



Effect of Sugarcane Bagasse and Rice Husk Biochar for Remediating Heavy Metal Contaminated Soils and Enhancing Agro-Ecological Productivity

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Authors' Contribution

All authors equally contributed to the study and approved of the final manuscript.

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ABSTRACT

Heavy metal contamination of agricultural and industrial soils is becoming an issue of great concern for scientists and environmentalists. In recent years, biochar has emerged as an efficient and cost-effective tool for adsorption of heavy metals and reducing their availability to crop plants. Soil contaminated with cadmium and lead was treated with sugarcane bagasse and rice husk biochar at varying rates (0.5–2%). Effects on metal adsorption, soil properties, and nutrient availability were evaluated through incubation and pot experiments. Biochar application reduced cadmium and lead availability, with sugarcane bagasse biochar being more effective than rice husk biochar. At a 2% application rate, cadmium and lead concentrations decreased by over 60% compared to untreated soil. Soil pH increased significantly, supporting reduced metal mobility and improved soil health. Total organic carbon and nutrient availability, including nitrate, phosphorus, and potassium, were maximized at higher biochar rates. The results highlight sugarcane bagasse biochar's superior performance in remediating heavy metal-contaminated soils and enhancing agro-ecological productivity. This study demonstrates that sugarcane bagasse biochar effectively reduces heavy metal availability in soil while enhancing soil fertility and crop growth. Biochar at 2% is a sustainable solution for reclaiming polluted soils and improving agro-ecological productivity.

INTRODUCTION

In recent years, increase in industries and development in cities infrastructure is the main cause of pollution. Pollution causes serious problems in the ground water by mixing of industrial waste and sewage sludge in it (Selvi et al., 2012). After the manufacturing process, effluent is the flow out of waste water discharged (Bishat et al., 2012). Cd is taken as highly toxic for the living organisms and humans among all these metals, and it shows the biological activity in terrestrial and aquatic organisms (Chellaiah, 2018). Agriculture and industrial development have results in a higher concentration of Cd in agricultural soils (Bojorquez et al., 2016). Cadmium enters ecosystems with the help of different anthropogenic activities and emits to the environment (Abbas et al., 2018). Deposition of Cd in plants and in Cd polluted soil endangers to the health of

animals and humans due to its higher mobility level in contaminated soils (Chen et al., 2016). Under the natural conditions average range of Cd in soil is 0.1-0.2 mg kg⁻¹ while it ranges between 5 – 110 mg L⁻¹ in the water (Waseem et al., 2014). However, different sources add higher concentrations of Cd in soil and water bodies from where it enters in the food chain and poses serious threats to humans.

Soil heavy metal contamination is a very serious issue, which is cause of soil fertility degradation and it also reduces the crop yield and effects quality. It has worse effect on human health via food chain (Wang et al, 2017). It is very important to develop such heavy metal pollution repair technology which behave as environment friendly technology. (Sugihiro et al, 2013). In contrast to organic pollutants, soil microorganisms cannot degrade the heavy

metals hence these metals reside in soil for prolong period of time, due to which there is an increased difficulty in reparation. The new material, biochar has porous structure, larger surface area and abundant surface functional groups which help it to repair heavy metal pollutants very effectively (Jin, 2011).

In Pakistan, in water samples the concentration of Cd (from different locations of the country) ranges between 0.001 to 0.21 mg L⁻¹ (Lone et al., 2003; Manzoor et al., 2006) that might be added to water bodies with effluents discharged from marble, steel, aluminum and metal plating industries as well as from mining sites (Tariq et al., 2006). Cadmium is considered highly carcinogenic and inorganic Cd is categorized in Group 2A of the elements carcinogenic to human beings while organic Cd is categorized among group 3 (Tchounwou et al., 2012). Surface water of different locations in Pakistan contained 0.2 mg L⁻¹ Cd (Nazif et al., 2006, Mastoi et al., 2008)). Cd contents in water is being collected from different areas of KPK ranged between 0.002 to 0.09 mg L⁻¹ while for the water samples collected from Malir River, in Sindh ranged between 0.002 to 0.07 mg L⁻¹ that are quite higher as compared to WHO limits (Haq et al., 2005). Permissible limits of Cd for soil and food samples are 0.8 and 0.02 mg kg⁻¹ (WHO, 1996). According to WHO, permissible limit of Cd in drinking water is 0.005 mg kg⁻¹ (WHO, 2004).

Lead is major environmental pollutant, and many studies have showed that lead is provoking health issues. Lead toxicity to humans can be a source of variety of health problems like mild metabolic effects under minor toxicity levels to convulsions, renal failure, coma and even death under severe toxicity is observed (Papanikolaou et al., 2005). Pb contents up to 0.01 mg L⁻¹ is considered to be safe as per WHO standards. In Pakistan, many of the samples collected from ground water sources from all around the country were found to have Pb contents higher than the WHO permissible limit and ranged from less than 0.001 to 4.7 mg L⁻¹ in different regions (Waseem et al., 2014). WHO permissible limits for Pb are 85 and 10 mg kg⁻¹ for soil and food commodities, respectively (WHO, 1996), while for drinking water the permissible limits of Pb is 0.05 mg kg⁻¹.

Biochar is such kind of material which is considered as insoluble, stable, highly aromatic and carbon rich solid (material) which is synthesized by the abandoned biomass by keeping the hypoxia condition and by keeping high temperature slow pyrolysis (usually <700°C) (Sohi et al., 2010; Li et al., 2011; Xu et al., 2017). It was already proven that biochar behaves as promoter which fixes the heavy metal pollution in the environment (Singh et al., 2010; Liu et al., 2014). The abandoned biomass consists of wood, agriculture waste, animal and plant waste. Biochar behaving as a new type of environmental functional material that shows great potential in soil improvement, pollution repair and better way of utilization of waste biomass resources, it has a major hot topic in agriculture science and environmental amendment. Many studies have shown various type of pyrolytic temperatures and raw materials that could affect the ash content, carbon content. Ash and mineral elements; aromatic hydrocarbons and single carbon or carbon with graphite

structure are major composition of Biochar. Biochar consists of more than 60 % carbon content and also include other elements such as H, O, N, S etc. Among biochar properties chemical composition and pyrolysis temperature of raw material makes big difference. with increase in temperature, volatile components of biochar decreases and the content of hydrogen and oxygen also affected while carbon and ash content increase gradually (Cao et al, 2010).

The lignin and cellulose in plant residue biochar is decomposed into smaller molecules during the process of dewatering and polymerization, due to which H/C and O/C is decreased. However, animal fecal and sludge biochar do not contain any lignocellulosic compounds, due to which they have low amount of carbon content as compared to plant residues. The aromaticity of plant residue biochar is significantly higher as compared to remaining two and the aromatic structure of biochar is used as a π -electron donor or acceptor, forming pollutant- π bond with pollutants, further impacting the adsorption effect of biochar on pollutants (Chen et al, 2016).

The major reason for reduction of heavy metal activities is, exchange adsorption of biochar surface which decreases the availability. Heavy metal retention becomes very stronger with the increased amount of cation exchange. (Lehmann, 2006; Reesa et al, 2014). Electrostatic interaction between negative charge groups on biochar surface and positive charges in the soil which define the nature of ion exchange. By this type of reaction having lower adsorption energy belongs to nonspecific adsorption and shows obvious reversibility. The cationic π function totally relay over the aromatization of biochar. With the increase in π conjugate aromatic structure the greater negative charge in π orbital changes, hence the electron losing ability of functional groups increases and adsorption becomes more significant (Li et al, 2017). Co-precipitation can effectively affect the activities of heavy metals to degradation due to adsorption and dissolution - precipitation of mineral constituent. The pH of soil is increased by the addition of biochar (Reesa et al, 2014), The reaction of heavy metal ions with -OH, PO₄³⁻, CO₃²⁻ can give hydroxide, carbonate or phosphate precipitation, which effectively reduces the heavy metal pollutant. Complexation is significantly used for heavy metal ions with strong affinity. On the adsorption of heavy metal, the reaction (of heavy metal) with oxygenic functional groups like hydroxyl group and amino group makes a great contribution (Xu et al, 2017; Li et al, 2017). The larger surface area and higher surface energy are helpful for biochar to strongly absorb the heavy metal pollutants and remove them from the soil.

Higher amounts of cadmium (Cd) and lead (Pb) in water and soil can lead to the food chain contamination and pose serious threats for humans. Removal of these metals from soil is a challenge and requires the use of new technologies and tools. Use of biochar for this purpose can act like a potential cost-effective and an economical method for removing heavy metals from soil. The study was carried out to overcome these challenges in agroecosystem with following objective: to estimate the effect of sugarcane bagasse and rice husk biochar on

adsorption and availability of lead and cadmium in contaminated agricultural soil.

MATERIALS AND METHODS

Soil Sampling and Collection

Soil samples were collected from different location of Hattar industrial area and suitable soil that was already contaminated with cadmium and lead was selected for experimentation. After selection of the soil, it was collected in bulk (0-15 cm depth) with a spade and brought to PMAS-Arid Agriculture University Rawalpindi. Soil was air-dried, and grounded. This soil was passed through a 2mm sieve for removal of stones, roots and other unwanted material. A sub-sample was taken for physical and chemical analysis.

Biochar Preparation

Biochar was prepared from sugarcane bagasse and rice husk. Feedstocks were collected, air-dried and handpicked for removing any unwanted material. After that both feedstocks were grinded separately and put in air-tight metal containers. These containers were put in furnace and temperature was raised to 450 °C slowly. Feed stock was heated at this temperature for at least 2 hours after which the furnace was switched off. The containers were left to cool down and then feed stock was inspected whether it is completely converted to biochar or not. Biochar prepared was stored in air tight containers until further use. Subsamples were collected for characterization of biochar.

Chemical Analysis of Biochar

To offer the basic features of biochar and to identify possible metal ion application in concern of stabilization, physical and chemical characteristics of both biochar were determined in accordance with Abdelhafez et al. (2014). The average of the particle size and surface area was measured as part of the particle characterization investigation. pH and electrical conductivity (EC) were determined in chemical studies by soaking the biochar in distilled water and heating it for 5 minutes. After that, the pH and EC values in the suspension were measured after it was cooled down. (Shinogi et al., 2003).

Adsorption Experiment

To estimate adsorption efficiency and adsorption capacity of sugar cane bagasse biochar (SCB) and rice husk biochar (RHB) an adsorption experiment was conducted as devised by Wang et al., (2015), 5 g soil was taken in centrifuge tube and 0.025, 0.05, 0.75 and 0.1 g of SCB and RHB were added separately to represent 0.5, 1, 1.5 and 2 % biochar application. After that 50 ml of 0.01 M HCl were added. Centrifuge tubes were closed tightly and then contents were shaken for 12 hours at 22 °C at 180 rpm on a reciprocating shaker. After that the contents were centrifuged for 10 minutes at 3000 rpm. The supernatant was collected and concentration of lead and cadmium were estimated with atomic absorption spectrophotometer.

Adsorption efficiency (η_s) of biochar was estimated by using following formula

$$\eta_s (\%) = \frac{C_0 - C_t}{C_t} \times 100$$

Adsorption capacity (Q_s) of biochar was calculated by following formula

$$Q_s (mg/kg) = (C_0 - C_t) \times (m_0 \div m_t)$$

Where,

C_0 = concentration of heavy metal in supernatant of control soil

C_t = concentration of heavy metal in supernatant of biochar treated soil

m_0 = weight of soil taken for experiment i.e. 5 g

m_t = weight of biochar added to each tube

Incubation Experiment

An incubation study of 90 days was conducted to estimate the effects of sugarcane bagasse biochar (SCB) and rice husk biochar (RHB) on absorption of lead (Pb) and cadmium (Cd) from soil. One kg soil was put into plastic containers and designated treatments were applied. The treatments include T₁: Control; T₂: SCB (0.5%); T₃: SCB (1%); T₄: SCB (1.5%); T₅: SCB (2%); T₆: RHB (0.5%); T₇: RHB (1%); T₈: RHB (1.5%) and T₉: RHB (2%). All treatments were replicated three times that gave rise to a total of 27 experimental units. Experimental units were incubated at 22 °C in an incubator where these experimental units were placed under completely randomized (CRD) arrangement. The moisture of the soil was maintained at 50 % field capacity throughout the experiment. Soil samples were collected at day 1, 30, 60 and 90 days and analyzed for soil pH, electrical conductivity, total organic carbon, nitrates, available P, available K and available concentrations of Pb and Cd.

Pot Experiment

Soil was put into earthen pots of 5 kg capacity. Both biochar mixed thoroughly according to the designated treatments. The treatments include: T₁: Control; T₂: SCB (0.5 %); T₃: SCB (1 %); T₄: SCB (1.5 %); T₅: SCB (2%); T₆: RHB (0.5 %); T₇: RHB (1 %); T₈: RHB (1.5 %); T₉: RHB (2%). Each treatment was replicated three times that will give rise to a total of 27 experimental units. After application of treatments, eight wheat seeds of wheat variety "Zincol" were sown in each pot and pots were irrigated with equal amount of water. Irrigation was carried out whenever required to avoid drought stress. After maturity, plants were harvested and soil samples were collected from each pot and analyzed for soil physiochemical analysis and macro nutrient analysis

Soil Analysis

In the case of pH analysis, 10 g of soil sample was taken in the flask and 50 mL of DW was added to it. Shaking was done for half hour on a mechanical shaker and then pH was recorded by with a calibrated meter (Jackson, 1973). Soil suspension (1:5) was used to determine EC of the soil while using a conductivity meter (Jackson, 1973). The Walkley-Black method was used to note down the amount of organic carbon from the soil (1947). In a 500 mL conical flask, 1 g dirt was added to 10 ml of 1 N potassium dichromate solution. 20 mL of condensate Sulfuric acid was added to the mixture, which was then be left for 30 minutes. With distilled water, the volume was made 200 mL and added orthophosphoric acid. A few drops of diphenyl amine indicator were added, and titration with 0.5 N ferrous sulphate solution was performed until the

green endpoint is reached. The contents of the TOC were determined by using the formula. Nitrate-N was determined by the method of Vendrell and Zupancic (1990). A flask was filled with 10 g soil and 20 mL distilled water was added. After 1 hour of shaking, filtration was done. A test tube was filled with 0.5 mL of filtrate and 1 mL of salicylic acid. Tubes were left for 1 hour for color development and then reading was taken at 410 nm on a spectrophotometer. Availability of phosphorus was determined by taking 5 g soil in a flask and then adding 100 mL of 0.5 M sodium bicarbonate to it. Shaking was done for half hour and then contents were filtered. Ten mL of this filtrate was taken and 1.0 mL of 5.0 N sulfuric acid solution was added to it. Volume was made to 40 mL with distilled water and then ascorbic acid reagent (8 mL) was blended for color development. Reading was noted at wavelength of 882 nm with a spec. In a 50 mL tube, 5.0 g soil was placed. And 33.0 mL of 1 N ammonium acetate solution was added to it. Solution was mixed for about 5 minutes and then centrifuged for 10 minutes to obtain a clear supernatant. The supernatant was diluted to 100 mL by adding ammonium acetate solution. Flame photometer was used to obtain standard curve and then measured the concentration of potassium in soil samples (Rhoades, 1982).

Available heavy metal (Pb and Cd) concentrations were measured by method devised by Soltanpour and Schwab (1977). In an Erlenmeyer flask, 10 g soil was placed, and 20 mL of ABDTPA solution was added. Shaking was done for 1 hours and then the suspension was filtered through a Whatman's No. 42 filter paper. Metal concentration was measured from the filtrate with atomic absorption spectrophotometer.

Statistical Analysis

Research data for different treatments were subjected to ANOVA by using completely randomized design (CRD) under 2 factor factorial arrangement for pot experiment. Treatment means were compared with least significant difference (LSD) test at 5 % level of probability (Steel and Torrie, 1986).

RESULTS

Study 1: Metal Adsorption by Biochar

Adsorption experiment, conducted to estimate the effect of biochar type and application rate on metal availability in soil solution, depicted that both biochar provided great efficiency and capacity for metal adsorption and removal.

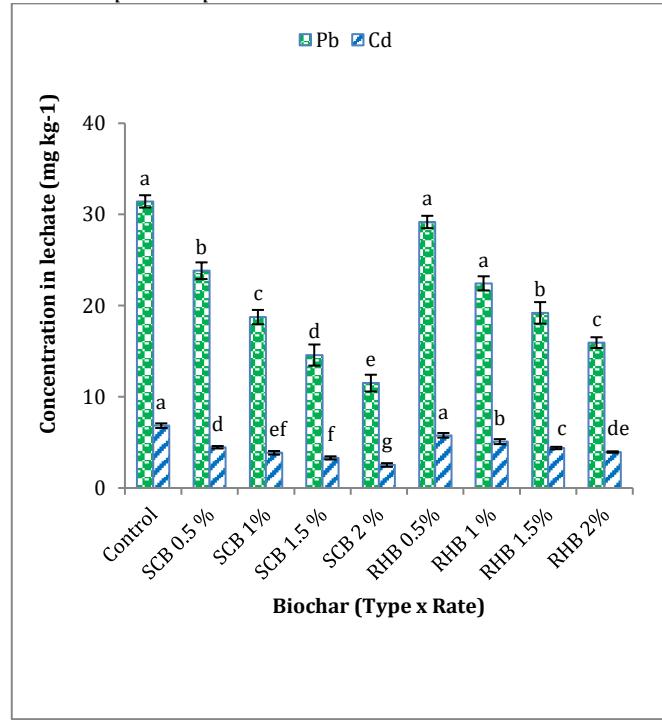
Metal Contents in Solution (mg kg⁻¹)

Data for heavy metal concentration in solution during the adsorption experiment and effect of biochar types is discussed in Fig 1. It was observed that release Pb and Cd concentrations in the released from soil into the solution varied significantly for biochar type as well as application rate. Concentration of Pb in control was 31.43 while for Cd its value was 6.83 mg kg⁻¹. Concentrations of Pb and Cd in soil solution were reduced significantly with application of 0.5 % SCB where respective values were 23.83 and 4.47 mg kg⁻¹. These values were also significantly less as compared to Pb and Cd concentration observed for the soil treated with 0.5 % RHB where respective values were 29.17 and 5.78 mg kg⁻¹ (Fig 1).

At 1 % RHB application, Pb concentration in solution was 22.44 mg kg⁻¹ that was statistically similar to the value observed for 0.5 % RHB. However, Cd contents were reduced significantly to 5.09 mg kg⁻¹ as compared to the value observed for 0.5 % RHB. On the other hand, application of 1 % SCB significantly reduced Pb and Cd concentrations in solution and respective values were 18.74 and 3.86 mg kg⁻¹. At 1.5 and 2 % SCB application, concentration of Pb in solution was 14.58 and 11.50 mg kg⁻¹ that were significantly less as compared to the values of 19.20 and 15.94 mg kg⁻¹ observed for 1.5 and 2 % RHB treated soil. Similar trend was observed for Cd contents in solution. SCB application at 1.5 and 2 % reduced its concentrations to 3.30 and 2.52 mg kg⁻¹ while for application of RHB at these rates Cd contents in the solution were 4.39 and 3.94 mg kg⁻¹ that were significantly different from each other (Fig 1).

Figure 1

Concentration of heavy metals detected on solution during the adsorption experiment



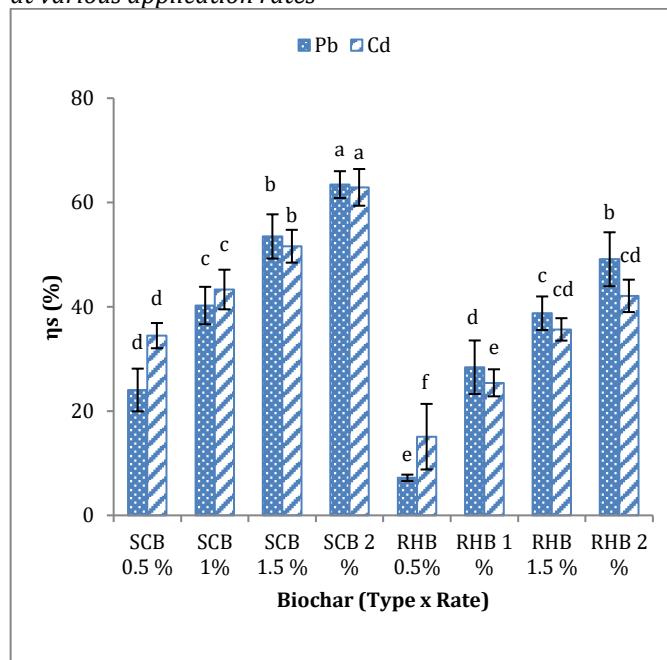
Adsorption Efficiency of Biochar, η_s (%)

Adsorption efficiency of SCB and RHB at various application rates is presented in Fig 2. It was observed that SCB had more adsorption efficiency as compared to RHB. Moreover, adsorption efficiency of both biochar increased with increasing rate of application. Maximum adsorption efficiency for Pb and Cd was observed for SCB application at 2 % where respective values were 63.43 and 62.90 %. RHB application at 2 % had adsorption efficiency of 49.11 and 42.09 % for Pb and Cd that were significantly less as compared to its counterpart in SCB. Adsorption efficiency of RHB for Cd at 2 % was statistically similar to the value of 35.66 % observed for 1.5 % RHB. However, at 1.5 %, adsorption efficiency for RHB was 38.76 % that was significantly less as compared to the value produced with 2 % RHB application. At application level of 1.5 %, SCB showed adsorption efficiency of 53.28 and 51.61 % for Pb and Cd that were significantly higher from the values

observed for 1.5% RHB. Similar trend was observed for 1 and 0.5 % SCB application. Application of 1 % SCB had an adsorption efficiency of 40.23 and 43.31 % for Pb and Cd while for 1 % RHB respective values were 28.4 and 25.41 % that were significantly less as compared to the adsorption efficiency of 1 % SCB. At 0.5 % application rate, SCB showed minimum adsorption efficiencies of 24.04 and 34.46 % for Pb and Cd. However, these values were significantly higher as compared to its counterpart i.e. 0.5 % RHB where adsorption efficiencies for Pb and Cd were 7.2 and 15.09 %, respectively (Fig 2).

Figure 2

Adsorption efficiency of SCB and RHB for lead and cadmium at various application rates

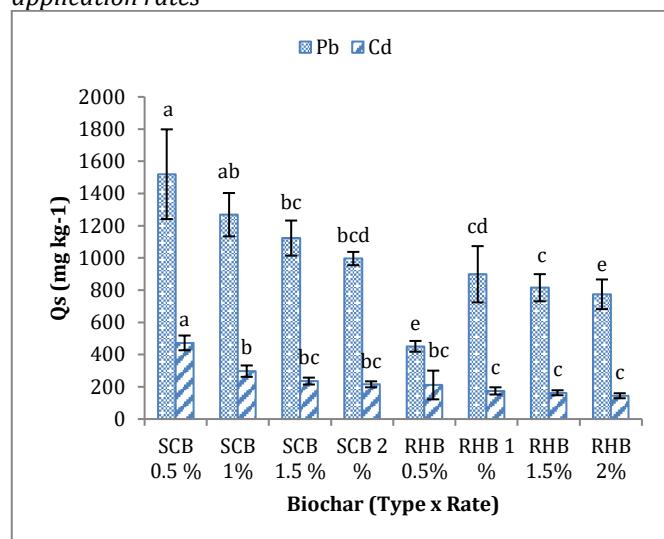


Adsorption Capacity of Biochar, Q_s (mg kg⁻¹)

Adsorption capacity of SCB and RHB varied significantly among biochar types as well as with application rates (Figure 3). Maximum adsorption capacity for Pb and Cd was observed for 0.5 % SCB where respective values were 1520 and 473 mg kg⁻¹ while for 0.5 % RHB these values were 451 and 211 mg kg⁻¹ that were significantly less as compared to 0.5 % SCB. At 1 % application rate adsorption capacity for Pb was 1269 that was slightly less as compared to the value produced for 0.5 % SCB. Adsorption capacity of 1 % SCB for Cd was 297 mg kg⁻¹ while for 1.5 and 2 % SCB respective values were 236 and 216 mg kg⁻¹ that were statistically similar from each other. In case of Pb, adsorption capacity of 1123 mg kg⁻¹ for SCB was observed that was slightly higher as compared to the value of 996 mg kg⁻¹ for 2 % SCB application (Fig 3). On the other hand, application of 1 % RHB adsorption capacity for Pb was 899 mg kg⁻¹ that was slightly higher as compared to the value of 815 mg kg⁻¹ for 1.5 % RHB application. However, for 2 % RHB, adsorption capacity for Pb was 774 mg kg⁻¹ that was significantly less as compared to the rest of the application levels of RHB. It was interesting to observe that RHB application at 1, 1.5 and 2 % levels adsorption capacity for Cd remained statistically similar and respective values were 175, 163 and 145 mg kg⁻¹ (Fig 3).

Figure 3

Adsorption capacity of SCB and RHB for Pb and Cd at various application rates



Study 2: Incubation Experiment for Estimating the Effects on Biochars on Soil Properties and Availability of Nutrients and Heavy Metals

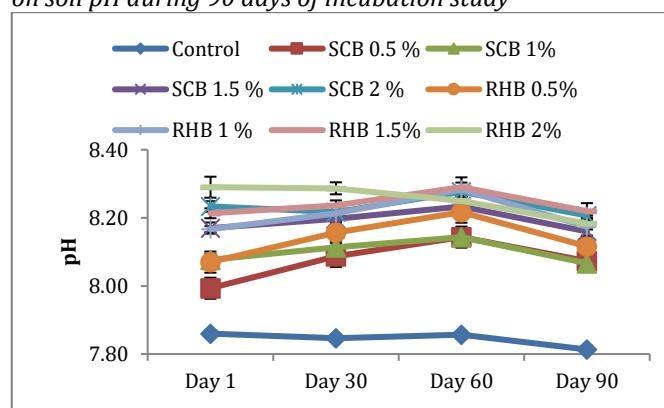
Various soil parameters studies in the incubation experiment showed significant effects of biochar type as well as application rates on soil chemical properties, nutrient release and heavy metal availability.

Soil pH

The pH value of Sugar Cane Biochar (SCB) in control was 7.86 on day 1 and the value reduced to 7.81 after 90 days of incubation (Fig 4). At 0.5% SCB the pH increased from 7.99 to 8.07 while, at 1% SCB the pH value was recorded as 8.07 at day 1 which is the same to that of day 90. However, there was an increased-on day 30 and day 60. Here pH remained unchanged during 90 days when 1.5% SCB was applied. We found the similar case when applied 2% of SCB. There was significant difference in the pH value of control when Rice Husk Biochar (RHB) was applied. At 0.5% RHB the pH increased from day 1 to day 60 of incubation (8.07-8.22) and reduced to 8.11 after 90 days of incubations. Similar case was found when 1% RHB was applied. At 1.5 the pH value was 8.21 on day 1 and the value was 8.29 on day 60. After 90 days of incubation, the pH value reduced to some extent (8.22) (Fig 4).

Figure 4

Effect of SCB and RHB biochar application at various rates on soil pH during 90 days of incubation study

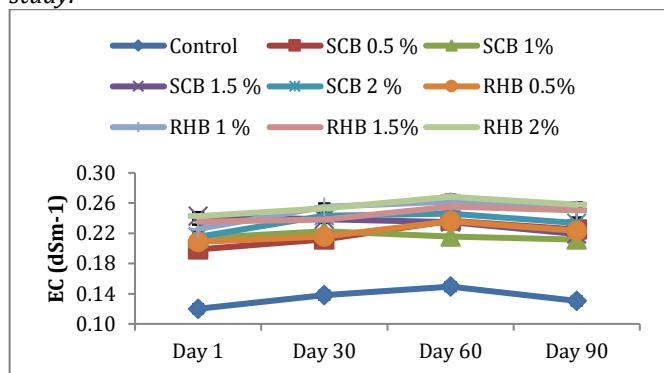


Soil Electrical Conductivity (dSm⁻¹)

The EC value of the soil amendment with SCB in control remained the same after 90 days of incubation (Day 1 = 0.12 dS m⁻¹ Day 90 = 0.13 dS m⁻¹) (Fig 5). From day 1 to day 60 of incubation, the value of EC increased from 0.20 dS m⁻¹ to 0.23 dS m⁻¹ when soil was amended with 0.5% SCB but, the value reduced to 0.22 dS m⁻¹ after 90 days of incubation. At 1% of the SCB, the EC value of soil was recorded at same day 1 and day 90 i.e. 0.21 dS m⁻¹ and 0.21 dS m⁻¹ respectively. However, a little increase was observed on day 30 and day 60. The pH value varied between 0.24 dS m⁻¹ to 0.25 dS m⁻¹ when 1.5% of SCB was applied to the soil. Similarly, the increase in EC value was observed from day 1 to day 90 of incubation when 2% of SCB was applied. The EC value of the soil amendment with RHB in control remained almost the same after 90 days of incubation. The EC value increased from 0.21 – 0.24 dS m⁻¹ after 60 days of incubation and reduced to 0.22 dS m⁻¹ after 90 days of incubation when the soil was supplemented with 0.5% of RHB. At 1%, 1.5% and 2% RHB added to soil, the EC values on day 1 were 0.22, 0.23 and 0.24 dS m⁻¹ while, on day 90 these values were 0.25, 0.25 and 0.26 dS m⁻¹, respectively (Fig 5).

Figure 5

Effect of SCB and RHB biochar application at various rates on soil electrical conductivity during 90 days of incubation study.



Total Organic Carbon (%)

After 90 days of incubation, the total organic carbon (TOC) decreased to 0.4% which was 0.54% at day 1 of incubation in control soil. Gradual increase in TOC was observed when 0.5% SCB applied to the soil (0.66-0.78%). In all the other concentrations used, the TOC increased from the 1st day of incubation to the 90 days of incubation (Fig 6).

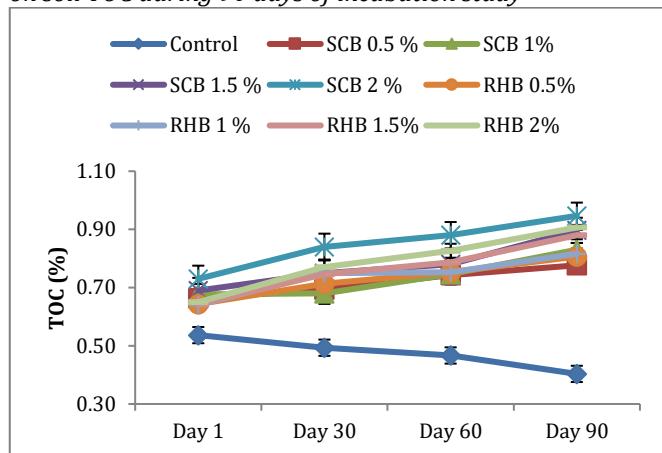
With 0.5% of SCB application to soil, the TOC was 0.66% and 0.78% on day 1 and day 90 of incubation. The TOC increased from 0.68 to 0.83 on day 1 till the 90th day of incubation when 1% SCB was supplied to the soil. Similarly, at 1.5% SCB the TOC value at day 1 and day 90 were 0.69% and 0.73%, respectively, and at 2% SCB these values were 0.73% and 0.94%.

In control soil with 0% RHB the TOC was 0.54% on day 1 and the value decreased to 0.40% after 90th day of incubation. A gradual increase was observed when 1% of RHB was applied to the soil. On day 1 the TOC was 0.68% and day 90% the TOC was 0.82%. A similar case was found when 1.5% and 2% RHB was applied to the soil. On day 1 the TOC value at 1.5% RHB was 0.64% and day 90 of

incubation the value was 0.88%. Similarly, at 2% RHB in soil, the TOC value increased from 0.65% to 0.91% till 90 days of incubation (Fig 6).

Figure 6

Effect of SCB and RHB biochar application at various rates on soil TOC during 90 days of incubation study

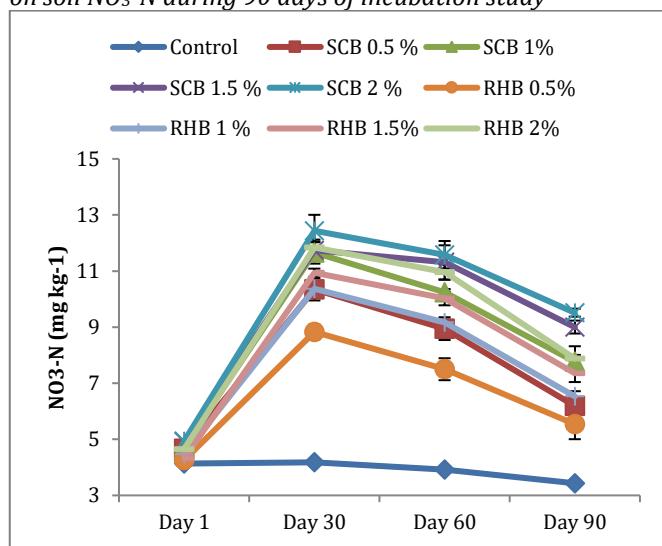


Soil NO₃-Nitroegn (mg kg⁻¹)

In control soil, the nitrogen content was 4.4 mg kg⁻¹ on day 1st while after 90 days of incubation the value was 3.43 mg kg⁻¹ (Fig 7). The N content was enhanced in soil when supplemented with 1%, 1.5% and 2% SCB till 60 days of incubation. The highest value (11.58 mg kg⁻¹) was recorded in soil supplemented with 2% SCB. After 60 days of incubation, a significant reduction started in N content. In soil supplemented with 0.5% RHB the value of N content increased from 4.25-8.95 mg kg⁻¹ till 60 day of incubation and after 60 days, the reduction in N contents was observed (5.54 mg kg⁻¹). N content increase was observed in soil amended with 1%, 1.5% and 2% RHB and the values observed after 90 days were 6.53, 7.35, 7.88 mg kg⁻¹, respectively (Fig 7).

Figure 7

Effect of SCB and RHB biochar application at various rates on soil NO₃-N during 90 days of incubation study



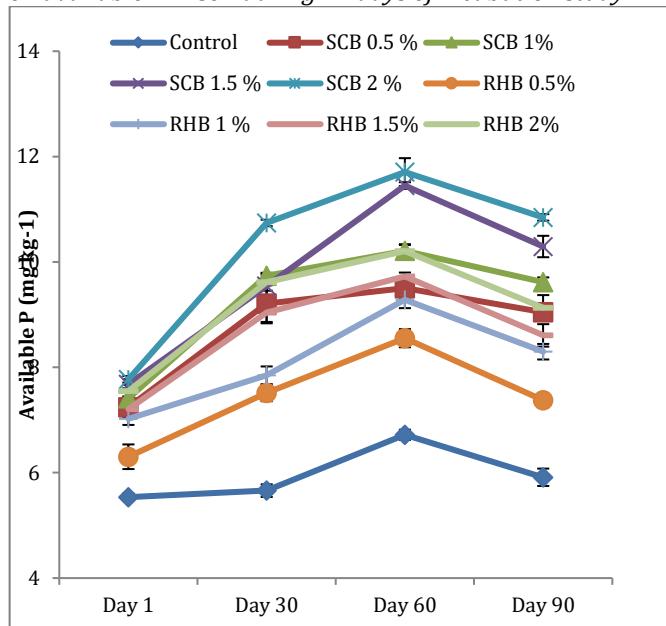
Available Phosphorus (mg kg⁻¹)

In control soil phosphate content were recorded as 5.53 mg kg⁻¹ on 1st day of incubation which increased to 6.72 mg kg⁻¹ till day 60, and on 90 day of incubation the value

was 5.91 mg kg⁻¹ (Fig 8). The P content was increased in soil with application of 0.5, 1, 1.5 and 2% SCB to soil till day 60 of incubation while, a little reduction was observed till 90th day of incubation. In soil supplemented with 0.5% RHB the P content value was 6.3 mg kg⁻¹ on day 1 of incubation which showed an increase till day 60 (8.55 mg kg⁻¹) of incubation and after 90th day of incubation, the value reduced to 7.37 mg kg⁻¹. The same trend was observed for the rest of RHB concentrations applied to the soil. The P content after 60 day of incubation was 10.21 mg kg⁻¹ which was 7.77 mg kg⁻¹ on 1st day when the soil was supplied with 2% RHB. After 90 days of incubation, this concentration reduced to 9.13 mg kg⁻¹ (Fig 8).

Figure 8

Effect of SCB and RHB biochar application at various rates on available P in soil during 90 days of incubation study

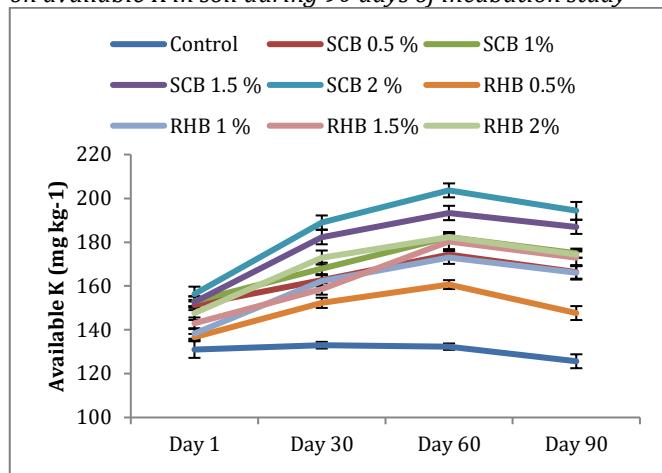


Available Potassium (mg kg⁻¹)

In control soil, the potassium concentration was 131 mg kg⁻¹ on 1st day which reduced to 126 mg kg⁻¹ after 90 days of incubation (Fig 9). However, in different treatments, different response to biochar application was observed. Soil with 0.5% SCB contained 151 mg kg⁻¹ K which increased to 166 mg kg⁻¹ after 90 days of incubation. Soil supplemented with 1% SCB the K content increased to 175 mg kg⁻¹ after 90 days of incubation which were 151 mg kg⁻¹ on 1st day. Similarly, with the addition of 1.0% SCB to soil increased the K content from 153 mg kg⁻¹ to 175 mg kg⁻¹ from 1st day to day 90. In treatment (1.5% and 2% SCB), the K value increased till 60 days of incubation and some reduction was observed on 90th day of incubation (Fig 9). With all the application of different concentrations of RHB to the soil (0.55, 1% 1.5% and 2%), a significant increase in K was observed after 60 days of incubation and a little reduction was observed from the 60 days of incubation till 90 days of incubation. On 1st day, day 30, day 60 and day 90 of incubation, the K contents were 156 mg kg⁻¹, 173 mg kg⁻¹, 182 mg kg⁻¹ and 174 mg kg⁻¹, respectively, when the soil was supplemented with 2% RHB.

Figure 9

Effect of SCB and RHB biochar application at various rates on available K in soil during 90 days of incubation study

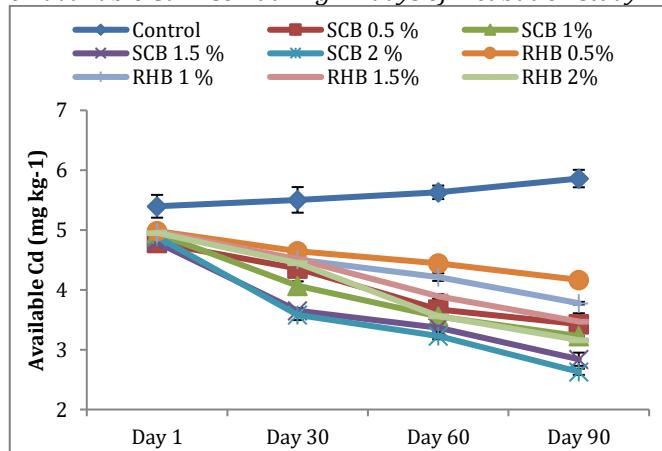


Available Cadmium (Cd) (mg kg⁻¹)

An increase was observed in Cd content throughout the incubation period. On day one the value was 4.9 mg kg⁻¹ which increased to 5.36 mg kg⁻¹ after 90 days of incubation (Fig 10). A gradual decrease was observed in Cd concentration after 90 days of incubation with the application of all the SCB concentrations. Cd content was recorded as 4.28 mg kg⁻¹ at day 1st of incubation which reduced to 2.93 mg kg⁻¹ after 90 days of incubation when the soil was amended with 0.5% SCB. Similarly, the Cd content in soil was reduced to 2.13 from 4.38 after 90 days of incubation when supplemented with 2% SCB. A similar trend of reduction of Cd content was also observed when soil was supplemented with RHB. Cd content at 0.5% was 4.48 mg kg⁻¹ on 1st day of incubation which reduced to 3.67 mg kg⁻¹ after 90 days of incubation. On day 1st of incubation, the Cd value was 4.45 mg kg⁻¹ and reduced to 2.66 mg kg⁻¹ after 90 days of incubation when the soil was amended with 2% RHB (Fig 10).

Figure 10

Effect of SCB and RHB biochar application at various rates on available Cd in soil during 90 days of incubation study



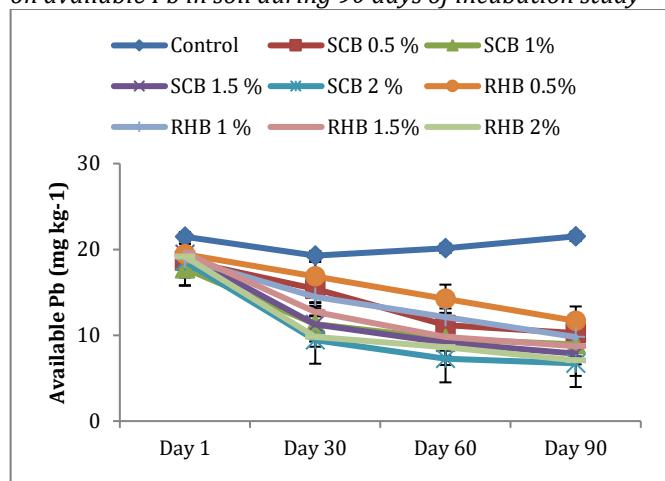
Available Lead (Pb) (mg kg⁻¹)

Lead content in soil was determined as 21.48 mg kg⁻¹ at day 1st of incubation which remain the same throughout the entire period of incubation (90 days = 21.53 mg kg⁻¹). At 0.5% SCB concentration, the Pb content was 18.69 at day 1st which was reduced to 10.25 after 90 days of

incubation (Fig 11). The Pb concentration was decreased to 6.72 (after 90 days) from 18.59 (day 1st) when SCB was supplied at 2% to the soil. Similarly, the same reduction pattern was observed when the soil was supplemented with RHB. Pb content was noted as 19.44 mg kg⁻¹ at 1st day of incubation which was reduced to 11.68 mg kg⁻¹ after 90 days of incubation when the soil was supplemented with 0.5% of RHB. At 2% RHB supplementation to the soil, the Pb content was reduced to 7.07 mg kg⁻¹ (day 90) from 19.17 mg kg⁻¹ (day 1st). Same pattern was observed for RHB when added to the soil at rate of 1% and 1.5% (Fig 11).

Figure 11

Effect of SCB and RHB biochar application at various rates on available Pb in soil during 90 days of incubation study



Study 3: Estimating the Effects of SCB and RHB Biochar Application at Various Rates on Soil Attributes: Pot Experiment

Soil pH

Application of different types and rates of biochar showed significant effect on soil pH (Table 1). Soil pH for SCB was 8.11 that were significantly higher as compared to the value of 8.07 observed for RHB application. Application rates of biochar also showed an increase in soil pH with increasing dosage. pH of control soil was 7.9 that was increased to 8.02, 8.13 and 8.19 at application levels of 0.5, 1 and 1.5 % biochar. Maximum increase in soil pH was observed with application of 2 % biochar where the value was 8.22 that was significantly higher than pH of soil receiving other application levels (Table 1).

Table 1

Effect of SCB and RHB biochar on soil pH

Rate	Biochar Type		Mean
	SCB	RHB	
Control	7.90 e	7.90 e	7.90 d
0.5 %	8.08 d	7.95 e	8.02 c
1 %	8.15 bc	8.12 cd	8.13 b
1.5 %	8.21 ab	8.18 abc	8.19 a
2 %	8.24 a	8.21 ab	8.22 a
Mean	8.11 a	8.07 b	

Soil Electrical Conductivity (dS m⁻¹)

Application of SCB and RHB at various dosages also showed significant variation in soil EC (Table 2). Application rates showed significant increase in EC of soil with increasing dosage. Control soil showed a value of 0.14 dS m⁻¹ that was increased to 0.18 dS m⁻¹ with application

of 0.5 % biochar. It was increased to a value of 0.22 dS m⁻¹ when 1 % biochar was applied. Further increase in EC of soil was observed at 1.5 and 2 % levels and respective values were 0.26 and 0.28 dS m⁻¹. However, these values were statistically similar to each other. On the other hand, biochar type also showed significant variation in soil EC where the value for SCB was 0.23 dS m⁻¹ that was significantly higher from the value of 0.2 dS m⁻¹ for RHB application (Table 2).

Table 2

Effect of SCB and RHB biochar on soil electrical conductivity (dS m⁻¹)

Rate	Biochar Type		Mean
	SCB	RHB	
Control	0.14 f	0.14 f	0.14 d
0.5 %	0.19 e	0.16 f	0.18 c
1 %	0.24 cd	0.19 e	0.22 b
1.5 %	0.29 a	0.23 d	0.26 a
2 %	0.29 a	0.26 bc	0.28 a
Mean	0.23 a	0.20 b	

Total Organic Carbon (%)

Table 3, shows the effect of various doses of SCB and RHB on TOC of soil in pot experiment. It was observed that type of biochar showed no significant difference for TOC in soil. In this case TOC for SCB application was 0.73 % while for RHB application it was 0.74 % and both of these values were statistically at par. On the other hand, increasing dosage of biochar showed significant increase in TOC of soil. In control pots TOC content were 0.59 % that were increased significantly to 0.68 % for application of 0.5 % biochar. At 1 % biochar application TOC contents were further increased significantly to 0.73 % while for 1.5 % application levels its value was 0.81 % that was significantly higher from the values observed for previous application rates. Maximum TOC contents were observed for 2% biochar application where its value was 0.85 % that was significantly higher from the rest of the application rate (Table 3).

Table 3

Effect of SCB and RHB biochar on soil total organic carbon (%)

Rate	Biochar Type		Mean
	SCB	RHB	
Control	0.59 d	0.59 d	0.59 e
0.5 %	0.67 c	0.69 c	0.68 d
1 %	0.72 bc	0.75 b	0.73 c
1.5 %	0.81 a	0.81 a	0.81 b
2 %	0.85 a	0.86 a	0.85 a
Mean	0.73 a	0.74 a	

Available Cadmium (Cd) (mg kg⁻¹)

Soil Cd contents showed no variation for biochar type but application rates significantly reduced availability of Cd in soil with increasing dosage (Table 4). Available Cd contents in soil treated with SCB were 2.42 mg kg⁻¹ that was statistically at par with the value of 2.38 mg kg⁻¹ for RHB application. Application rates of biochar showed significant variation for available Cd contents in soil. Its value in control soil was 3.32 mg kg⁻¹ that was reduced to 2.63 mg kg⁻¹ with 0.5 % biochar application. Further reduction in available Cd content was observed at 1 and 1.5 % biochar application where respective values were 2.31 and 1.98 mg kg⁻¹. These values were significantly less as compared to the previous dosages. Maximum reduction

in available Cd was observed when 2 % biochar was applied where its value was 1.77 mg kg^{-1} . This value was significantly less than the rest of the application rates (Table 4).

Table 4

Effect of SCB and RHB biochar on soil available cadmium content (mg kg^{-1})

Rate	Biochar Type		Mean
	SCB	RHB	
Control	3.32 a	3.32 a	3.32 a
0.5 %	2.61 bc	2.66 b	2.63 b
1 %	2.38 cd	2.23 de	2.31 c
1.5 %	2.03 ef	1.94 fg	1.98 d
2 %	1.77 g	1.77 g	1.77 e
Mean	2.42 a	2.38 a	

Available Lead (Pb) (mg kg^{-1})

Table 5 presents the data for the effect of SCB and RHB application at various rates on available Pb contents in soil. It was observed that type of biochar showed no significant difference for available Pb contents and values for SCB and RHB were 10.79 and 10.59 mg kg^{-1} that were statistically at par. However, application rates of biochar showed significant reduction in availability of Pb with increasing application rate. Available Pb contents in control soil were 15.42 mg kg^{-1} that were reduced significantly to 12.06 mg kg^{-1} with application of 0.5 % biochar. It was further reduced to 10.14 % at application level of 1 % while for 1.5 % biochar it was reduced to 8.85 mg kg^{-1} . This value was significantly less than available Pb contents in 1 % biochar treated soil. Minimum value of 7 mg kg^{-1} for available Pb contents were observed for 2 % biochar application and this value was significantly less as compared to the rest of the application rates (Table 5).

Table 5

Effect of SCB and RHB biochar on soil available lead contents (mg kg^{-1})

Rate	Biochar Type		Mean
	SCB	RHB	
Control	15.42 a	15.42 a	15.42 a
0.5 %	11.77 b	12.35 b	12.06 b
1 %	10.16 c	10.11 c	10.14 c
1.5 %	9.26 cd	8.44 de	8.85 d
2 %	7.34 ef	6.66 f	7.00 e
Mean	10.79 a	10.59 a	

DISCUSSION

Adsorption capacity of biochar depends mainly on its high surface area due to its porous particles. Moreover, oxygen containing functional groups at the surface of biochar particles acts as charged surface and increases its adsorption efficiency and capacity (Clemente et al., 2015; Najar et al., 2015; Colla et al., 2014). The ability of biochar to adsorb heavy metal ions is affected by type of feedstock used, pyrolysis temperature and application rate of biochar (Wang et al., 2017). In the present study biochar application showed significant variation for heavy metal adsorption when biochars prepared from different feedstocks were applied at various rates. These results are supported by Xu and Zhao (2013) who found that biochar prepared from peanut straw performed better as compared to canola straw biochar in terms of copper and lead adsorption. Ding et al. (2014) also reported that bagasse biochar possesses high potential for removal of Pb

from aqueous solutions by adsorbing it to its surface that are in complete accordance with the results of the present study. Li et al. (2013) have reported a decrease in availability of Cd in soil after application of biochar and concluded that adsorption of Cd ions on biochar particles was the main reason of reduced availability. It is also reported in several studies that little amounts of biochar have high metal adsorption capacity and are considered more cost effective and environmentally friendly as compared to larger amounts as high dosage might increase soil pH and may lead to a reduction in plant biomass production (Beesley et al., 2011; Li et al., 2011). This also favors the findings of present study where highest adsorption capacity was observed for 0.5 % SCB application. Ahmad et al. (2014) suggested that ion exchange, precipitations and electrostatic interactions (including anion and cation metallic attractions) are chief mechanisms for removal of heavy metals from solution. Moreover, higher occurrence of oxygen containing functional groups and cation release make biochar more efficient absorbent. The results of the present study are in complete accordance with those reported by Ni and Weng (2015) who found that biochar addition to soil showed adsorption efficiencies of 68 and 73 % for Pb and Cd with application rate of 8 % biochar while higher application rates showed reduction in metal adsorption efficiency.

Application of biochar is known to improve soil properties. A number of studies have reported an increase in soil pH, TOC, nutrient release and reduction in availability of heavy metals after biochar application. Presence of basic cations like K^+ , Ca^{+2} and Mg^{+2} decrease exchangeable Al^{+3} and H^+ ions in soil thus increasing its pH (Zwieten et al., 2010). Application of biochar to an acidic soil having an initial pH of 4.5 increased its pH to 6 that matches the results of the present study (Galinato et al., 2011). Increase in pH is also reported by Nigussie et al. (2012) and Southavong et al. (2012) who concluded that presence of alkaline earth metal carbonates in biochar is the main reason of an increase in soil pH after biochar application. Wang and Liu (2015) have reported an increase in soil pH with an increasing application rate during 90 days in incubation that is in complete accordance with the results of the present study. Bamminger et al. (2014) have reported an increase of 20 to 40 % in soil organic carbon contents that supports the results of the present study. Wang and Liu (2015) also reported an increase of 25-54 % for organic carbon and 4-12 % in total nitrogen contents of soil after application of 1 % wheat straw biochar that matches our results for TOC and $\text{NO}_3\text{-N}$ contents. Similar results were reported by Mandal et al., (2015) who observed an increase of 1.74 % in TOC of soil after application of biochar. The results reported by Kaur and Sharma (2015) are in complete accordance with the results reported in present study where application of 2, 3, 5 and 10 % biochar increased TOC contents of soil up to 150, 155, 165 and 175 g kg^{-1} and showed a gradual increase in TOC with increasing application rate. Pandian et al. (2016) also supports these findings and reported that application of 5 t ha^{-1} maize and red gram stalk biochar increased TOC up to 4.8 g kg^{-1} . It was also reported that after initial application mineralization of organic carbon was increased at the start

of the incubation however, TOC contents reached a constant value at later incubation intervals that is in complete accordance with the results of the present study (Rajagopal, 2018)

Many factors may contribute to higher nutrient concentrations in biochar amended soils. Biochar itself acts as a fertilizer and releases significant amounts of macro as well as micro nutrients (Knoblauch et al., 2021). Moreover, increasing CEC and overall surface area of soil also play its part in nutrient retention and losses through leaching, volatilization and conversion to gaseous forms is reduced that ultimately results in higher nutrient concentration as compared to unamended soils (Hossain et al., 2020; Hagemann et al., 2017; Yao et al., 2012).

Liang et al., (2006) reported that biochar have more negative surface charge, high charge density and surface area hence imparts the soil with greater cation adsorption capacity and can be a great tool to reduce metal availability in contaminated soils. Liu et al (2015) have reported similar results and observed that concentration of Cd, Cu and Zn were reduced with increasing biochar dosage during 1 year incubation experiment. Jiang et al., (2012) also reported that increasing rate of rice straw biochar significantly reduced Pb contents in soil in incubation experiment that supports the results of the present study.

Biochar has great potential to alter soil chemical properties due to which it is used as a sustainable amendment (Lehmann et al. 2011; Glaser, Lehmann, and Zech 2002). Alteration in soil chemical properties is due to biochar composition and it can easily give raise to soil pH. There are so many studies which showed great evidence regarding the enhancement of soil pH because of biochar application specifically in acidic soil (Song et al. 2018; Peng et al. 2011). Soil pH got risen due to ash content in biochar which behave as main contributor for this kind of enhancement. Ash content have ability to increase the pyrolysis temperature and mineral content of feedstock which is used for biochar production (Rafiq et al. 2016).

Carbon reserves of soil can be enhanced by the biochar application, which leads to clasp (hold) the soil nutrients and soil fertility improvement. There is mineralization of native organic carbon for short term, which increases the soil carbon sequestration and improves the crop growth and yield (Ouyang et al. 2014; Song et al. 2018). Zhan et al. (2021) after the experiment based on four-year duration, an increase of 27.5% in total nitrogen content, 75.5 % rise in carbon content of soil and 50.5% improvement in peanut yield with the addition of biochar. Many studies stated that application of biochar could show decrease in nitrogen leaching and also enhances the nitrogen retention, also effects the total nitrogen content of soil by biochar addition in soil. (Chan et al. 2008; Major et al. 2012). Biochar (because of porous nature) has capacity of absorbing ammonium and nitrates, due to which it can enhance storage capacity of nitrate and ammonium and also give rise to immobilization of nitrogen.

On the other hand, volatilization of ammonium is reduced and increase in bioavailability of nitrogen in concern of crop growth and yield occurs (Rondon et al. 2007). Nitrogen leaching is decreased by the suitable rates of biochar application. It retains the soil nitrogen and stops

the ammonium volatilization (Rondon et al. 2007). As the mineral phosphorus resources are reducing, application of biochar plays vital role to phosphorus recycling from agriculture waste such as sewage sludge, manure. Biochar addition to soil increases the availability of phosphorus leads to high concentration of phosphorus in its ash content (Zhai et al. 2015). Soil sorption capacity influences the increase in availability of phosphorus. After the biochar addition, with the minimum sorption capacity phosphorus availability can become higher, such as alkaline soil is being compared to acidic composition soil (Zhai et al. 2015).

By applying the biochar, phosphorus availability decreased in alkaline soil and in case of acidic soil its availability got enhanced (Chintala et al. 2014). In most soils cation exchange capacity got raised after the biochar application. Certain changes in soil cation exchange capacity and concentration of acid and basic cations potentially impact nutrient availability and ultimately crop yield is affected. (Martinsen et al. 2015).

In recent years research, biochar application has gained much attention because of its additional effect on soil microbes and also controls the microbial activity. Biochar has strong mutual interaction with soil minerals because of which its application enhances the microbial population (Zhang et al. 2014; Pokharel, Ma, and Chang 2020). To increase the soil C sinks, biochar has strongly been recommended. It is resistant to biotic and abiotic degradation. Because of its influence on availability of nitrogen, carbon and physiochemical properties of soil, it can alter microbial abundance, biomass structure and may shows changes into soil organic carbon cycle (Zhang et al. 2014). Moreover, large specific surface area of biochar adsorbs enough amount of substrate that affects enzymatic activity of soil. After the application of biochar, there is increase in enzymatic activity of soil which is primarily attributed to high microbial activity, due to the labile source of carbon from biochar (Ouyang et al. 2014).

CONCLUSION

This study highlights the potential of biochar, particularly sugarcane bagasse biochar, as an effective amendment for remediating heavy metal-contaminated soils and enhancing soil quality. Biochar application reduced the availability of cadmium and lead while significantly improving soil pH, organic carbon content, and nutrient release. Sugarcane bagasse biochar outperformed rice husk biochar in metal adsorption efficiency and its impact on soil fertility. The findings suggest that biochar, applied at rates up to 2%, can sustainably reclaim polluted soils, promote crop growth, and support agro-ecological productivity. These results provide a pathway for improving soil health and achieving sustainable agriculture in contaminated areas.

Authors' contributions

sHuma N and Khan N M performed the experiments, analyzed the data, prepared figures and tables, authored and reviewed drafts of the article. Mujtaba G and Naz S and Khan M A assisted in performing the experiments and performing plant analysis. Yaseen A, Ali M and Nawaz S improved the written language. Huma N, Khan N M and

Mujtaba G conceived and designed the experiments and approved the final draft. Naaz S and Nawaz S also helped

in using IBM SPSS Statistical Package. All authors read and approved the final manuscript.

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