



# Phosphorus-loaded biochar changes soil heavy metals availability and uptake potential of maize (*Zea mays* L.) plants

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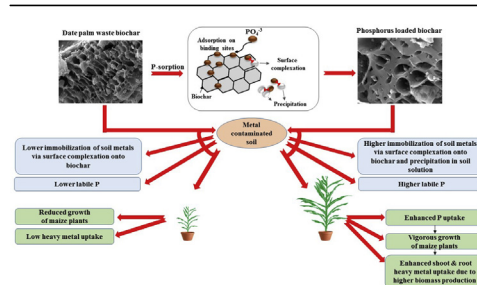
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## HIGHLIGHTS

- Phosphorus (P) was affectedly loaded onto date palm waste derived biochar (BC).
- P-loaded BC (BCP) increased soil labile P, and decreased soil labile heavy metals.
- BC and BCP application transformed the potentially available metal species to more stable ones.
- BCP application enhanced plant growth parameters and uptake of P.
- Calculated indices showed that BCP was not feasible for phytoextraction by maize.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 22 August 2017

Received in revised form

24 November 2017

Accepted 26 November 2017

Available online 28 November 2017

Handling Editor: J. de Boer

### Keywords:

Biochar

Immobilization

Uptake

Fractionation

Phytostabilization

## ABSTRACT

Biochar (BC) was produced by pyrolyzing the date palm leaf waste at 600 °C and then loaded with phosphorus (P) via sorption process. Greenhouse pot experiment was conducted to investigate the application effects of BC and P-loaded biochar (BCP) on growth and availability of P and heavy metals to maize (*Zea mays* L.) plants grown in contaminated mining soil. The treatments consisted of BC and BCP (at application rates of 5, 10, 20, and 30 g kg<sup>-1</sup> of soil), recommended NK and NPK, and a control (no amendment). Sorption experiment showed that Langmuir predicted maximum P sorption capacity of BC was 13.71 mg g<sup>-1</sup>. Applying BCP increased the soil available P, while BC and BCP significantly decreased the soil labile heavy metals compared to control. Likewise, heavy metals in exchangeable and reducible fractions were transformed to more stable fraction with BC and BCP applications. The highest application rate of BCP (3%) was most effective treatment in enhancing plant growth parameters (shoot and root lengths and dry matter) and uptake of P and heavy metals by 2–3 folds. However, based on metal uptake and phytoextraction indices, total heavy metals extraction by maize plants was very small for practical application. It could be concluded that using P-loaded biochar as a soil additive may be considered a promising tool to immobilize heavy metals in contaminated mining areas, while positive effects on the biomass growth of plants may assist the stabilization of contaminated areas affected by wind and water erosion.

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## 1. Introduction

The steady expansion of the population has reached its maximum globally, disturbing the relationship between the agricultural area and the rate of population increase. Anthropogenic activities due to extensive agricultural and industrial progression are polluting the planet Earth drastically by organic and inorganic contaminants. Though the increase in the agricultural area in arid regions is not at all noticeable, it is eroded as a result of urban encroachment, some desertification processes, soil salinization, soil pollution and erosion due to water and winds (Dregne, 2002). Among the various anthropogenic contaminants, heavy metals due to their non-biodegradable nature and long-term persistence in soils, are resulting in land degradation, and toxicity for plants, humans, and animals (Bolan et al., 2014). Heavy metals and metalloids can be found in the earth naturally, nevertheless, industrialization and urbanization have introduced a huge amount of heavy metals into the soil. One of the major source introducing heavy metals to the soil is mining industries (Ok et al., 2011; Reglero et al., 2008; Al-Farraj et al., 2013). Waste generations from mining activities are deteriorating the ecosystem profoundly (Bolan et al., 2004; Mench et al., 2010). A number of studies demonstrated Cd, Pb, Cu and Zn as the main predominant contaminants in mining area soil. In this context, heavy metals are considered to have been posing a serious threat to the ecosystem compartments even after many years from their induction to the environment, mainly due to their non-biodegradable nature (Vangronsveld et al., 2009). Therefore, it is a devastating necessity to find a sustainable remediation strategy for reducing mobility and phytoavailability of heavy metals to the plant grown on polluted mining soils.

Among various remediation techniques to remove pollutants from the environment, application of immobilizing agents to stabilize heavy metals in contaminated soils is the widely accepted technique due to its simplicity and economic feasibility. However, immobilizing agent selection is of great importance to achieve high immobilization efficiency, low cost, and feasibility. Moreover, safe disposal of the loaded sorbent after sorption process is also very critical. Biochar (BC) has been recently recommended as a sorbent for metals removal from aqueous solutions and as an immobilizing agent to stabilize metals in contaminated soils. Biochar is a solid product obtained by pyrolysis of waste biomass (Liu et al., 2011; Xu et al., 2013). Soil application of biochar not only enhances carbon sequestration for hundreds of years but also possesses a high capacity to adsorb potential pollutants in soil through electrostatic interactions, precipitation, ion exchange, complexation and chemisorption (Lehmann, 2007; Beesley et al., 2011; Yuan and Xu, 2011). Therefore, application of biochar to restore soil is getting a huge attention of the researchers nowadays. Though a number of studies reported that biochar was effective in decreasing heavy metals accumulation in the plants, it may not always perform correspondingly due to its huge heterogeneity in properties.

On the other hand, phytoremediation technique, due to its low cost has also been employed since last two decades to rehabilitate partially contaminated soil (Paz-Ferreiro et al., 2014). There are two processes involved in heavy metal phytoremediation: 1) enhanced heavy metal phytoextraction and uptake by aboveground biomass, and 2) increased metal phytostabilization through metal accumulation and stabilization in plant roots. Phytoremediation techniques generally involve the use of *hyperaccumulator* plant species to improve soil quality via translocating the heavy metals into various organs of the plant without deteriorating soil structure, fertility and biological activities (Ebbs et al., 1997; Salt et al., 1995). However, due to their specificity towards particular contaminant, low biomass production, and slow growth rate, *hyperaccumulator* plants do not provide an efficient solution to decontaminate the polluted

soils (Lasat, 2002). In literature, there are attempts to introduce the agronomic crops such as maize (*Zea mays* L.) for phytoremediation purposes. The easy cultivation, high biomass production, rapid growth, extensive fibrous rooting system, abundant seed production and resistance to adverse environmental conditions make maize crop a potential candidate to accumulate and remove heavy metals from soil. Many researchers have reported that maize plants are heavy metal tolerant and have considerable ability to accumulate metals in the above-ground biomass (Hernandez-Allica et al., 2008; Murakami and Ae, 2009; Zhang et al., 2008; Ebbs et al., 1997; Meers et al., 2005). Furthermore, if supplied with an exogenous nutrient source such as phosphorus (P), nitrogen or potassium, the improved growth of maize plants may enhance metal accumulation in the aboveground biomass due to the exertion of extra nutrients, which in turn may serve as a significant tool to remediate polluted soils. Among various essential nutrients to improve maize crop production, P is the major yield-limiting nutrient (Onasanya et al., 2009). The P is associated with many components of metabolism such as nucleic acids, sugar phosphates, coenzymes, nucleotides, and phospholipids. It is involved in enzymatic reactions and physiological processes in the plants. The P is of substantial importance due to its critical role in root development, tolerance induction and immobile nature in the soil (Onasanya et al., 2009; Ahmad et al., 2017a). Therefore, exogenous P application to maize crop can enhance its production as well as tolerance to the stresses.

Joint application of organic amendments (as a source of exogenous nutrients and immobilization agents) and phytoremediation was shown to increase plant growth and subsequently, assisting phytoremediation (Wang et al., 2012; Wei et al., 2011). However, joint application of biochar (organic amendment) and phytoremediation using different plant species has already been attempted but did not prove an efficient solution because biochar reduced the bioavailability of heavy metals in soil (Houben et al., 2013; Fellet et al., 2014). The combined application of biochar and phytoextraction may result in contrast effects, because biochar decreases soil metal availability on one hand, and increases phytoextraction potential of plants, on the other hand, resulting in enhanced soluble heavy metals uptake and accumulation in plant organs. Application of a nutrient enriched biochar to a high biomass production crop in a contaminated soil may enhance uptake potential of the crop by promoting its growth. Hence, we hypothesized that modification of biochar by loading it with nutrients such as P and applying the P-loaded biochar to the maize crop in a contaminated soil may alter soil heavy metals mobility and phytoremediation potential of the maize plants. Paz-Ferreiro et al. (2014) reviewed the potential of combining both phytoremediators and biochar and suggested that research is needed to confirm the sustainability of using phytoremediation in combination with biochar as remediation tools. To our knowledge, there is no available information about the application of nutrients-loaded biochar to assist growth and metal uptake potential of agronomic crops. Therefore, the objectives of this study were: (i) to assess the effects of biochar and P-loaded biochar on soil heavy metals extractability in a contaminated mining soil, and (ii) to elucidate the application effects of biochar and P-loaded biochar on growth and metal extraction potential of maize plants.

## 2. Materials and methods

### 2.1. Production of biochar and P-loaded biochar amendments

Date palm tree waste was collected from the agricultural farms in Riyadh city, Saudi Arabia, dried in air, leaves were separated, washed with de-ionized water to remove dust and impurities, and

cut into small pieces (2.5–5 cm), grinded using a mechanical grinder, sieved through 0.6 mm screen and stored. Date palm leaf waste biomass (BM) taken in a crucible with a loose cover and pyrolyzed (5 °C per min) at 600 °C for 3 h in a tube furnace (Carbolite, type 3216, UK) to produce biochar. Next day, the crucibles were taken out and kept in a desiccator for 30 min to remove moisture. The resulted material was weighed and washed several times with de-ionized water to remove soluble salts. Produced biochar was dried in an oven at 80 °C, stored in air-tight containers and labeled as BC.

A solution containing 200 mg P L<sup>-1</sup> was prepared in de-ionized water (18.2 MΩ cm<sup>-1</sup> resistivity; Milli-Q, Germany) using analytical grade KH<sub>2</sub>PO<sub>4</sub> (Loba Chemie, India). Biochar was added to the solution at the rate of 10 g L<sup>-1</sup> and shaken on a mechanical shaker at 150 rpm for 24 h to reach equilibrium. Biochar was separated from the solution by centrifugation at 3500 rpm for 20 min, washed thrice with de-ionized water to remove surplus phosphorus (not sorbed onto biochar), dried in an oven at 65 °C and stored in airtight containers with BCP label. The BM, produced BC and BCP were subjected to proximate, ultimate and chemical analyses.

## 2.2. Characterization of biochar and P-loaded biochar amendments

The BC and BCP were subjected to proximate analysis such as moisture, ash contents, and volatile matter, following the standard method of ASTM D1762-84 (ASTM, 1989). While resident matter (fixed carbon) was calculated with a different method. Electrical conductivity (EC) and pH of the materials were determined in 1:10 (1 g material in 10 mL de-ionized water). The cation exchange capacity was measured by NH<sub>4</sub><sup>+</sup>–Na<sup>+</sup> method. Total P was extracted following US EPA (1998) procedure using microwave digestion (MARS, CEM Corporation, USA). Available P was extracted using AB-DTPA extraction method (Soltanpour and Schwab, 1977). The P was analyzed by a molybdate-ascorbic acid method using UV/VIS spectrophotometer (Lambda EZ 150, PerkinElmer, USA) (Mussa et al., 2009). In this method, ascorbic acid, H<sub>2</sub>SO<sub>4</sub>, ammonium molybdate and antimony potassium tartrate were used to develop a color reagent. After adjusting the pH and developing the color in the sample, the absorbance was measured at 880 nm using UV/VIS spectrophotometer.

To observe surface morphology and structural changes induced by physio-chemical modifications, the materials were analyzed using scanning electron microscope (SEM; EFI S50 Inspect, Netherlands). Samples were spread on aluminum stubs coated with adhesive carbon tapes (12 mm; PELCO, UK) and coated with nano-gold particles for 60 s using 108 Auto/SE Sputter Coater (Ted Pella Inc. USA). Images were taken in the range of 2000–300× magnification at an acceleration voltage of 30 kV under high vacuum. Thermal stability of the materials was analyzed using thermogravimetric analyzer (DTG-60H, Shimadzu, Japan). Weight loss of the materials was recorded with the rise in temperature from 0 to 1100 °C. The composition of structural and functional groups of the materials was determined using Fourier transforms infrared spectroscopy (FTIR) (Bruker Alpha-Eco ATR-FTIR, Bruker Optics Inc.). To analyze various mineralogical phases of the produced materials, X-ray diffractometer (MAXima\_X XRD-7000, Shimadzu, Japan) was used with 30 mA Cu Kα radiation at the scan speed of 2° min<sup>-1</sup> in continuous scan mode. Surface area, total pore volume, and pore diameter were analyzed through Brunauer–Emmett–Teller (BET) method using surface area and porosity analyzer (TriStar II 3020, Micromeritics, USA).

The amount of P sorbed onto biochar matrix in BCP was calculated using the following equation (Ok et al., 2007):

$$q_e = \left[ \frac{C_0 - C_e}{m} \right] \times v \quad (1)$$

where C<sub>0</sub> and C<sub>e</sub> are the initial and equilibrium concentrations (in mg L<sup>-1</sup>) of the phosphorus, m is the mass of material (in g), and v is the volume of solution (in L).

The amount of total P in BC subtracted from the amount of total P in BCP also given the amount of sorbed amount of P onto biochar matrix during equilibrium batch. A non-linear form of Freundlich (Eq. (2)) and Langmuir (Eq. (3)) models were applied to fit the experimental sorption isotherms (Ahmad et al., 2013, 2017b).

$$q_e = K_F C_e^{1/n} \quad (2)$$

$$q_e = \frac{Q_L C_e K_L}{1 + K_L C_e} \quad (3)$$

where Q<sub>L</sub> is the Langmuir maximum adsorption capacity (mg g<sup>-1</sup>) and K<sub>L</sub> is the Langmuir sorption equilibrium constant (L mg<sup>-1</sup>), K<sub>F</sub> is the Freundlich affinity capacity parameter (L g<sup>-1</sup>), 1/n is the Freundlich component related to linearity.

## 2.3. Soil samples collection, processing, and analyses

Soil samples were collected from gold mining area called Mahad Adh Dahab (Cradle of Gold), Saudi Arabia. It is located about 380 km in Northern East of Jeddah (23°30'30" N, 40°51'30" E), in the West-central part of Arabian Shield. Due to mining activities, the area is polluted with heavy metals particularly with lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn). Therefore, soil samples were collected from different locations in Mahad Adh Dahab at the depth of 0–30 cm and mixed to form a composite soil sample. The composite sample was brought to the laboratory, air dried, grinded, passed with 2 mm screen and stored in polyethylene bags for further analyses. The processed soil sample was subjected to routine and heavy metal analyses with three replications. The soil was found to be sandy loam in texture, deficient in organic matter (0.46%) with active organic carbon contents of 312 mg kg<sup>-1</sup>, pH of 7.23 and EC of 3.01 d S m<sup>-1</sup>. The lime contents as analyzed with acid neutralization method (Allison and Moodie, 1965) were 4.51%. Total heavy metals (Cd, Pb, Mn, Cu, Pb, and Zn) and P in the soil samples were extracted following the procedure developed by US-EPA (1998) using microwave digestion (MARS, CEM Corporation, USA). Available heavy metals and P in the soil were extracted using AB-DTPA extraction method (Soltanpour and Schwab, 1977). The P concentration in the extracts was analyzed by a molybdate-ascorbic acid method using UV/VIS spectrophotometer, and heavy metals were analyzed using Inductivity coupled plasma optical emission spectrophotometer (ICP-OES, PerkinElmer Optima 4300 DV, USA). Total and AB-DTPA extracted P and metals are presented in Table 1. The soil was highly contaminated with Cu, Fe, Mn, Pb and Zn-containing a total of 3474, 25962, 1413, 1360 and 11239 mg kg<sup>-1</sup>, respectively, while marginally contaminated with Cd (27.8). AB-DTPA extracted P was much lower (7.6 mg kg<sup>-1</sup>) than total P in the soil (388 mg kg<sup>-1</sup>) indicating that a major portion of the P was non-extractable due to precipitation and/or fixation.

## 2.4. Soil incubation with biochar and P-loaded biochar amendments

One kg of processed Mahad Adh Dahab area soil was thoroughly mixed with the BC and BCP at application rates of 5, 10, 20, and 30 g kg<sup>-1</sup> of soil, recommended NK (60–45 kg ha<sup>-1</sup>) and NPK (60–60–45 kg ha<sup>-1</sup>), and a control (no amendment). The amended

**Table 1**  
Total and extractable amounts of heavy metals and phosphorus (P) of the soil collected from Mahd Adh Dahab (mining area).

	Cd mg kg <sup>-1</sup>	Cu mg kg <sup>-1</sup>	Fe mg kg <sup>-1</sup>	Mn mg kg <sup>-1</sup>	Pb mg kg <sup>-1</sup>	Zn mg kg <sup>-1</sup>	P mg kg <sup>-1</sup>	EC dS m <sup>-1</sup>	pH	OM <sup>a</sup> g kg <sup>-1</sup>	AOC <sup>b</sup> mg kg <sup>-1</sup>	CaCO <sub>3</sub> g kg <sup>-1</sup>	CEC cmol kg <sup>-1</sup>	Texture
Total	27.8 ± 2.3	3474 ± 17.9	25962 ± 51	1413 ± 15.9	1360 ± 11.6	11239 ± 37.8	388 ± 13	3.01 ± 0.02	7.23 ± 0.11	4.6 ± 0.21	312 ± 11.57	45.1 ± 0.69	7.92 ± 0.23	Sandy loam
Extractable	11.8 ± 1.2	194.2 ± 6.1	9.7 ± 0.52	72.9 ± 3.25	67.3 ± 6.24	231.7 ± 13.5	7.6 ± 0.89	—	—	—	—	—	—	—

<sup>a</sup> Organic matter.

<sup>b</sup> Active organic carbon.

and un-amended (control) soils were moistened at 50% of soil saturation percentage, filled in airtight containers and kept under dark for 45 days at room temperature (23 ± 2 °C). Moisture contents of the samples were maintained according to weight loss on regular basis.

## 2.5. Greenhouse pot experiment

After 45 days of incubation, soil from the containers was transferred into pots. The pots were arranged in RCBD (randomized complete block design) in the greenhouse with three repeats. Maize (*Zea mays* L.) was used as an experimental crop. About 10 corn seeds were sown in each pot. The pots were watered daily to maintain the moisture contents by observing daily weight loss. Thinning was performed when seedlings were grown to 10 cm length by maintaining four healthier plants per pot. The recommended dose of N and K was applied to all the pots (excluding control) in 2 splits, 1st right after thinning and 2nd after 2 weeks of sowing.

## 2.6. Harvesting and analyses

Maize plants were harvested with their roots after 30 days of sowing. Plants were washed with tap water to remove debris, with EDTA solution to remove entrapped metals and finally with de-ionized water. Extra water was wiped out gently with tissue paper and roots were separated from the shoots. Fresh weight and length of shoots and roots were recorded and samples were kept in paper bags for air dryness. Dry weight was recorded after complete dryness and samples were ground with mortar and pestle. Heavy metals and P in plant tissues were extracted with the procedure reported by Wolf (1982) and analyzed by inductivity coupled plasma optical emission spectrophotometer. Soil within the pots was air dried, crushed gently and subjected to further routine analyses. Available and total P and metals were extracted and determined by the procedure described earlier. Various soil heavy metals fractions were determined using sequential extraction procedure developed by Tessier et al. (1979).

## 2.7. Phytoextraction indices

To ensure phytoextraction and phytostabilization ability, the bio-concentration factor (BCF) in shoots and roots was calculated as the follow (Zhuang et al., 2007):

$$\text{Bio-concentration factor (BCF)} = \frac{\text{metal concentration in plant shoot or root}}{\text{metal concentration in soil}}$$

Additionally, translocation factor (TF), which indicates the ability of *Zea maize* plants to translocate heavy metals from the roots to the shoots, was calculated by the following formula (Zacchini et al., 2009):

$$\text{Translocation factor (TF)} = \frac{\text{metal concentration in aerial parts}}{\text{metal concentration in roots}}$$

## 2.8. Statistics

Statistics 8.01 program was used to statistically analyze the collected data. Least significant difference (LSD) test with 5% probability level was used to compare the influence of various treatments on maize crop production (Steel et al., 1997).



**Table 2**

Proximate, chemical, elemental composition, surface characteristics of the date palm tree leaf waste biomass (BM), its derived biochar (BC) and phosphorus loaded biochar (BCP).

Material		BM	BC	BCP
Yield	%	–	41.32 ± 0.61	–
Moisture	%	4.98 ± 0.004	1.01 ± 0.09	1.00 ± 0.08
Mobile matter	%	62.22 ± 2.32	8.93 ± 1.13	10.06 ± 1.18
Resident matter	%	24.47 ± 2.36	51.39 ± 0.70	50.11 ± 0.61
Ash	%	8.33 ± 0.01	38.68 ± 0.34	38.98 ± 1.98
pH	–	5.94 ± 0.00	10.23 ± 0.01	7.04 ± 0.02
EC	(dS m <sup>-1</sup> )	3.24 ± 0.00	5.55 ± 0.01	0.48 ± 0.00
CEC	(cmol kg <sup>-1</sup> )	70.3 ± 1.07	39.86 ± 0.93	31.29 ± 1.21
Total P	(mg kg <sup>-1</sup> )	1161.95 ± 13	1173.22 ± 9.52	4277.13 ± 17
Available P	(mg kg <sup>-1</sup> )	66.85 ± 3.82	76.88 ± 3.92	3116.67 ± 11
N	%	3.2	0	0
C	%	48.46	76.23	79.12
H	%	9.73	3.36	1.85
S	%	0	0	0
O	%	38.61	20.4	19.03
H/C	–	2.39	0.53	0.28
O/C	–	0.6	0.2	0.18
Surface area	m <sup>2</sup> g <sup>-1</sup>	0.46	164.73	151.25
Pore size	nm	24.565	4.919	4.13
Total volume in pores	cm <sup>3</sup> g <sup>-1</sup>	0.00019	0.09746	0.09231

### 3. Results and discussion

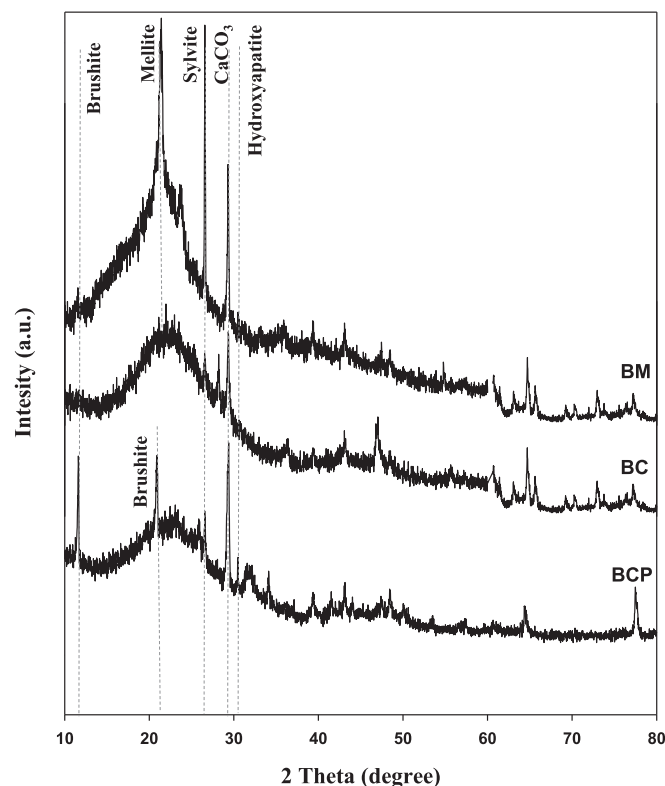
#### 3.1. Characterization of biochar and P-loaded biochar

Proximate and chemical analyses of date palm leaf waste biomass derived BC and BCP are shown in Table 2. On the basis of dry weight, pyrolysis gave 41.32% yield of BC. Moisture contents decreased with pyrolysis from 4.98 to 1.01% in BC and 1.00% in BCP due to thermalization. Pyrolysis increased the resident matter and ash contents, while decreased mobile matter. Increase in ash contents from 8.33% in BM to 38.68% in BC and 38.98% in BCP might be due to the formation of mineral compounds and condensation (Lehmann, 2007). Due to a thermal process, mobile matter decreased from 62.22% in BM to 8.93% in BC and 10.06% in BCP. Increase in resident matter from 24.47% in BM to 51.39% in BC and 50.11% in BCP was probably due to the enhanced degree of aromaticity. The pH of the BC increased almost 2 times (10.23) of pH of the BM (5.94), which could be due to lower acidic functional groups and concentration of basic functional groups during pyrolysis process (Ahmad et al., 2017c; Mukherjee et al., 2011). When BC was loaded with P, the pH of BCP decreased 3 units from the pH of BC, which might be due to shaking, washing and filtration which resulted in the loss of basic ions (such as Cl<sup>-</sup>). This is also indicated with lower EC of the BCP compared to BC. Due to loss of surface functional groups and increased carbon aromaticity, cation exchange capacity (CEC) was decreased from 70.30 cmol kg<sup>-1</sup> in BM to 39.86 cmol kg<sup>-1</sup> in BC and 31.29 cmol kg<sup>-1</sup> in BCP. Total P was 1173.22 mg kg<sup>-1</sup> in BC, which reached to 4277.13 mg kg<sup>-1</sup> when equilibrated with P solution for loading, indicating 3.104 mg of P has been sorbed onto one g of BC. Similarly, available P was found to be 76.88 mg kg<sup>-1</sup> in BC, while it reached to 3116.67 mg kg<sup>-1</sup> in BCP suggesting 3.039 mg of P sorbed onto one g of BC.

Elemental composition and BET surface area parameters of the amendments are presented in Table 2. Total C contents were increased in biochar materials (76.23% in BC and 79.12% in BCP) compared to BM (48.46%). That might be due to the increased degree of carbonization during pyrolysis (Wang et al., 2012). Other elements such as H, N, and O decreased with the pyrolysis due to loss of O-containing functional groups by dehydration and due to volatilization, respectively (Al-Wabel et al., 2014; Ahmad et al., 2014a). BCP exhibited lowest H/C and O/C molar ratios of 0.28 and 0.18, respectively, followed by BC, indicating lower polarity and

highest aromaticity. BC showed the highest surface area of 164.73 m<sup>2</sup> g<sup>-1</sup> and total volume in the pore of 0.09746 cm<sup>3</sup> g<sup>-1</sup>. BM exhibited minimum surface area (0.46 m<sup>2</sup> g<sup>-1</sup>) and total volume in pores (0.00019 cm<sup>3</sup> g<sup>-1</sup>), while maximum pore size (24.565 nm).

Thermally induced changes in the surface morphology of the BC and BCP amendments were accessed through scanning electron microscope (SEM). Images obtained through SEM are presented in Fig. S1a–d (Supplementary material). BM showed smooth and crystalline structure (Fig. S1a), which became amorphous when



**Fig. 1.** XRD patterns of date palm tree leaf waste biomass (BM), and its derived biochar (BC) and phosphorus loaded biochar (BCP).

pyrolyzed (Fig. S1b). Due to volatilization of organic compounds and loss of volatiles, BC structures became porous with channels (Usman et al., 2015). White amorphous particles of P are visible on the surface and in the channels of BC confirming the sorption of P while loading process (Fig. S1c and d). Mineralogy of the amendment materials as analyzed by XRD patterns is shown in Fig. 1. Various inorganic minerals were observed in the materials. A peak of C containing mineral i.e. mellite ( $\text{Al}_2[\text{C}_6(\text{COO})_6] \cdot 16\text{H}_2\text{O}$ ) appeared at  $2\theta = 21.42$  was present in BM, which disappeared during pyrolysis in BC and BCP (Ahmad et al., 2017c; Usman et al., 2015). A peak at  $2\theta = 26.56$  was designated as sylvite (KCl), which was more prominent in BM compared to BC. Calcite ( $\text{CaCO}_3$ ) was also observed at  $2\theta = 29.3$  in BM which reduced in BC and increased again in BCP. A peak of brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) at  $2\theta = 11.62$  and  $20.92$  and a peak of hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) at  $2\theta = 30.50$  in BCP was detected in BCP, which were absent in BM and BC. Surface functional groups on the amendments investigated through FTIR spectra in the range of  $600\text{--}4000\text{ cm}^{-1}$  are shown in Fig. S2. A broadband around  $3300\text{ cm}^{-1}$  represents  $-\text{OH}$  stretches of H-bounded water molecules and other volatile functional groups in BM, which was disappeared in BC and BCP suggesting a loss of moisture and other volatiles during pyrolysis as also indicated by proximate analysis. A band at  $2916.52\text{ cm}^{-1}$  was designated as C–H stretches, while at  $2847.18\text{ cm}^{-1}$  was designated as O–H and C–H stretch in BM, which was vanished in BC and BCP. These stretches represent the presence of aliphatic compounds and polar functional groups in BM, while absence in BC and BCP (Jouiad et al., 2015; Usman et al., 2015).

A band at  $1605.12\text{ cm}^{-1}$  indicated O–H stretches of carboxylic groups in BM, which lost during pyrolysis. Polysaccharide cellulose was identified with a band at  $1046.28\text{ cm}^{-1}$  of C–O stretches. A band at  $799.50\text{ cm}^{-1}$  was credited as C–H aromatics, which became sharp in BC and BCP. A shoulder peak at  $982\text{ cm}^{-1}$  in BCP was ascribed as  $\text{PO}_4^{3-}$  stretches. Thermal degradability of the amendments as assessed through TGA thermograms is shown in Fig. S3. Maximum weight loss of 81.14% was shown by BM, while BC and BCP showed a similar weight loss of 62.63%. Weight loss around temperature  $200\text{ }^\circ\text{C}$  in BM and BC was due to loss of non-structural free water. Curves around  $300$  and  $400\text{ }^\circ\text{C}$  in BM and BC represents weight loss due to decomposition of cellulosic and hemicellulosic compounds (Yang et al., 2007). Complete weight loss of the materials occurred at a temperature around  $900\text{ }^\circ\text{C}$  for BM and at  $1010\text{ }^\circ\text{C}$  for BC and BCP due to degradation of lignin (Hernández-Mena et al., 2014).

### 3.2. Mechanism of P sorption onto biochar

Due to lower anion exchange capacity and net negatively charged surface area, binding of  $\text{PO}_4^{3-}$  ions with negatively charged functional groups (hydroxyls, carbonyls, and carboxyls) on the surface of biochar is very unusual (Chun et al., 2004). Nevertheless, the  $\text{PO}_4^{3-}$  ions might form bridge bonds with the residual charge of ligand bonded divalent cations ( $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$ ) resulting in  $\text{PO}_4^{3-}$  sorption onto the biochar (Hale et al., 2013; Mukherjee et al., 2011). The divalent cations can either form bridge bonds on the surface of biochar, or can be released from the biochar and bond with  $\text{PO}_4^{3-}$  ions and get precipitated. Furthermore, the presence of free active sites (binding sites) on the surface of the biochar may result in sorption of  $\text{PO}_4^{3-}$  ions onto the biochar matrix (Hale et al., 2013). Non-linear forms of Langmuir and Freundlich isotherms were employed on the sorption data. The Langmuir isotherm predicted that maximum sorption capacity ( $Q_L$ ) of the biochar for P was  $11.43\text{ mg g}^{-1}$  (Table S1). The best fitted Freundlich isotherm ( $R^2 = 0.93$ ) suggested that P was sorbed onto biochar matrix via multilayer chemisorption (Ahmad et al., 2017d). The occurrence of chemisorption was confirmed by the XRD analysis of the BCP, which indicated the formation of the new mineral phases (brushite and hydroxyapatite) due to binding of  $\text{PO}_4^{3-}$  ions with divalent  $\text{Ca}^{2+}$  either on the surface of biochar and in the form of precipitation (Fig. 1). Moreover, the presence of  $\text{PO}_4^{3-}$  ions on the surface of the biochar was also observed in FTIR spectra of the BCP, confirming the binding of the  $\text{PO}_4^{3-}$ –P onto the biochar surface. The procedure to load P onto biochar, possible mechanism of P sorption onto biochar and application of the produced materials have been represented in Fig. S4.

### 3.3. Soil pH and available forms of phosphorus

Changes in the AB-DTPA extracted (plant available) P concentration with various amendments applications are presented in Fig. 2a. No significant differences ( $p > 0.05$ ) were observed on AB-DTPA extracted soil P under control, NK, all BC treatment and 0.5% BCP. Due to the presence of P, NPK and BCP significantly ( $p < 0.05$ ) increased AB-DTPA extracted P compared to control. Application of 1, 2 and 3% BCP increased P availability by 61.92, 74.67 and 79.42% compared to control. Formation of exchangeable monodentate complexes between anionic P and surfaces of biochar in BCP could be a reason for more P availability in soil (Sparks, 1995). Another reason for increased P availability could be the mineralization of P

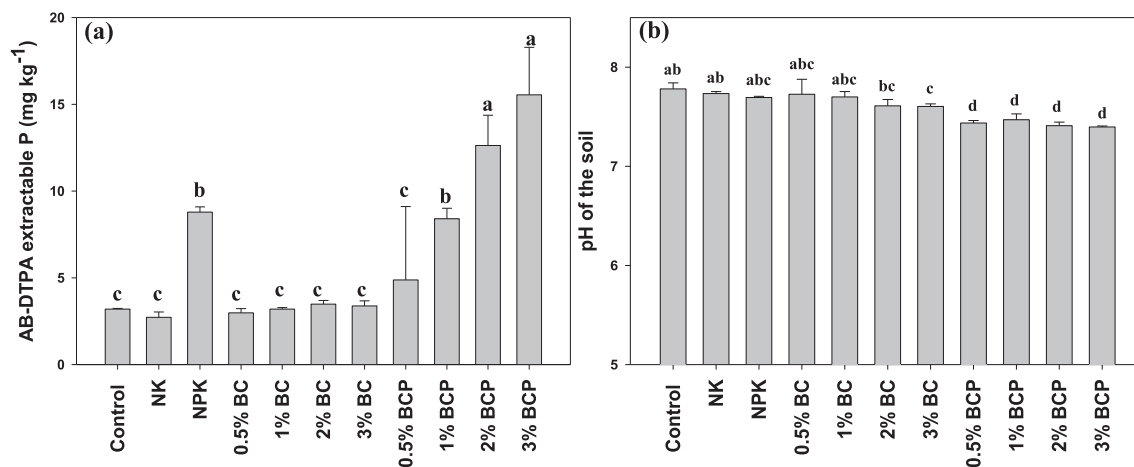
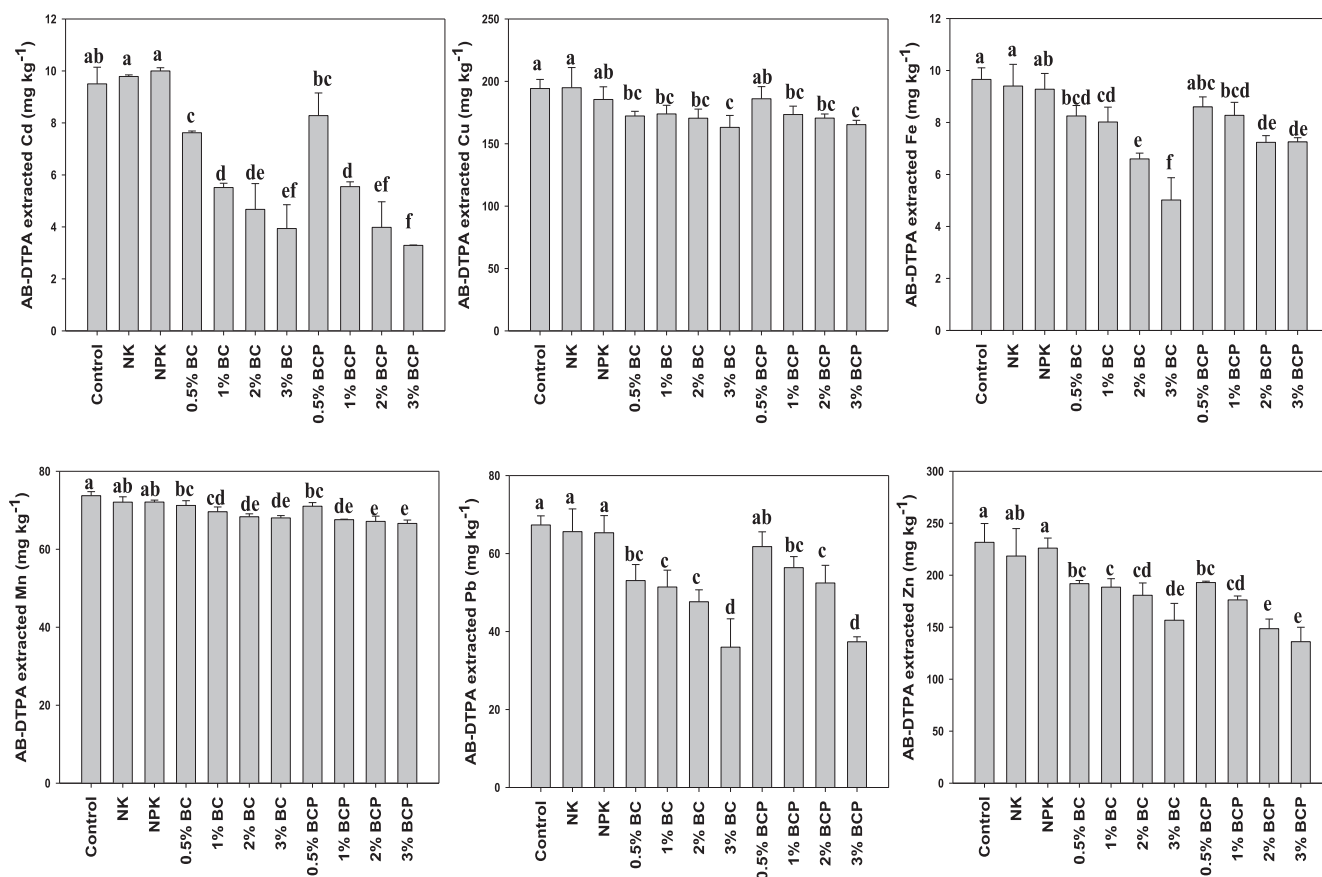


Fig. 2. Amendments (NK, NPK, BC: date palm tree leaf waste derived biochar, and BCP: phosphorus loaded biochar) effects on (a): availability of soil phosphorus (P) and (b): changes in soil pH. (Different letters above bars represents significant difference at  $p < 0.05$  among various treatments. Bars with same letters represent no significant difference).



**Fig. 3.** Amendments (NK, NPK, BC: date palm tree leaf waste derived biochar, and BCP: phosphorus loaded biochar) effects on the availability of heavy metals in soil. (Different letters above bars represents significant difference at  $p < 0.05$  among various treatment. Bars with same letters represent no significant difference).

during incubation of the soil. During incubation, due to phosphatase activity, P-bounded biochar might have mineralized and P was transformed to inorganic P. The P availability can also be influenced by various other factors such as soil pH, organic matter, and soluble/exchangeable Ca, Fe and Al in the soil (Smithson, 1999). Between the pH range of 6–7, P availability to the plants becomes maximum. As shown in Fig. 2b, higher BCP application rates i.e. 2 and 3%, shown lower pH, closer to 7 (7.41 and 7.39, respectively). After BCP application, desorption of P has also occurred due to pH changes and other soil reactions, resulting in higher P availability. During sorption process for P loading, some of the P was adsorbed on the surface by forming detachable complexes, which might also have desorbed easily (Khare et al., 2007). Higher P availability with higher BCP application rate might be due to more organic matter (biochar), which when mineralized, compete with P sorption sites in soil resulting in higher P concentration in the solution (Ohno et al., 2007; Ohno and Amirbahman, 2010). Therefore, in addition to desorption of P in the soil solution, biochar can decrease P fixation in soil resulting in enhanced P availability (Morales et al., 2013).

#### 3.4. Soil available forms and fractionation of heavy metals

The concentration of AB-DTPA extracted heavy metals in soil are shown in Fig. 3. To investigate the variation in the AB-DTPA extracted heavy metals as influenced by the application of various amendments, the heavy metals data was subjected to the statistical analysis (LSD test). All the treatments exhibited significant differences ( $p < 0.05$ ) in terms of plant available heavy metal

concentrations compared to control (Fig. 3). It was interesting to notice that BC and BCP showed a similar trend i.e. with an increase in the application rate of the BC and BCP, availability of the metals decreased proportionally. The 3% BCP application reduced soil metals concentrations slightly more than 3% of BC application that might be due to the binding of the surface bounded P in BCP to the heavy metals. Highest reduction in available Cd was observed when 3% of either BC (58.54%) or BCP (65.39%) were applied. Pb, Zn, Fe, Cu and Mn shown a reduction in availability by 46.53 and 44.51%, 32.34 and 41.27%, 48.05 and 24.93%, 16.21 and 14.88, and 7.75 and 9.65% compared to control under 3% BC and BCP application, respectively. These results revealed the potential of biochar in alleviating the adverse effects of heavy metals on the plant growth in polluted soil. Various mechanisms could be responsible for reducing soil available heavy metal concentration due to biochar application, including electrostatic interaction, adsorption of metals to biochar, surface precipitation, complexation, and ion metal exchange with alkali and alkaline earth cations, and physical sorption phenomena (Farooq et al., 2010; Ahmad et al., 2014b; Usman et al., 2013; Park et al., 2011; Cao et al., 2009; Tong et al., 2011). Due to high surface area and pore volume, biochar has high affinity towards heavy metals, mainly by physical sorption (Idrees et al., 2016). On the other hand, the formed various functional groups on biochars can interact with a range of heavy metals through complexation (Ma et al., 2010). Due to microbial activities in the soil, biochar degradation could produce carboxylic acids as a main resulting product by degradation of lignin and cellulose (Klinke et al., 2002). Such kind of products have been reported alleviating the detrimental effects of Pb, Cd and some other metals

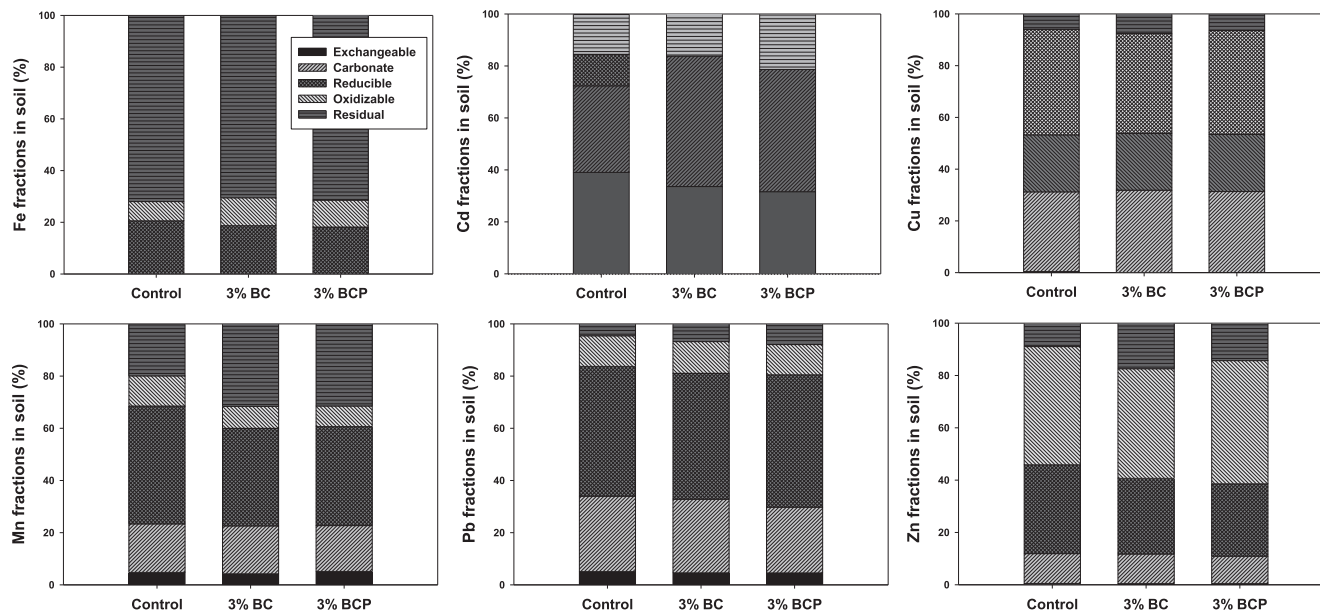


Fig. 4. Amendments (NK, NPK, BC: date palm tree leaf waste derived biochar, and BCP: phosphorus loaded biochar) effects on heavy metal fractions in soil.

by immobilizing them in metal hydroxide forms (Chen et al., 2003; Suanon et al., 2015). Additionally, the precipitation process has been reported to be of great importance in controlling soil availability of heavy metals through forming precipitates either in soil solution or on biochar surface (Ma et al., 2010; Yang et al., 2016). As expected in the current study, the highest total ( $4277.13 \text{ mg kg}^{-1}$ ) and available P ( $1173.22 \text{ mg kg}^{-1}$ ) was exhibited by P-loaded biochar (BCP), which served as P fertilizer in the soil and improved plant growth eventually resulting in higher metal uptake. As pH of the soil remained around 7.5, it therefore, did not affect the mobility of metals to larger extents (Fig. 2b).

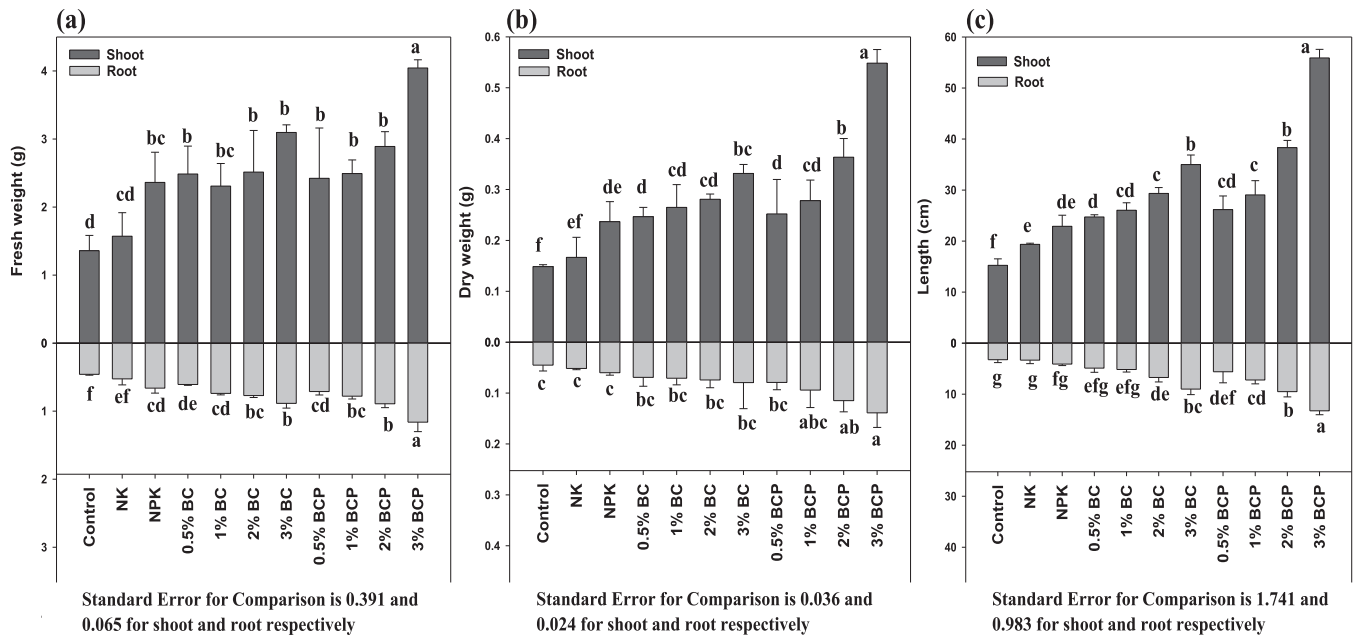
Our results are in agreement with Park et al. (2011), who found a significant reduction in  $\text{NH}_4\text{NO}_3$  extracted Cd, Cu and Pb concentrations in the soil under biochar application. Similarly, Al-Wabel et al. (2014) reported a significant decrease in  $\text{NH}_4\text{OAc}$  and AB-DTPA extracted soil heavy metals such as Fe, Mn Zn, Cd, Cu and Pb when biochar was applied at the rate of 1, 3 and 5% (w/w). Fellet et al. (2011) also reported the reduction in mobility and availability of heavy metals such as Zn, Pb, Cr and Cd under biochar application. Biochar might have changed readily available metals into geochemically stabilized forms resulting in immobilization of the metals (Ahmad et al., 2014a). Hence, it can potentially be used to enhance crop production even in the polluted soils.

Changes in soil heavy metal fractions under the application of various amendments are shown in Fig. 4. Heavy metal polluted soil amended with amendments (NK, NPK, BC and BCP) along with control was incubated for 45 days under dark, which might have changed the fractions of the metals. All the fractions in the bars are represented as a percentage of the total metal contents (sum of all the fractions). In the case of Cd, reducible metal fraction reduced to zero from 12.09% in control, under 3% BC and BCP treatments. Exchangeable fraction reduced from 39.03% in control to 33.59 and 31.61% under 3% BC and BCP applications, respectively, while carbonate and residual fraction increased from 33.35 to 15.54% in control to 50.22 and 16.19% under 3% BC and 47.03 and 21.37% under 3% BCP application, respectively. Exchangeable Mn fraction decreased to 4.25% in 3% BC, while increased to 5.3% in BCP application, from 4.75% in control. Carbonate, reducible and oxidizable

Mn fraction decreased, while residual fraction increased from 19.93% in control to 31.62 and 31.48% under 3% BC and BCP application, respectively. The exchangeable fraction of Pb decreased from 5.08% in control to 4.61 and 4.59% in 3% BC and BCP application, respectively. No significant changes were observed in carbonate bounded, reducible and oxidizable Pb fractions, while residual Pb fraction increased from 4.57% in control to 6.79 and 7.93% in 3% BC and BCP application, respectively. In Cu fractions, there were no significant differences in reducible and oxidizable fractions, while exchangeable fraction decreased from 0.41% in control to 0.06% and 0.11% under 3% BC and BCP application, respectively. Carbonate fraction of Cu increased from 30.81% in control to 31.82 and 31.23% under 3% BC and BCP application, respectively. Likewise, residual Cu fraction increased from 5.96% in control to 7.54 and 6.42% under 3% BC and BCP application, respectively. There were no significant variations in exchangeable, carbonate and residual Fe fractions. Reducible Fe fraction decreased from 20.57% in control to 18.69 and 18.17% under 3% BC and BCP application, respectively. Oxidizable Fe fraction increased from 7.41% in control to 10.71% in 3% BC and 10.4% in 3% BCP application.

In the case of Zn, carbonate bounded and exchangeable fraction did not show much variations. Reducible and oxidizable Zn fraction decreased, while residual fraction increased from 8.95% in control to 17.42 and 14.34% under 3% BC and BCP application, respectively. These results indicated an overall reduction in exchangeable and reducible metals fractions, while increment in residual metal fractions with the application of BC and BCP amendments. These results are in agreement with the results reported by Xu et al. (2014) and Zhu et al. (2015). As the exchangeable fraction is the available fraction, which can directly be taken up by the plants, therefore, reduction in exchangeable metal fractions under BC and BCP application suggested immobilization of the metals. Reducible and oxidizable fractions are termed as slow release fractions, which can be used by the plants when transformed to acid soluble fractions. Residual fraction is scarcely available to plants, as it is usually fixed in soil lattice (Chen, 2005). As the conversion of the exchangeable fraction to the residual fraction can reduce biological toxicity (Ahmad et al., 2012), hence, the results of the current study





**Fig. 5.** Amendments (NK, NPK, BC: date palm tree leaf waste derived biochar, and BCP: phosphorus loaded biochar) effects on (a) fresh weight in  $\text{g plant}^{-1}$ , (b) dry weight in  $\text{g plant}^{-1}$  and (c) length in  $\text{cm plant}^{-1}$  of shoot and root of maize crop grown on heavy metal polluted mining area soil in a greenhouse. (Different letters above bars represents significant difference at  $p < 0.05$  among various treatments. Bars with same letters represent no significant difference).

explored the potential of BC and BCP amendments to alleviate metal toxicity by reducing phyto-available fractions. Reduction in exchangeable metal fractions and an increase in residual metals fractions represents that physio-chemical changes have occurred when BC or BCP was applied to the soil (Yang et al., 2014). Due to large surface area and oxygen functional groups such as carboxyl and phenol hydroxyl, heavy metals can bind on the surface of the biochar via chelation and complexation resulting in decreased exchangeable fractions of the metals (Caporale et al., 2014). Additionally, biochar provided organic matter to the soil and increased its CEC resulting in enhanced complexation which also decreased exchangeable metal fractions (Abdel-Fattah et al., 2015). Application of biochar increased alkaline groups ( $\text{OH}^-$ ,  $\text{SiO}_3^{2-}$  and  $\text{CO}_3^{2-}$ ) in the soil solution, which created hydroxide, silicate, and carbonates of insoluble metals, eventually transforming exchangeable metal fractions to reducible, oxidizable and residual fractions (Xu et al., 2016; Loganathan et al., 2012). Transformation of exchangeable and reducible metal fractions to residual metal fraction resulted in

reduced mobility and phytoavailability of the metals in the contaminated mining soil.

### 3.5. Plant growth and phosphorus uptake

Results of the shoot and root fresh weights (g), dry weights (g) and lengths (cm) are shown in Fig. 5a–c. Results indicated that addition of BC and BCP significantly ( $p < 0.05$ ) increased length and fresh and dry weight of root and shoot. All the amendments showed significant differences (LSD test) from the control. The maximum fresh weight of the plant (shoot and root) was recorded in the treatments with the highest application rate of either BC or BCP i.e. 3%, followed by 2%, while lowest was observed in control (without any amendment). The 3% BCP application was found to be the best among all the amendments as it increased shoot fresh and dry weights by 66.42 and 72.90%, root fresh and dry weights by 60.54 and 67.53%, and shoot and root length by 72.70 and 75.52%, respectively, compared to control (Fig. 5). Followed by 3% BCP, 2%

**Table 3**

Amendments (NK, NPK, BC (date palm tree leaf waste derived biochar), and BCP (phosphorus loaded biochar)) effect on phosphorus concentration in root and shoot of maize plants, and uptake of phosphorus into root and shoot tissues on dry matter basis of maize plants.

Treatments	Concentration ( $\text{mg kg}^{-1}$ )		Uptake ( $\mu\text{g plant}^{-1}$ )	
	Shoot	Root	Shoot	Root
Control	5137.2 ± 39 c	2047.1 ± 84 e	765.47 ± 22 e	91.46 ± 2.0 d
NK	5151.7 ± 33 c	2024.7 ± 81 e	882.79 ± 21 e	103.79 ± 3.4 dc
NPK	5438.9 ± 88 c	2105.0 ± 49 e	1303.1 ± 24 de	125.10 ± 3.5 dc
0.5% BC	5402.3 ± 68 c	2368.8 ± 91 de	1322.6 ± 19 cde	155.95 ± 4.2 cd
1% BC	5427.5 ± 56 c	2570.4 ± 77 cde	1403.8 ± 37 cd	177.08 ± 5.9 cd
2% BC	5705.5 ± 67 bc	3022.5 ± 67 cd	1600.1 ± 27 cd	221.34 ± 5.8 bcd
3% BC	5937.7 ± 31 bc	3105.1 ± 56 bcd	1962.3 ± 26 c	242.59 ± 16.0 bcd
0.5% BCP	5558.8 ± 42 c	3342.4 ± 60 bc	1368.7 ± 21 cde	257.38 ± 5.1 bc
1% BCP	5846.2 ± 61 bc	3886.7 ± 65 b	1566.3 ± 23 cd	344.02 ± 5.6 b
2% BCP	7511.0 ± 89 ab	4824.7 ± 32 a	2740.5 ± 25 b	551.97 ± 8.9 a
3% BCP	8065.3 ± 83 a	5044.6 ± 75 a	4423.3 ± 32 a	704.35 ± 15.9 a

Different letters represents significant difference at  $p < 0.05$  among various treatments. The same letters represent no significant difference.

BCP exhibited highest dry weights and lengths of shoot and root compared to other treatments. No significant differences ( $p > 0.05$ ) were observed among 2% BC, 3% BC, 0.5% BCP, 1% BCP and 2% BCP in terms of shoot fresh weights. Rajkovich et al. (2012) reported an increase of 30–43% in biomass of corn plants with different biochar applications. Uzoma et al. (2011) reported 98–50% increase in maize grain yield under 20 and 15 t ha<sup>-1</sup> of biochar application, respectively. According to Zhang et al. (2012), biochar application rate of 20 and 40 t ha<sup>-1</sup> increased maize crop yield by 15.8 and 7.3%, respectively.

Vigorous growth under BCP application was due to higher P availability as is indicated by root and shoot P concentrations and uptake. The BC application did not significantly increase P concentration in shoots and roots, with exception of 2 and 3% BC application resulting in a slight increase (9.96 and 13.48%, respectively) in shoot P compared to control (Table 3). Whereas application of BCP significantly improved the concentration of P in shoot compared to control. As application rate of the BCP increased, P concentration in shoot tissues increased proportionally. The 1, 2 and 3% BCP application increased shoot P concentration by 12.13, 31.60 and 36.31%, respectively, compared to control. Similarly, BCP also significantly increased root P concentration than control. The 1, 2 and 3% BCP application rates increased root P concentration by 47.33, 57.57 and 59.42%, respectively than control. Likewise, maximum P uptake in shoots and roots was observed in 3% BCP application (4423.3 and 704.4  $\mu\text{g plant}^{-1}$ , respectively) followed by 2% BCP (2740.5 and 551.9  $\mu\text{g plant}^{-1}$ , respectively) as presented in Table 3. Best performance of BCP in terms of shoot and root P concentration and uptake was due to P release from the biochar matrix to soil solution which ultimately taken up by the plants. As P was loaded affectedly, anionic P particles also produced exchangeable monodentate complexes with the bidentate complexes on the surface of biochar. These exchangeable monodentate complexes enhanced P available pool in the soil which eventually

taken up the maize plant (Sparks, 1995). In BC, due to low P concentration, anionic P might have formed bidentate complexes at the sorption sites of the surface of BC which hindered the P availability to the roots of plants (Sparks, 1995). Higher P uptake to shoot and root in BCP application resulted in vigorous plant growth producing more dry matter. It was very interesting to notice that P uptake of shoots and roots of maize plants was significantly higher than the recommended NPK fertilization. Therefore it can be stated that application of P-loaded biochar to the agronomic crops could serve as a cost-effective fertilization strategy as compared to commercial fertilizers.

### 3.6. Heavy metals concentration and uptake by maize plants

Heavy metals concentrations in shoot and root of the maize plants are shown in Table 4. The results showed that the applied amendments did not cause significant differences ( $p > 0.05$ ) in the concentrations of heavy metals in shoots. Similarly, with the application of the amendments, there were no significant differences in the root concentrations of Cd, Fe, and Zn compared to control soil. On the contrary, significant differences ( $p > 0.05$ ) were observed among various amendments on the concentrations of Cu, Mn, and Pb in the roots of the plant. It was observed that the BC and BCP showed significant decreases in the shoots concentrations of these three metals compared to control soil, with the most significant differences at higher application rate. Generally, the highest reduction was observed in 3% BCP application. By adding BC at 3%, concentrations of Cu, Mn, and Pb in the roots of maize plants decreased from 472.66, 260.70, and 176.28 mg kg<sup>-1</sup> to 325.88, 193.23, and 136.06 mg kg<sup>-1</sup>, respectively. This reduction in metals (Cu, Pb, and Mn) concentration in roots of maize plants was might be due to plant selectivity and metal immobilization in the soil, as indicated by a reduction in AB-DTPA extracted soil heavy metal levels (Lu et al., 2012). The Cu and Pb concentrations might have

**Table 4**  
Amendments (NK, NPK, BC: date palm tree leaf waste derived biochar, and BCP: phosphorus loaded biochar) effects on heavy metals concentration and uptake into shoot and root tissues of maize plant grown in greenhouse on heavy metal polluted mining area soil.

Amendments	Cu		Fe		Mn		Pb		Zn		Cd	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
<b>Concentration (mg kg<sup>-1</sup>)</b>												
Control	26.79 a	472.7 ab	353.6 a	9558 a	48.80 a	260.7 ab	30.05 a	176.3 a	138.4 a	1445 a	2.90 a	7.61 a
NK	27.55 a	488.9 a	361.1 a	9562 a	37.78 a	281.6 a	26.70 a	173.2 a	134.6 a	1434 a	2.85 a	7.46 a
NPK	28.61 a	489.9 a	368.5 a	9522 a	40.34 a	278.9 a	32.56 a	174.5 a	136.1 a	1460 a	3.77 a	7.78 a
0.5% BC	22.43 a	481.9 ab	369.9 a	9555 a	42.79 a	265.2 ab	29.60 a	171.7 a	139.6 a	1432 a	3.29 a	8.13 a
1% BC	25.20 a	428.6 abcd	325.0 a	9521 a	36.40 a	252.5 ab	32.70 a	167.8 ab	135.2 a	1402 a	2.52 a	7.43 a
2% BC	24.05 a	404.8 bcde	329.5 a	9504 a	41.64 a	258.5 ab	31.02 a	153.9 ab	130.8 a	1361 a	2.10 a	7.13 a
3% BC	23.41 a	393.2 cdef	327.9 a	9486 a	41.51 a	243.0 ab	29.01 a	152.8 ab	130.2 a	1400 a	2.70 a	6.31 a
0.5% BCP	26.55 a	441.2 abc	367.6 a	9539 a	40.56 a	266.3 ab	29.61 a	163.3 ab	148.4 a	1377 a	3.51 a	6.77 a
1% BCP	25.65 a	351.3 def	338.6 a	9547 a	45.00 a	223.6 ab	30.55 a	150.6 ab	143.2 a	1361 a	3.22 a	5.75 a
2% BCP	23.29 a	343.9 ef	320.1 a	9504 a	39.16 a	208.8 ab	28.15 a	145.1 ab	127.0 a	1342 a	2.20 a	5.35 a
3% BCP	22.14 a	325.9 f	302.6 a	9471 a	34.35 a	193.2 b	26.21 a	136.1 b	131.4 a	1336 a	2.15 a	5.25 a
<b>Uptake (<math>\mu\text{g plant}^{-1}</math>)</b>												
3% BCP												
Control	4.01 d	20.84 b	52.48 d	425.04 c	7.260 b	12.20 b	4.48 c	7.893 c	20.52 d	67.26 c	0.44 a	0.36 a
NK	4.70 cd	25.27 ab	61.99 cd	493.57 c	6.530 b	14.49 ab	4.40 c	8.987 c	22.77 cd	74.42 c	0.49 a	0.38 a
NPK	6.85 bcd	29.49 ab	86.36 bcd	570.03 c	9.460 ab	16.80 ab	7.78 bc	10.44 bc	33.68 bcd	87.51 c	0.96 a	0.47 a
0.5% BC	5.49 bcd	33.16 ab	90.75 bcd	668.18 bc	10.69 ab	18.64 ab	7.25 bc	11.58 bc	35.19 bcd	99.32 bc	0.79 a	0.54 a
1% BC	6.65 bcd	30.33 ab	82.87 bcd	676.66 bc	9.960 ab	17.73 ab	8.86 b	11.85 bc	36.32 bcd	99.42 bc	0.66 a	0.54 a
2% BC	6.78 bcd	29.50 ab	92.69 bcd	708.13 bc	11.69 ab	19.78 ab	8.82 b	11.67 bc	36.90 bcd	101.98 bc	0.59 a	0.53 a
3% BC	7.76 bc	33.33 ab	108.89 b	733.19 bc	13.85 ab	16.82 ab	9.60 b	11.79 bc	43.24 bcd	99.22 bc	0.88 a	0.41 a
0.5% BCP	6.68 bcd	34.41 ab	94.04 bc	755.75 bc	10.35 ab	20.87 ab	6.71 bc	12.66 abc	38.11 bcd	107.87 bc	0.95 a	0.54 a
1% BCP	6.97 bcd	31.61 ab	92.99 bc	892.3 bc	13.09 ab	20.50 ab	8.41 bc	13.91 abc	40.27 bcd	128.27 abc	0.88 a	0.51 a
2% BCP	8.62 b	40.31 ab	114.1 b	1111.1 ab	14.69 ab	24.29 ab	10.1 b	16.44 ab	46.27 b	152.54 ab	0.76 a	0.64 a
3% BCP	12.3 a	58.10 a	166.1 a	1331.6 a	19.26 a	27.18 a	14.5 a	19.02 a	72.59 a	186.06 a	1.21 a	0.73 a

Different letters represents significant difference at  $p < 0.05$  among various treatment. The same letters represent no significant difference.

immobilized by the O-containing functional groups of the biochar (Uchimiya et al., 2011). Immobilization of heavy metals with biochar applications has already been reported (Ahmad et al., 2016; Jones et al., 2016; Ok et al., 2015). Therefore, the 3% BCP application was more effective in promoting plant growth, enhancing P concentration, and reducing metal concentrations in root and shoot tissues of maize plants.

On the other hand, the success of phytoremediation process (including phytoextraction and phytostabilization) is dependent upon both the biomass of roots and shoots grown and their uptake of heavy metals. Therefore, metals uptake by roots and shoots of maize plants was calculated and presented in Table 4.

The results showed that heavy metals uptake to shoot and root significantly ( $p < 0.05$ ) increased with the BC and BCP application compared to control (except for Cd). Maximum heavy metal uptake to shoot and root was observed in 2 and 3% BCP application, which is related to higher dry matter production due to more P uptake. Application of 2 and 3% BCP increased shoot uptake of Cu by 115 and 206%, Fe by 117 and 216%, Mn by 102 and 165%, Pb by 125 and 222%, Zn by 125 and 254%, and Cd by 75 and 177%, respectively, compared to control, while similar amendments increased root uptake of Cu by 93 and 179%, Fe by 161 and 213%, Mn by 99 and 123%, Pb by 108 and 141%, Zn by 127 and 177%, and Cd 79 and 104%, respectively, compared to control. Overall, 3% BCP application improved phytoextraction ability of the maize plant by 2–3 folds through enhancing plant growth due to P fertilization. These results suggest the possibility of using biochar in combination with nutrient supplements for phytoremediation of heavy metals using maize crop. These results were in agreement with the findings of Houben et al. (2013) and Fellet et al. (2014). Houben et al. (2013) in their study observed phytostabilization of Cd and Zn by *Brassica napus* plant under biochar application. Fellet et al. (2014) observed higher Pb uptake by different plants under biochar application due to enhancement in plant growth, thus suggesting phytostabilization. Application of biochar often results in improved soil water holding capacity, nutrient retention, microbial community, CEC and responses of plants to diseases. These improved characteristics eventually result in increased plant yield, suggesting the potential of the biochar as phytoextractant (Graber et al., 2010; Paz-Ferreiro et al., 2014). Results of the current study were in contrast with the findings of some previous studies which suggested reduced heavy metals uptake under biochar application that might be attributed to dilution effect of increased plant growth, immobilization of metals in soil and stable metal-organic complexes formation (Glaser et al., 2015; Xu et al., 2016; Beesley et al., 2013; Park et al., 2011; Namgay et al., 2010).

It has been established that phytoextraction indices for plant species such as TF and BCF with values greater than unity are usually considered as *hyperaccumulators*, while TF and BCF values less than unity are considered as *excluders* (Steve and Zhao, 2003; Usman et al., 2013; Lu et al., 2014). The calculated phytoextraction indices in the current study are shown in Table S2. The results revealed that the TF and BCF values for BC and BCP amendments are less than unity, suggesting that phytoextraction is not feasible for the maize plants in the current study in spite of higher biomass production (Steve and Zhao, 2003). The percent accumulation of the metals by shoot and root of the maize plants is reported in Table S3. Results indicated that a major portion of the metals was accumulated in the roots, compared to shoots. On average, above 82, 88, 63, 60, 73 and 44% of the Cu, Fe, Mn, Pb, Zn and Cd absorbed by the plant were accumulated in the roots, respectively. Accumulation of heavy metals in roots indicated reduced translocation of metals, (as indicated by TF and BCF values in Table S2), suggesting phytostabilization to a lower extent. Phytostabilization is the ability of a plant species to accumulate a significant proportion of the

heavy metals in roots, thus preventing nearby or underground water contamination (Vamerali et al., 2009). Fellet et al. (2014) and Karami et al. (2011) reported that application of biochar was suitable for phytostabilization of some heavy metals. Therefore, based on our results, it can be stated that P-loaded biochar can efficiently be applied for heavy metals immobilization in the soil, but it is not feasible for phytoextraction by maize plants in a contaminated mining area soil.

#### 4. Conclusion

Biochar (BC) and P-loaded biochar (BCP) after sorption from polluted aqueous solution were applied to a heavy metal contaminated mining area soil in a greenhouse pot experiment to investigate the effects of these amendments on soil heavy metal lability and, growth and phytoextraction of maize (*Zea mays* L.) plants. Results revealed that 3% BCP application performed outclass in immobilizing soil heavy metals (Cu, Fe, Mn, Pb, Zn, and Pb) and improving plant growth. The application of BCP increased soil available (AB-DTPA extracted) concentrations of P, while application of BC and BCP decreased the soil available heavy metals concentrations significantly. Enhanced metal immobilization in soil with BCP application could be due to surface complexation onto biochar, and metal precipitation with P in the soil solution. Exchangeable and reducible heavy metals fractions (potentially available) were transformed into residual metal fractions (more stable) resulting in reduced mobility of the metals in soil. Additionally, application of BCP improved plant growth significantly by enhancing root and shoot lengths and dry matter. Therefore, it could be concluded that application of P-loaded biochar to a contaminated soil can (i) solve the disposal issue of the loaded sorbents after sorption, (ii) immobilize the heavy metals in polluted soil and (iii) serve as P fertilizer to boost up crop growth for more biomass production, subsequently helping the stabilization of contaminated areas affected by wind and water erosion. However, based on phytoextraction indices, total heavy metals extraction by maize plants was very small for the practical application. Therefore, future studies are needed to investigate the interaction between biochars and a wide range of plants including phytoremediators and agronomic crops as remediation tools in terms of phytostabilization and phytoextraction.

#### Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research, King Saud University for funding this work through the international research group project IRG-14-14.

#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2017.11.156>.

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