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RESIDUAL EFFECT OF BIOCHAR ON GROWTH, ANTIOXIDANT DEFENCE AND CADMIUM (Cd) ACCUMULATION IN RICE IN A Cd CONTAMINATED SALINE SOIL

Farhan Hafeez[1], Muhammad Rizwan[2,*], Muhammad Saqib[3], Tahira Yasmeen[2], Shafaqat Ali[2], Tahir Abbas[2], Muhammad Zia-ur-Rehman[3] and Muhammad Farooq Qayyum[4]

[1]Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Tobe Camp University Road Abbottabad 22060 Pakistan. [2]Department of Environmental Sciences and Engineering, Government College University, Allama Iqbal Road, 38000 Faisalabad, Pakistan. [3]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan. [4] Department of Soil Sciences, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan.
***Corresponding author: mrazi1532@yahoo.com**

Both salinity and cadmium (Cd) contamination of soils are the most critical issues of global food security. Soil application of biochar could decrease toxic metal and sodium (Na) uptake by plants in the season, but its long-term residual effects on metal uptake in latter crops need evaluation. A pot experiment with rice was conducted under ambient conditions in a Cd-contaminated soil which was used in an earlier experiment on wheat where soil was treated with different rates of biochar (0, 3, and 5% w/w) and irrigated with water having different salinity levels (0, 25, and 50 mM). Further treatments were not applied in the current study. The results revealed that plant growth and photosynthesis reduced while Cd and Na concentrations and oxidative stress were increased in plants under combined Cd and salinity. Biochar application reduced the Cd and Na concentrations and increased the potassium (K) concentrations in plants while diminished the oxidative stress in salt-stressed rice. Biochar positively affected the plant growth and physiology in a soil treated with 0, and 25 mM salt stress. However, plant growth and photosynthesis were reduced with biochar amendments under 50 mM NaCl stress. Soil AB-DTPA extractable Cd was higher in salinized soil without biochar addition, while biochar reduced the Cd extractability. It can be concluded that all biochar amendments had a significant residual effect on decreasing Cd and Na uptake while plant growth was negatively affected at higher biochar and salinity levels.
Keywords: Biochar, residual effect, heavy metal stress, salinity, physiology.

INTRODUCTION

In present era, crop production is negatively affected due to abiotic stresses including heavy metals and salinity (Murtaza *et al.,* 2015; Rizwan *et al.,* 2016a). Soil contamination with cadmium (Cd) has affected the production of good quality food worldwide (Gallego *et al.,* 2012; Rizwan *et al.,* 2012). Crop plants could absorb Cd easily via roots which can translocate to the aerial parts even in plants grown in moderately Cd-contaminated (Rehman *et al.,* 2015; Rizwan *et al.,* 2017). Excess of Cd not only had negative effects on the quality of foods but also reduces the growth and yield of plants through different mechanism such as reduction in nutrient uptake, photosynthesis and overproduction of reactive oxygen species (ROS) (Nagajyoti *et al.,* 2010).
Soil salinization is another adverse environmental factor involved in limiting the growth and productivity of crops across the globe (Hussain *et al.,* 2015; Abbasi *et al.,* 2016; Mohamed *et al.,* 2017). Agricultural soils mainly irrigated with wastewater and applied with sewage sludges may simultaneously be contaminated with toxic metals and salinity (Bauddh and Singh 2012; Rehman *et al.,* 2018). Previous studies showed that salinity increased the Cd uptake in plants whereas reduced the plant growth (Shafi *et al.,* 2009; Rady *et al.,* 2016). It was also observed that both Cd toxicity and salinity caused oxidative stress in crops (Abbasi *et al.,* 2016; Zhang *et al.,* 2016; Abbas *et al.,* 2017a).
Rice is one of the main cereals and is the staple food for over 50% of the world population (Rizwan *et al.,* 2016b). Available evidences have shown that the rice plant has the ability to accumulate Cd which negatively affects the rice growth and quality (Gao *et al.,* 2016; Rehman *et al.,* 2017). Therefore, minimization of Cd in edible parts of the crops is necessary mainly to reduce its negative effects on humans.
Different amendments could be used for the purpose of reducing Cd stress and salinity in crops (Ok *et al.,* 2015; Rizwan *et al.,* 2015). Biochar, a product of organic material under lower O_2 environment, as soil amendment has been increasingly used to improve the soil fertility, heavy metal toxicity and salt stress in plants (Akhtar *et al.,* 2015a; Bian *et al.,* 2016; Rizwan *et al.,* 2016c; Abbas *et al.,* 2017a). Biochar could also improve the plant growth by increasing the

bioavailability of mineral nutrients and improving soil chemical, biological, and physical properties (Rizwan *et al.,* 2016c; Saifullah *et al.,* 2018). In salt-affected as well as metal-contaminated soils, biochar amendment could reduce metals and Na accumulation by plants (Akhtar *et al.,* 2015a; Yousaf *et al.,* 2016; Ali *et al.,* 2017). In a previous experiment, it has been shown that biochar supply alleviated the Cd stress and salinity in wheat by decreasing Cd, Na uptake in wheat as well as by diminishing soil bioavailable Cd (Abbas *et al.,* 2017b). Furthermore, the information about the fate of the decreased soil bioavailable Cd is needed before recommendation of biochar in degraded soils. Akhtar *et al.* (2015b) have shown the positive impacts of biochar on reducing the Na accumulation in salt-stressed wheat. Therefore, the study of residual impacts of biochar in plants could provide the information regarding the application of biochar in saline soils aiming to decrease the metal accumulation in crops.

The purpose of the current study was to evaluate the residual impacts of biochar on Cd and Na accumulation, plant growth and physiology of pot-grown rice under combined Cd and salinity stress. Rice was selected as a latter crop as wheat-rice system prevails in large cultivated areas of Pakistan and probably across the world.

MATERIALS AND METHODS

Materials and treatments: Soil selected in the current experiment was also used for the wheat cultivation previously (Abbas *et al.,* 2017b). Briefly, soil was collected from an agricultural field (0-20 cm) receiving raw effluent since the last 30 years and thus contaminated with Cd with total and bioavailable Cd concentrations of 2.86 and 0.41 mg kg^{-1}. Detailed physicochemical characteristics of the studied soil have been given previously (Abbas *et al.,* 2017b). Soil texture was sandy clay loam containing sand, silt, and clay of 44, 25, and 31% respectively. pH, electrical conductivity (EC) (dS m^{-1}), and cation exchange capacity (CEC) ($cmol_c$ kg^{-1}) of soil were 7.33, 2.97, and 4.96 respectively. In soil, total metal concentrations were determined by the method of Amacher *et al.* (1996), bioavailable metals by the method of Soltanpour (1985), EC and sodium adsorption ratio (SAR) were determined with standard protocols (US Salinity Lab. Staff 1954; Page *et al.,* 1982).

After the harvest of wheat (Abbas et al., 2017b), the same pots were used for the present experiment. There were six treatments including three rates of biochar (0, 3.0, and 5.0% w/w) applied in all possible combination with three levels of NaCl salinity (0, 25, and 50 mM). The salinity was developed by irrigation of NaCl solution. Biochar was prepared by heating rice straw at 450 ºC for two hours. The biochar, as characterized by using standard procedures (Qayyum *et al.,* 2015), had pH 10.0, volatile matter 24%, ash content 22.5%, EC 2.4 dS m^{-1}, C 42.3%, N 1.5%, P 0.3%, K 2.54% and Na 1.1%. The salinity developed in biochar + NaCl treatments was slightly higher than the respective NaCl treatments alone. Without biochar supply, the average EC levels were 3.22, 8.43, and 11.24 dS m^{-1} in 0, 25, and 50 mM NaCl treatments respectively.

Experimental setup: The experiment was performed under ambient environmental conditions. 30-day-old rice seedlings (cv. Kainat) were transplanted in pots containing soil (3.0 kg). First of all, 6 seedlings were transferred per pot following CRD (completely randomized design) with 4 replications and finally four seedlings were retained in each pot at 10^{th} day of sowing. No further biochar or salinity was added during this experiment and tap water was used as a source of irrigation where a specific level of water (1-2 cm) was maintained in each pot throughout the experimental duration. Fertilizers such as N as urea, P as DAP, and K as SOP were applied by considering 120-50-25 kg NPK ha^{-1}. Half of the N and whole of P and K were used at 15^{th} day of transplanting while remaining half of N was applied at 35^{th} day of transplanting.

Physiological measurements: The upper fully expanded fresh leaves of rice were selected for the estimation of chlorophyll *a*, chlorophyll *b* concentrations as well as gas exchange parameters (water use efficiency (WUE), photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr)) at 70^{th} day of transplanting. The extraction with 85% v/v acetone was done for the measurement of chlorophyll contents and then the standard equations were used for calculations (Lichtenthaler, 1987). Gas exchange attributes were recorded by using a portable Infra-Red Gas Analyzer (IRGA).

Electrolyte leakage (EL), Malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) levels in samples were estimated as described by Abbas *et al.* (2017b) at 70^{th} day of transplanting. Briefly, EL was determined by recording both initial and final ECs of solution by placing the samples in water for 2h at 32 °C which was termed as EC1 and then the same samples were heated at 121 °C for 20 min termed as EC2 and finally formula was used for calculations of EL (Dionisio-Sese and Tobita, 1998).

$$EL = (EC_1/EC_2) \times 100 \qquad (1)$$

A method given by Heath and Packer (1968) with further modifications latter (Dhindsa *et al.,* 1981; Zhang and Kirkham, 1994) was employed for the estimation of leaf MDA content with the help of thiobarbituric acid (TBA). For the determination of leaf H_2O_2 concentration, 3 ml of 50 mM phosphate buffer adjusted at specified pH (6.5) were used in samples of 50 mg. After this, centrifugation was done (30 min, 6,000g and 4 °C), then 1.0 ml of 0.1% titanium sulfate in H_2SO_4 (20%, v/v) was added in supernatent and centrifugation (6,000g, and 4 °C) was done for 20 min. H_2O_2 concentration was determined by taking the absorption at 410 nm and then calculations were done by using extinction coefficient (0.28 µmol^{-1} cm^{-1}). Antioxidant enzymes such as ssuperoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities were determined by the methods described earlier (Aebi, 1984; Zhang 1992).

Plant harvesting and growth parameters: After 70 days of growth, plants were harvested and plant height, number of tillers were recorded. Plants were separated into parts and then thoroughly washed with water, and dry weights were recorded after oven drying (70 °C) for about 72 h.

Soil and plant analysis after harvesting: The Cd in shoots and roots was determined by digesting the tissues in HNO_3-$HClO_4$ (3:1, v:v) and then taking the readings with the help of atomic absorption spectrophotometer (Ryan *et al.,* 2001). Both Na and K concentrations in samples were determined by dissolving the ashed material in HNO_3 and then using the flame photometer. Soil sampling was done after harvesting the rice, oven dried (40 °C), and sieved (2 mm). After this, soil AB-DTPA extractable Cd, EC, and pH were determined as demonstrated previously (section materials and treatments).

Statistical analyses: All the data were analyzed statistically at 5% probability level with the help of One-way ANOVA with SPSS for windows. The multiple comparison of means was done by Tukey's HSD post hoc test. The combined impacts of BC and salt treatments were analyzed with the help of Two-way ANOVA.

RESULTS

Plant dry weight and photosynthesis: Salinity caused reduction in plant height, number of tillers per plant, shoot and dry weight of roots than control (Figure 1 and 2). Overall, shoot length, shoot and root dry weights were reduced by 8, 20%, and 13, 35%, and 25 and 34% in 25 and 50 mM salt treatment than control respectively. Without salt stress, plant height increased by 8.5%, and 20% and number of tillers per plant increased by 37, and 75% in 3.0 and 5.0% biochar than control plants, respectively (Figure 1 and 2).

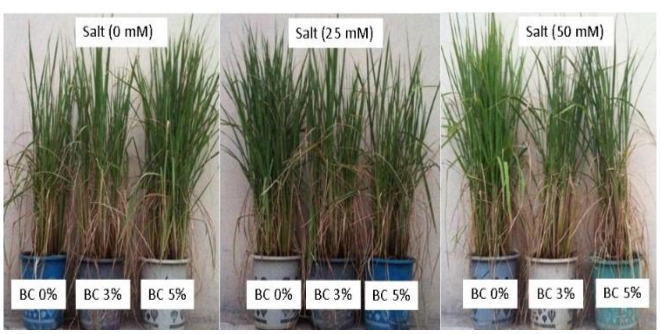

Figure 1. A pictorial view of experiment at 70 days of growth.

In 5.0% biochar without salt stress, dry weights of shoot and root were enhanced by 72 and 86% than control respectively. Under 25 mM salt stress, biochar application increased plant growth and shoot biomass in 25 mM + 3.0% biochar than respective salt stress whereas root biomass increase in 5.0% biochar under the same salt level. At 50 mM salt stress, biochar reduced these parameters at all levels compared to respective salt stress alone. Overall, the highest growth and dry weights were obtained with 5.0% biochar while lowest growth and dry weights were obtained in 50 mM NaCl + 5.0% biochar treatments.

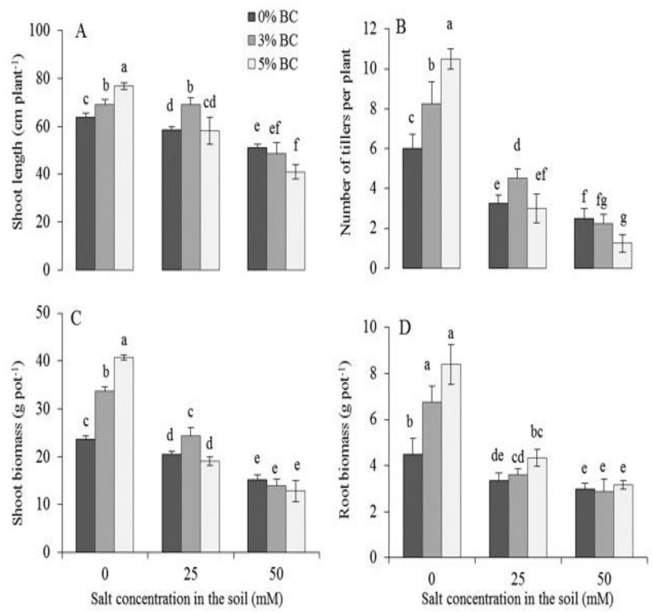

Figure 2. Shoot length (A), number of tillers per plant (B), shoot dry weight (C), and root dry weight (D) of rice under either alone or combined Cd and salt stress and treated with biochar. All values are means of four replicates with SD. Different letters indicate significant differences among treatments at p < 0.05.

Soil salinity diminished chlorophyll *a* concentration by 15, and 33%, whereas chlorophyll *b* concentration by 25, and 41% in 25 and 50 mM NaCl (Figure 3A, 3B). Biochar supply enhanced chlorophyll concentrations in 0 and 25 mM salt stress whereas slightly reduced the chlorophyll contents in higher salt (50 mM) treatment. The highest chlorophyll contents were observed in 5.0% biochar while the lowest contents were observed in 50 mM NaCl + 5.0% biochar treatment. Salt stress reduced the Pn by 20.5%, and 40%, Gs by 26%, and 49.5%, Tr by 24%, and 42%, and WUE by 16.5 and 37% in 25 and 50 mM salt stress than control plants, respectively (Figure 3C-F). Biochar treatments increased these parameters in 0 and 25 mM salt stress than respective treatments without biochar whereas slight reduction in these parameters were observed at all biochar levels under 50 mM NaCl than control. At 5.0% biochar treatment alone, Pn, Gs, Tr as well as WUE were enhanced by 68, 64, 72, and 61.5% over control plants, respectively.

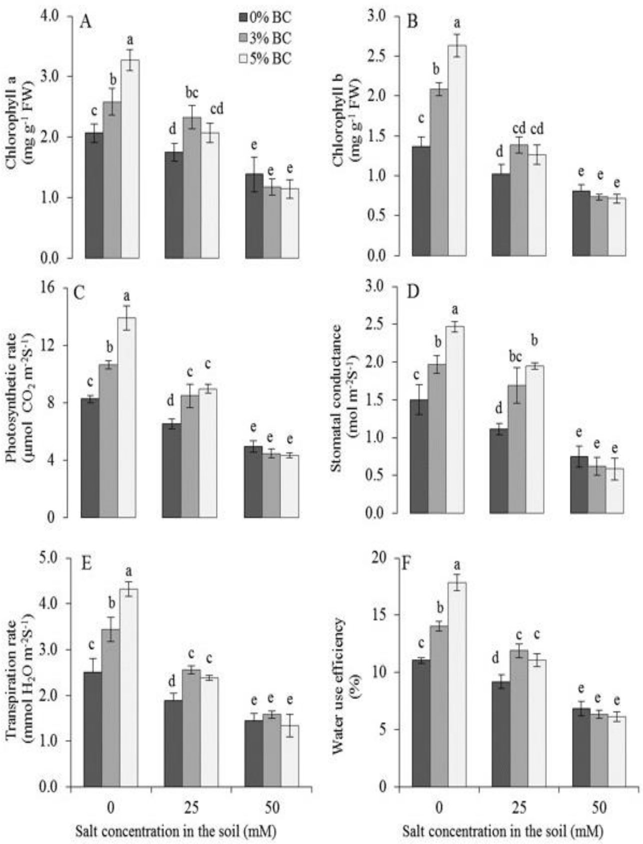

Figure 3. Chlorophyll concentration (A-B) and gas exchange attributes (C-F) of rice under either alone or combined Cd and salt stress and treated with biochar. All values are means of four replicates with SD. Different letters indicate significant differences among treatments at p < 0.05.

Cadmium, Na, and K in plants: Soil salinity increased the Cd and Na whereas reduced the K concentrations in shoots and roots (Figure 4). Under salt stress alone, Cd concentration enhanced in shoots by 20, and 36% and by 15 and 30% in roots with 25 and 50 mM NaCl treatments than control, respectively. At 50 mM salt stress, Na contents increased by 22.3 and 15.5 times in shoots and roots than control plants, respectively. K concentrations were reduced by 45%, and 40% in shoots and roots with 50 mM NaCl than control treatment, respectively. Biochar reduced the Cd and Na under salt treatments than respective salt treatments alone. The lowest Cd and Na contents were found in 5.0% biochar whereas the highest contents of these elements were found in 50 mM NaCl treatment alone. At 50 mM NaCl, Cd concentrations reduced by 20%, and 31% in shoots and by 14.5%, and 29% in roots in 3.0 and 5.0% biochar treatments than NaCl treatment alone, respectively. At 5.0% biochar, Na concentrations in shoot reduced by 36, and 35% and in roots reduced by 30.5, and 39% in 25, and 50 mM NaCl than respective salt treatments alone. K concentrations were gradually increased in shoots and roots with increasing biochar levels than control being maximum in 5.0% biochar treatment and minimum in 50 mM NaCl treatments.

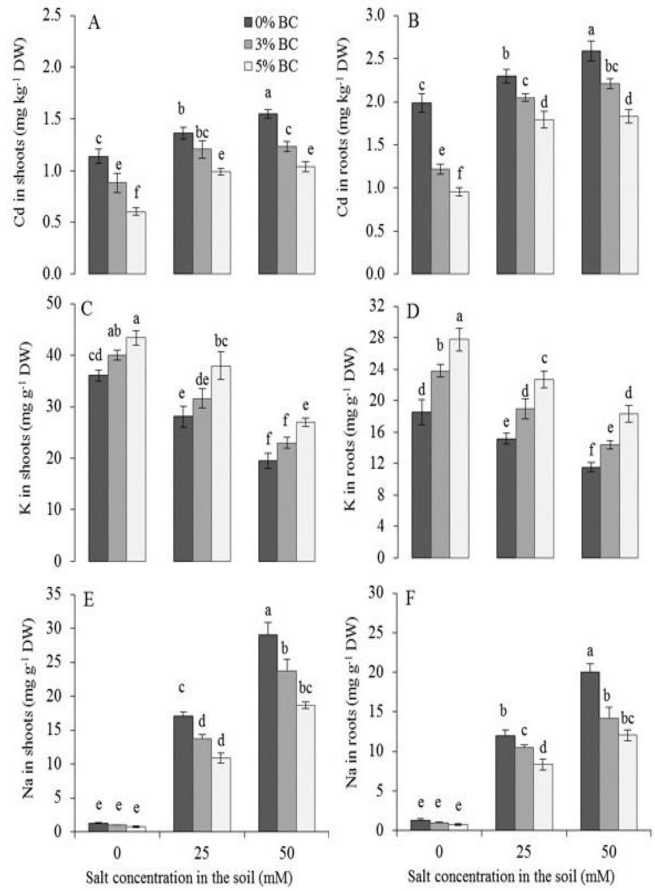

Figure 4. Cadmium (Cd), potassium (K), and sodium (Na) concentrations in shoot and roots of rice under either alone or combined Cd and salt stress and treated with biochar. All values are means of four replicates with SD. Different letters indicate significant differences among treatments at p < 0.05.

Oxidative stress and antioxidant enzymes: Oxidative stress was found to be increased in shoots under salt stress (Figure 5). MDA contents increased by 23, and 49.5% and EL enhanced by 20, and 51%, and H_2O_2 concentrations were enhanced by 22, and 60% in 25 and 50 mM NaCl over control, respectively. Biochar application significantly ($p < 0.05$) diminished these parameters in shoots under 0 and 25 mM salt treatments whereas slightly reduced in higher NaCl (50 mM) levels. The lowest concentrations of MDA, EL, and H_2O_2 were found in 5.0% biochar and the highest concentrations were reported in 50 mM NaCl. At 5.0% biochar, MDA contents reduced by 45, and 42%, EL contents reduced by 35, and 38% and H_2O_2 contents reduced by 63, and 44% in 0 and 25 mM salt treatments respectively than salt treatments alone. Salinity diminished SOD, CAT activities while improved POD activity under salinity (Figure 5D-F). Biochar treatments increased the SOD, and CAT and reduced the POD activity than control.

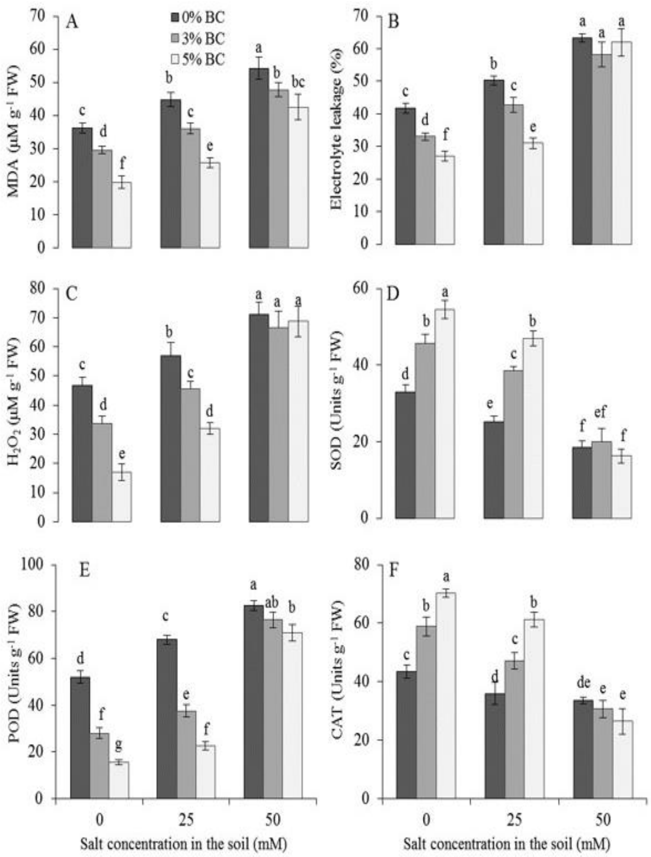

Figure 5. Malondialdehyde contents (A), electrolyte leakage (B) and H_2O_2 (C) and antioxidant enzyme activities (D-F) in rice leaves under either alone or combined Cd and salt stress and treated with biochar. All values are means of four replicates with SD. Different letters indicate significant differences among treatments at $p < 0.05$.

Soil pH, EC and AB-DTPA-extractable Cd: Data related to pH, EC and bioavailable soil Cd is presented in Table 1. Salt stress increased the pH of soil and biochar treatments further increased the pH than the NaCl treatment. The highest pH was found in 5.0% biochar + 50 mM NaCl whereas lowest pH value was found in control. EC of the soil increased with increasing NaCl levels whereas biochar further increased the EC under 0 and 50 mM NaCl and reduced under 25 mM NaCl than respective treatments without biochar. Salt stress increased soil bioavailable Cd than control. Biochar treatments reduced the soil Cd than the treatments without biochar. The lowest Cd was found in 5.0% biochar treatments whereas the highest Cd concentration was found in 50 mM NaCl treatment.

Table 1. Post-harvest soil pH, electrical conductivity (EC), and AB-DTPA extractable Cd.

Salt (mM)	Biochar (%)	Soil pH	EC (dS m^{-1})	Cd (mg kg^{-1})
0	0	7.31 ± 0.04d	3.17 ± 0.10f	0.34 ± 0.01bc
	3	7.51 ± 0.02b	4.07 ± 0.09e	0.23 ± 0.02d
	5	7.64 ± 0.04a	4.65 ± 0.20d	0.18 ± 0.01e
25	0	7.36 ± 0.02d	8.31 ± 0.27b	0.43 ± 0.02a
	3	7.55 ± 0.03b	7.74 ± 0.20c	0.38 ± 0.01b
	5	7.61 ± 0.02a	7.64 ± 0.23c	0.31 ± 0.01c
50	0	7.40 ± 0.01c	11.09 ± 0.12a	0.47 ± 0.02a
	3	7.64 ± 0.02a	10.48 ± 0.31b	0.38 ± 0.01b
	5	7.68 ± 0.05a	11.30 ± 0.23a	0.34 ± 0.03bc

Values are means of 4 replicates with SD. Different letters indicate a significant difference among treatments at $p < 0.05$.

DISCUSSION

Biochar was added in the soil under Cd + salt stress in a previous study on wheat (Abbas *et al.,* 2017b) and residual impacts of biochar on rice were studied in the present experiment. Our results indicated that biochar had residual effects in alleviating the metal and salt stress in latter crops which mainly depend upon the biochar and salinity levels in the soil. Rice growth and photosynthesis was negatively affected under salt stress without biochar addition (Figure 1-3). It was reported that combined Cd and salinity reduced the growth parameters and biomass than the respective Cd and salt (Shafi *et al.,* 2009). The lower dry weight obtained may be due to the negative structural changes in rice seedlings in plants without biochar (Abbasi *et al.,* 2015). However, a number of studies have shown that biochar improved the plant growth under either salt stress (Lashari *et al.,* 2013) or Cd toxicity (Abbas *et al.,* 2017a). In a previous experiment, the application of biochar diminished the Cd as well as salt stress in wheat (Abbas *et al.,* 2017b). Here, in the current study the increase in rice biomass under lower biochar and salt stress indicated the residual effects of biochar in reducing the toxicity while higher biochar and salt stress reduced the plant biomass which might be due to higher EC of the soil under these conditions (Table 1).

Leaf photosynthesis was reduced under the stress while biochar addition improved the photosynthetic attributes in rice (Figure 3). Physiological disorders have been widely studied in plants under salinity (Akhtar *et al.,* 2015b; Abbasi *et al.,* 2016) and Cd toxicity (Younis *et al.,* 2016; Rizwan *et al.,* 2017a). Biochar supply enhanced the photosynthesis in rice which might be due to the reduction of metal accumulation in plants (Figure 4). Reduced Cd and Na contents in plants positively affected chloroplast ultra-structures which may result in increased photosynthesis in plants (Rizwan *et al.*, 2015, 2017a).

Cadmium and Na concentrations increased in plants while K concentration decreased under salt stress (Figure 4). Under salt stress, the increase in Cd and Na concentrations have been reported in soybean (Ashrafi *et al.*, 2014), amaranth (Xu *et al.*, 2017), and wheat (Abbas *et al.*, 2017b). Salinity may increase the ionic strength in soil and affects the osmoregulation which may play an important role in mobility of Cd in soil (Xu *et al.*, 2017). The higher Cd accumulation in rice under salt toxicity may be due to increase in soil soluble salt level which may play a role in mobilization of Cd in the soil (Ozkutlu *et al.*, 2007). Biochar presence in the soil reduced the Cd and Na and increased the K contents in rice seedlings (Figure 4). Residual biochar increased the Na^+ adsorption in the soil and reduced its accumulation in wheat (Akhtar *et al.*, 2015b). This higher Na adsorption on the biochar might be helpful in the reduction of osmotic stress in plants. Furthermore, higher K uptake by rice may alleviate Na stress by increasing K/Na ratio as suggested previously (Abbas *et al.*, 2017b). Submerged incubation decreased the Cd with Fe- and Mn-oxides and gradually increased the Cd soluble and residual fractions (Hsu *et al.*, 2015). The decrease in Cd contents in plants with biochar treatments may be due to changes in Cd fractions in the soil with biochar (Bian *et al.*, 2016). Biochar may decrease the release of Cd into the soil solution under submerged conditions.

Plant suffered from oxidative stress under Cd or salt stress which resulted in the overproduction of ROS and reduction of antioxidant enzyme activities which may cause the imbalance between the generation and elimination of ROS (Schutzendubel and Polle 2002). Cadmium and salt stress enhanced the production of EL, MDA and H_2O_2 concentrations and reduced leaf CAT, SOD, and POD activities (Figure 5). These results are similar with the studies demonstrating that Cd and salt stress caused the oxidative stress in maize (Abbasi *et al.*, 2016) and wheat (Shafi *et al.*, 2009) than control plants. Biochar diminished the oxidative stress as observed by the lower production of ROS and increased antioxidant enzyme activities in leaves (Figure 5). Published literature showed that the biochar supply caused the reduction in oxidative stress than control plants (Younis *et al.*, 2016; Abbas *et al.*, 2017a). This reduced oxidative stress might be due to the reduction in Cd and Na contents in plants than control (Figure 4).

Soil pH, EC, and bioavailable Cd were increased under salt stress whereas biochar supply further enhanced the pH and EC and diminished the soil bioavailable Cd (Table 1). The reduced Cd concentration in the soil with biochar has been demonstrated previously (Rizwan *et al.*, 2016b; Abbas *et al.*, 2017a). It has been shown that biochar contributes towards CEC of the soil which may mobilize the soil Cd (Rizwan *et al.*, 2016c). Biochar has a strong sorption capacity which might be an additional reason for decrease in AB-DTPA extractable Cd. It is known that some biochar sorb high amounts of Cd, and the sorption process is often irreversible with low desorbed fractions of Cd into aqueous solutions (Rizwan *et al.*, 2016c). Furthermore, biochar may also increase the soil moisture contents which may dilute the salts in the soil solution and as a result the osmotic stress may be alleviated (Akhtar *et al.*, 2015b). Our results, however, showed that the plant growth was reduced under higher biochar and salt stress (Figure 2). This reduced plant growth may be due to the higher EC of the soil in presence of biochar as shown in Table 1.

Conclusion: It could be concluded that the biochar soil amendment demonstrated a significant residual impact in decreasing the Cd and Na in rice under combined Cd and salt stress. Biochar application decreased the Cd and Na concentrations in plants which might be due to the decrease in bioavailable Cd in soil. However, higher levels of biochar (5.0% w/w in this study) and salinity were not effective in enhancing the plant growth and photosynthesis and cause an increase in EC of the soil than the salt stress alone. Thus, biochar levels should be carefully applied in saline soils aiming to increase the plant growth and reduce the toxicity of hazardous metals. Overall, long-term field studies are needed to verify the above results before the recommendation of biochar in soils simultaneously contaminated with toxic metals and salinity.

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