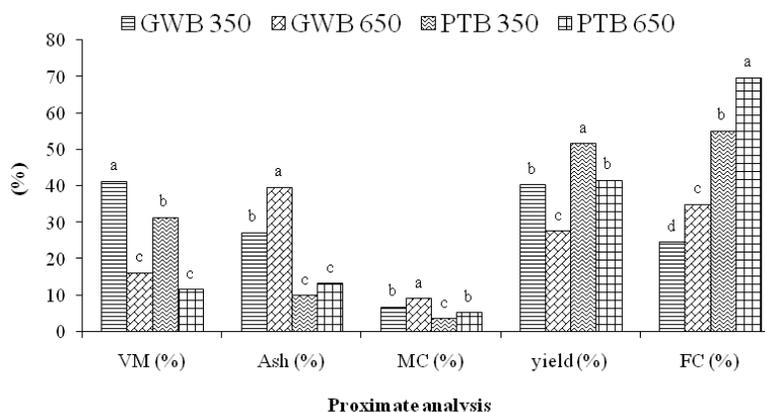


Fig. 1: Effect of feedstock and pyrolysis temperature on proximate analysis of biochar. Different letters on a parameter indicate significant difference among treatments at $\alpha \leq 0.05$ according to LSD

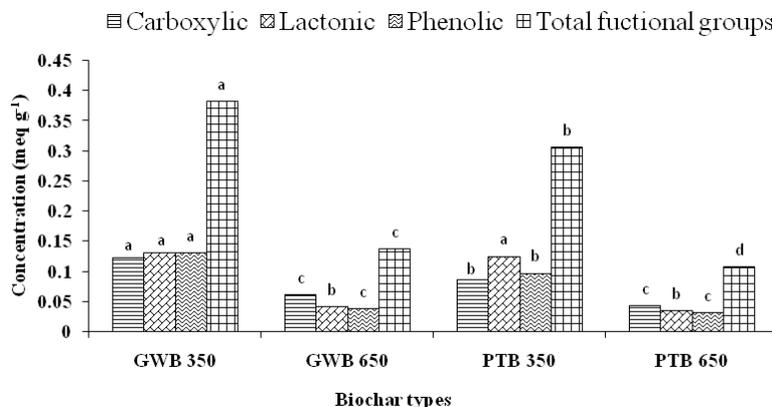


Fig. 2: Effect of feedstock and pyrolysis temperature on the concentration of oxygen containing functional groups present in biochar. Different letters indicate significant difference among treatments at $\alpha \leq 0.05$ according to LSD

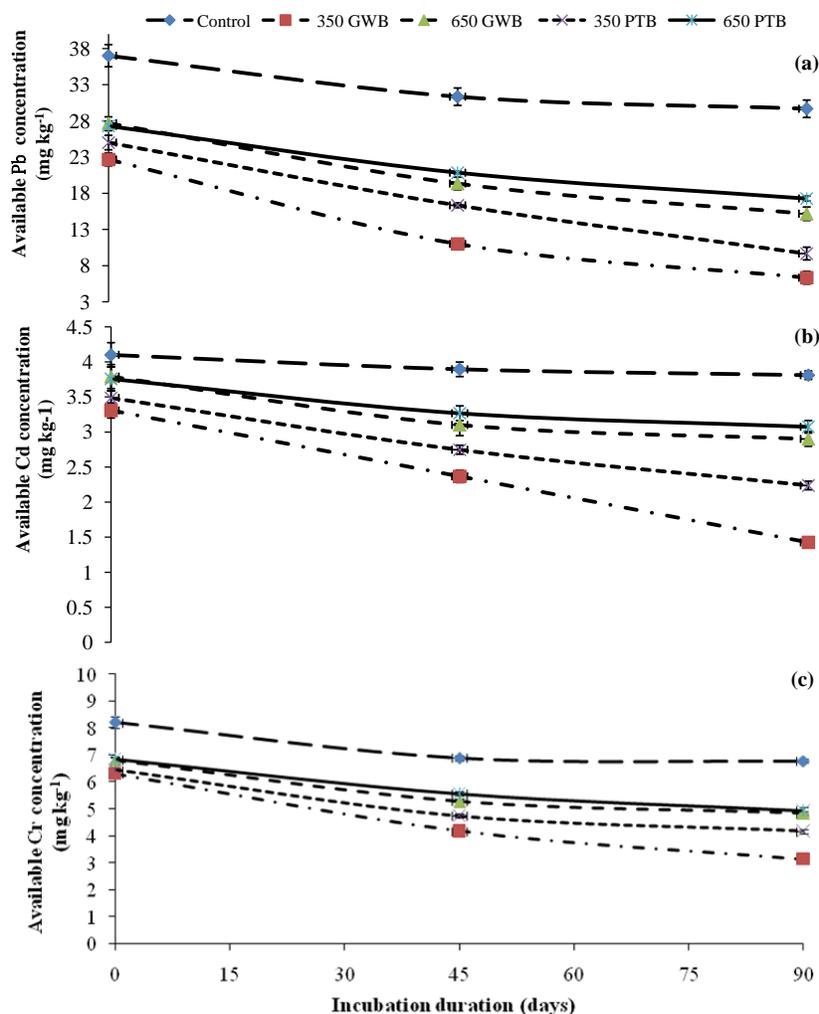


Fig. 3: Temporal changes in soil available Pb (a), Cd (b) and chromium (Cr) concentration (mg kg^{-1}) under the influence of different types of biochar (2% w/w). Error bars represent standard errors; all the treatments were replicated thrice ($n=3$)

Temporal Changes in Chromium (Cr) Fractionation

Low pyrolysis temperature produced biochars added to soil significantly ($P \leq 0.05$) decreased the total available Cr and Cr (VI) concentrations while Cr (III) was significantly ($P \leq 0.05$) increased as compared with high pyrolysis temperature produced biochars (Fig. 4). The maximum decrease in available Cr and Cr (VI) concentration (i.e., up to 55 and 67%) respectively, occurred after 90 days as compared with control when GW biochar produced at 350°C was added to soil. In contrast, Cr (III) concentrations increased up to 54% as compared with control.

Temporal Changes in Soil Properties

The soil pH was significantly ($P \leq 0.05$) decreased by addition of low pyrolysis temperature produced biochar as compared with control at 45 days and then

increased and approached the pH of the control soil (Table 3). Addition of high pyrolysis temperature produced biochar to soil significantly ($P \leq 0.05$) enhanced the soil pH at 45 days and then it decreased and approached to pH of the control soil up to 90 days. The maximum value of soil EC was measured for the GW biochar produced at 650°C was added to soil after 90 days of incubation, which was 29% higher as compared with the control (Table 3). Low pyrolysis temperature produced biochar addition to soil resulted in significantly ($P \leq 0.05$) higher values of CEC as compared with high pyrolysis temperature produced biochar. The maximum value of soil CEC was measured where GW biochar produced at 350°C was added to soil after 90 days of incubation duration, which was up to 34% higher as compared with the control (Table 3). The maximum value of soil TOC content was measured where PT biochar produced at 650°C was added to soil, which was 68% higher as compared with the control treatment (Table 3).

Table 2: Effect of feedstock and pyrolysis temperature on the physicochemical properties of biochar

Feedstock	Pyrolysis Temperature					
	350°C		650°C		650°C	
	Surface area (m ² g ⁻¹)		CEC* (c mol _c kg ⁻¹)		pH	
Green waste	023.81 ± 2.77 c	309.58 ± 18.56 a	43.74 ± 05.23 a	30.41 ± 02.37 b	08.01 ± 0.16 c	010.34 ± 0.45 a
Popular Twigs	019.25 ± 2.57 c	215.92 ± 06.30 a	31.25 ± 04.36 b	27.91 ± 03.34 b	07.38 ± 0.20 d	009.38 ± 0.19 b

*stands cation exchange capacity

Values are mean of the three replicates ± standard deviation (n=3). Different letters under each parameter indicate significant difference among treatments at α=0.05 according to the LSD test

Table 3: Effect of biochar types on soil chemical properties after different incubation duration

Biochar Types	Incubation Duration					
	0 Days	45 Days	90 Days	0 Days	45 Days	90 Days
	pH			Cation exchange capacity (cmol _c kg ⁻¹)		
Control	7.97 ± 0.025 ef	7.98 ± 0.066 ef	8.00 ± 0.050 ef	14.47 ± 0.600 fg	14.31 ± 0.550 g	14.13 ± 0.327 g
*GWB350°C	8.06 ± 0.070 d-f	7.88 ± 0.055 f	7.95 ± 0.035 ef	17.96 ± 0.970 cd	20.44 ± 0.996 ab	21.90 ± 0.795 a
GWB650°C	8.46 ± 0.081 a	8.44 ± 0.108 ab	8.25 ± 0.060 c	16.33 ± 0.299 d-f	17.34 ± 0.575 c-e	18.12 ± 0.712 cd
**PTB350°C	7.93 ± 0.040 ef	7.90 ± 0.036 f	7.91 ± 0.040 ef	17.45 ± 0.450 c-e	18.43 ± 0.485 c	19.05 ± 0.810 bc
PTB650°C	8.25 ± 0.060 bc	8.19 ± 0.068 cd	8.11 ± 0.055 c-e	16.00 ± 0.310 e-g	17.39 ± 0.624 c-e	18.11 ± 0.378 cd
	Electrical conductivity (dS m ⁻¹)			Organic carbon (g kg ⁻¹)		
Control	1.33 ± 0.040 i	1.36 ± 0.047 hi	1.34 ± 0.061 i	4.30 ± 0.364 g	4.31 ± 0.522 g	4.23 ± 0.336 g
GWB350°C	1.46 ± 0.070 f-i	1.64 ± 0.070 b-e	1.74 ± 0.056 a-c	6.86 ± 0.375 ef	6.14 ± 0.224 f	6.73 ± 0.233 f
GWB650°C	1.63 ± 0.072 c-f	1.81 ± 0.050 ab	1.85 ± 0.066 a	8.36 ± 0.375 cd	7.94 ± 0.316 de	8.46 ± 0.307 b-d
PTB350°C	1.40 ± 0.066 g-i	1.48 ± 0.050 e-i	1.52 ± 0.045 d-h	9.31 ± 0.260 bc	8.74 ± 0.325 b-d	9.60 ± 0.316 b
PTB650°C	1.55 ± 0.060 d-g	1.67 ± 0.060 a-d	1.73 ± 0.059 a	13.72 ± 0.633 a	13.02 ± 0.456 a	13.70 ± 0.540 a

*Stands green waste biochar

**Stands popular twigs biochar

Values are mean of the three replicates ± standard deviation (n=3). Different letters under each parameter indicate significant difference among treatments at P<0.05 according to Tukey's-HSD test

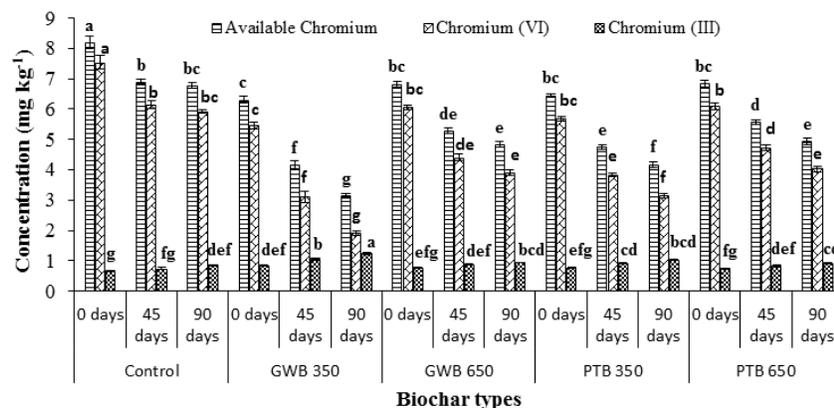


Fig. 4: Fractionation of available chromium concentration in soil as influenced by different types of biochar addition on temporal basis (2% w/w basis). Different letters within the graph indicate significant difference among treatments at P ≤ 0.05 according to Tukey's-HSD test

Discussion

In this project, we produced four different biochars by using two feedstocks (i.e. green waste, GW and popular twigs, PT) and two pyrolysis temperatures (i.e. 350 and 650°C). There were significant differences in the nutritional concentrations, elemental and molar ratios, physicochemical properties and oxygen-containing functional groups present in all four types of biochar. After production and characterization of different biochars an incubation

experiment was conducted to select the most effective biochar for maximum immobilization of the heavy metals (HM) i.e. Pb, Cd and Cr in soil system.

Results revealed that statistically higher concentration of the nutrients N, P and K were measured in the biochar produced at low pyrolysis temperature of 350°C. Some nutrients in feedstock become volatilized at high pyrolysis temperatures; for example, N starts to volatilize at ~200°C (Steiner *et al.*, 2008), while other nutrients are incorporated to aromatic rings at high pyrolysis temperatures and thus

they become unavailable (Knoepp *et al.*, 2005). Moreover, biochar produced from GW feedstock showed significantly higher concentration of nutrients such as N, P, K, S and O as compared with that produced from PT feedstock.

Significant variations in physicochemical properties of biochar due to different feedstock types and pyrolysis temperatures have already been reported by Shinogi and Kanri (2003) and Deluca *et al.* (2009). High values of H:C, O:C and (O+N):C molar ratios of a biochar could speak for more attraction with polar compounds (Wang *et al.*, 2005) which in turn would facilitate complexation of heavy metals (HM) in the soil. In this study, all four types of biochars had H:C and O:C molar ratios within threshold limits, reported by Spokas (2010) and Schimmelpfennig and Glaser (2012). However, maximum values of H:C, O:C and (O+N):C molar ratios were measured in the biochar produced at low pyrolysis temperature from both GW and PT feedstocks.

Increase in surface area of biochar with increasing pyrolysis temperature could be attributed to abundance of micro pores increasing the overall porosity and surface area of the biochar (Lehmann and Joseph, 2009). According to Uchimiya *et al.* (2011b), woody feedstocks contain more lignin content, resistant to break down during pyrolysis as compared with non-woody feedstocks, producing biochar with low surface area. Increasing basicity of biochar due to increase in temperature has also been found by Chun *et al.* (2004). High pH and the associated lower concentration of oxygen-containing functional groups could result into less complexation ability of biochar. Lower amount of functional groups could even conceal the advantage of large surface area at higher pyrolysis. This finding still speaks for the suitability of low pyrolysis temperature.

Presence of oxygen-containing functional groups is a very important property of biochar regarding remediation of heavy metals contaminated soil (Uchimiya *et al.*, 2011a). Effectiveness of low temperature pyrolyzed biochars in bio-complexing of heavy metals could mainly be attributed to this factor of abundance in functional groups. Results of decreasing the concentration of oxygen-containing functional groups with increase in temperature of pyrolysis, mainly due to volatilization of oxygen are found in line with those of Uchimiya *et al.* (2011a, 2011b); and Han *et al.* (2013).

High oxygen-containing functional groups at low temperature causes increased CEC (Wu *et al.*, 2012) and charge density and thus leading to retention of cations (Liang *et al.*, 2006). Similarly, higher values of CEC for GW biochar could again be attributed to greater surface area and higher concentrations of oxygen-containing functional groups. Soil total organic C concentrations were found maximum when high pyrolysis temperature-produced biochar was added to soil. Addition of biochar increases microbial activity in the soil (Saranya *et al.*, 2011), which may increase the organic matter content in the soil (Agbede *et al.*, 2008).

Improvements in soil properties such as CEC, surface area, pH and organic carbon content, due to biochar addition also play an important role in heavy metals immobilization in soil (Uchimiya *et al.*, 2010a; 2011b). The same was depicted from findings of our incubation experiment that addition of biochar to soil significantly decreased the available concentration of heavy metals; Pb, Cd and Cr. According to Uchimiya *et al.* (2011b), a soil having a high value of CEC and organic C content showed higher immobilization of HM due to greater exchange of cations (e.g. K, Ca and Mg) with HM on the biochar surface and more complexation of HM with organic matter. Maximum decrease in the available concentrations of heavy metals was occurred when HM spiked soil was treated with low pyrolysis temperature-produced biochars (i.e. GW and PT biochars produced at 350°C). Improvements in soil chemical properties by biochar addition as well as by biochar characteristics play a significant role in decreasing metals bioavailability in soil (Ahmad *et al.*, 2014).

Available concentrations of Cr and Cr (VI) found decreasing with addition of low pyrolysis temperature-produced biochars which could be related to high CEC and oxygen containing functional groups. The same finding has already been explained by Choppala *et al.* (2012) that more oxygen containing functional groups in low pyrolysis temperature-produced biochar could donate the π electrons to Cr (VI) and reduce it into Cr (III), thereby decreasing the concentration of toxic Cr (VI) or increasing less toxic and less mobile Cr (III). Available concentrations of HM were also significantly decreased with the passage of time in soil after biochar addition. According to Bian *et al.* (2014), new reactive sites on biochar surface are formed by oxidation with the passage of time leading also to an increase in the immobilization of HM with the passage of time.

Conclusion

Conclusively, green waste biochar produced at low pyrolysis temperature (i.e. 350°C) is more effective for the remediation of heavy metals (HM) polluted soil as compared with popular twigs biochar produced at high pyrolysis temperature (i.e. 650°C). Biochar produce at low pyrolysis temperature contains high concentration of oxygen-containing functional groups and has more CEC and ultimately caused more immobilization of HM in soil through surface complexation and precipitation mechanisms. Low pyrolysis temperature produce biochar addition to soil significantly improved the soil chemical properties that played an important role in HM immobilization in soil. Further, among feedstocks, green waste biochar showed maximum immobilization of heavy metals as compared PT biochar produced due to its better physicochemical especially surface area and CEC and oxygen containing functional groups characteristics.

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