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Influence of biochar amendment and foliar application of iron oxide nanoparticles on growth, photosynthesis, and cadmium accumulation in rice biomass

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Abstract

Purpose The majority studies used the biochar or nanoparticles alone in metal-contaminated soils while less is known about the combined use of these amendments in metal-contaminated soils. We aimed to explore the efficiency of iron oxide nanoparticles (Fe NPs) and biochar on cadmium (Cd) uptake in rice in pot trial.

Materials and methods An experiment was performed under ambient environmental conditions in a Cd-contaminated soil with and without biochar addition (1.0% *w/w*), and different Fe NP concentrations (0, 10, 20, 30 mg/L) were foliar sprayed at different time intervals (at 3rd, 4th, and 5th weeks of nursery transplantation in the pots) during the plant growth. After harvesting, rice growth, photosynthesis, Cd and Fe contents in rice tissues, and soil bioavailable Cd and soil pH were measured.

Results and discussion Iron NPs enhanced the dry weights of rice tissues, chlorophyll concentrations, and gas exchange characteristics and the impact of NPs was further increased when the biochar was applied along with NPs. Iron NPs significantly decreased the intake of Cd in rice shoots by 31 and 42% and in rice roots by 26 and 39% with the foliar spray of Fe NPs (30 mg/L) without and with biochar, respectively. Foliar spray of Fe NPs reduced the total Cd accumulation by shoots, whereas the total Cd accumulation in the roots increased. The co-presence of Fe NPs and Cd enhanced the Fe concentrations in shoots of rice by 52 and 33% and in roots of rice by 32 and 21% when 30 mg/L Fe NPs were supplied with and without biochar, respectively. The effects of Fe NPs were higher with biochar application than without biochar except Fe concentrations in rice seedlings where the opposite trend was observed.

Conclusions The increase in biomass, Fe concentrations in tissues, and decrease in Cd levels in plants clearly demonstrate that Fe NPs + biochar could be a promising technique for the utilization of Cd-contaminated soils in the future.

Keywords Biochar · Cadmium · Immobilization · Iron nanoparticles · pH · Rice

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1 Introduction

Heavy metals have persistence nature and are toxic to humans and other living things such as plants depending upon the increased environmental pollution level of these metals (Nagajyoti et al. 2010; Aziz et al. 2015; Chiao et al. 2019). The increased pollution of arable soils with cadmium (Cd) has become a vital issue that threatens food quality and human health (Rizwan et al. 2017a; Chiao et al. 2019). As compared to the other toxic metals, the Cd has received a greater attention due to its huge production and accumulation in soils mainly due to mining, urbanization, and industrialization (Rehman et al. 2015). The metal-polluted soils need to be covered by the cultivation of crops mainly to limit metal entrance to other environmental compartments. Rice (*Oryza sativa* L.) is considered as a major food crop for large part of the global population particularly in Asia. Rice can accumulate Cd and translocate it to the grains which might be a major source of Cd in people consuming rice or its products as a staple food (Xie et al. 2015; Chiao et al. 2019). Similarly, Cd causes several disorders in rice and other crops at levels of physiological, morphological, and molecular (Rizwan et al. 2017b; Huang et al. 2018a). Thus, due to nutritional value of rice, the decrease in Cd bioavailability in soils and its accumulation in rice is urgently needed.

Chemical stabilization of toxic heavy metals in the soil may occur by the application of soil amendments through several reactions such as sorption and biochemical mechanisms which has gained a considerable attention during the recent past (Zanuzzi et al. 2013). The immobilization of the heavy metals in the soil is an environmentally sustainable technique for the growth of crops (Zanuzzi et al. 2013; Rehman et al. 2017). Recently, the use of biochar as an amendment to minimize the availability of metals for plants and their accumulation by crops has been widely studied (Rizwan et al. 2016b; Suksabye et al. 2016; Abbas et al. 2018). Biochar is an organic material that is rich in carbon and is obtained by pyrolysis of materials in low oxygen conditions (Lehmann and Joseph 2009; Sigua et al. 2015). Numerous studies have reported that biochar has a great potential for metal immobilization in the soil and can decrease metal accumulation by plants depending upon types of metals and feedstocks, doses applied, and experimental conditions (Rizwan et al. 2016b; Suksabye et al. 2016). Published reports have depicted that the higher rates of biochar were more efficient in decreasing metal concentrations in crops (Abbas et al. 2017; Rizwan et al. 2018), but these biochar levels are not practicable under real conditions. Thus, there is need to evaluate the application of biochar with other materials for practical purposes. Rice straw is widely produced across the globe due to the growth of rice crop on a large area worldwide, and thus, there is tremendous scope for the production of biochar from the rice straw and application of this biochar for environmental remediation.

On the other hand, nanoparticles (NPs) are widely produced in various shapes, sizes, and types and are used in various industries such as medicine, electronics, and agriculture (Ma et al. 2015; Rizwan et al. 2017c). The possibility of NPs has been largely studied for the purpose of environmental remediation (Mohammadi et al. 2018; Sebastian et al. 2018). It has been depicted that silver NPs increased the salinity tolerance in wheat (Mohamed et al. 2017), zinc oxide (ZnO) NPs decreased the Cd concentration in grains of cereals (Hussain et al. 2018), iron oxide NPs reduced the Cd concentration in rice tissue (Sebastian et al. 2018). Although NPs minimized the metal intake by plants, but the combined use of NPs with other amendments has not been fully explored. In comparison with single biochar or NP amendments, the combined use of biochar and NPs may be a good option for decreasing the Cd or other metal accumulation by crops. For example, iron phosphate NPs supported biochar inhibit the Cd in different parts of cabbage (Qiao et al. 2017). Nano-hydroxyapatite supported with biochar decreased the lead (Pb) intake in cabbage (Yang et al. 2016). Nano zero valent iron (ZVI) combined with biochar decreased the Cd and arsenic (As) uptake in rice when grown in a co-contaminated soil (Qiao et al. 2018).

The information about to the combined application of biochar and Fe NP amendments and their potential impacts on Cd and Fe accumulation by rice is still very limited. Iron amendments reduced the Cd intake in rice seedlings (Bashir et al. 2018) because the increase in Fe concentrations in the plants can regulate Cd contents in plants. Thus, it is important to explore the potential impact of biochar combined with Fe NP amendments on Cd uptake by rice. It was hypothesized that the application of foliar Fe NPs and in combination with biochar soil amendment may decrease the Cd accumulation in rice by immobilizing the Cd in the soil in the presence of biochar and simultaneously increasing Fe concentrations in rice by NPs. Therefore, the aims of the current study were to explore the impact of biochar and Fe NPs on (i) plant height and dry biomass of rice, (ii) chlorophyll contents, (iii) the uptake and translocation of Cd and Fe in rice, and (iv) Cd bioavailability in the soil. This study provides important information regarding the safe use of NPs and biochar in agriculture and could benefit the use of nanotechnology in the remediation of metal-polluted arable lands.

2 Materials and methods

2.1 Materials

The experimental soil was sampled from the arable land, which was under cultivation of cereal crops and irrigated with city effluents as a main source of water to fulfill the water requirement of the crops. This field is located in Multan, Pakistan, as already described by Rehman et al. (2015). The soil contains toxic trace elements mainly Cd which is owing to the long-term use of the marginal quality water to irrigate the crops. Soil was sampled by selecting a depth of 0–20 cm from various points selected randomly from an area of 1 ha and all the soil samples were pooled and thoroughly mixed before further processes such as air-drying of the soil and sieving through 2 mm mesh and characterization for initial selected soil properties. A hydrometer method was used for the measurement of soil texture (Bouyoucos 1962). Soil pH (1:2.5 soil to water ratio), EC, sodium adsorption ratio, and soluble anions and cations were measured with the established procedures (US Salinity Lab. Staff methods 1954; Page et al. 1982). Plant-available trace elements were measured by using atomic absorption spectrophotometer after the extraction of soil with ammonium bicarbonate diethylene triamine pentaacetic acid (AB-DTPA) solution (Soltanpour 1985). Total concentrations of trace elements were evaluated by the protocol of Amacher (1996) in which soil was digested in concentrated HNO_3 and $HClO_3$ by heating the solution containing soil. Calcimeter (Moodie et al. 1959) and Walkley-Black methods (Walkley and Black 1934) were used to measure the $CaCO_3$ and soil organic matter contents, respectively. The selected characteristics of the experimental soil have been reported in the previous study by Ali et al. (2019). In brief, the soil texture was sandy loam containing sand, silt, and clay of 74, 17, and 9%. The soil pH, EC, and organic matter contents were 7.83 and 2.18 dS/m and 1.02%, respectively. The soil total and available Cd concentration were 7.86 and 1.32 mg/kg, respectively.

Rice straw biochar was prepared by pyrolyzing the selected feedstock at 450 °C and characterized as described in our previous study, and detailed properties of biochar are reported there (Abbas et al. 2017). The feedstock was collected from the agricultural field mainly irrigated with canal water. Biochar used in the current study contains 22.5% ash contents, 24% volatile matter, and 42.3% carbon contents. The pH of the selected biochar was 10 and EC value of 2.4 dS/m.

Iron oxide (II, III) NPs were of Alfa Aesar containing the following properties: purity, 97%; formula weight, 231.54; particle size, 50–100 nm APS powder; surface area, 20–50 m^2/g; melting point, 1538 °C; and density, 5.2 g/cm^3.

2.2 Experimental design

A pot study was conducted in the botanical garden under ambient environmental conditions. The day time average temperature and humidity were 34 °C and 58% at the start of the experiment and 39 °C and 68% at the end of the experiment, respectively. The rice variety selected was Kainat and the seeds were surface disinfected by dipping the seeds in sodium hypochlorite for about 2 min and then thoroughly washed to clean the seeds before sowing. Rice nursery was grown in the field for 25 days, and during this period, the seedlings were irrigated with tap water. A total of 5.0 kg of the selected soil was added in each plastic pot and pots were separated into two sets. The height of each pot was 20 cm and diameters of top and bottom were 18 and 15 cm. Thereafter, one set was used for the treatment of biochar at a rate of 1.0% in the soil termed as with biochar treatment and the second set was used with no biochar termed as without biochar treatment. The amount of biochar used in the current study was based on our previous experiment (Ali et al. 2019). The experiment was performed in completely randomized design with factorial arrangement. After this, rice nursery was transplanted with six seedlings in each pot in a complete randomize design, and finally, four seedlings were kept after 1 week of transplantation. The fertilizers comprised nitrogen (N), phosphorus (P), and potassium (K) that were used for the plants in the forms of urea, diammonium phosphate (DAP), and sulfate of potash (SOP) with concentrations of 250, 100, and 50 mg/pot. After 7 days of transplanting, total amount of P and K and half amount of N were given to the plants by dissolving in water, while remaining N was given after 3 weeks of transplanting. About 2 cm layer of H_2O was maintained in each pot over the entire experimental period. Different concentrations of Fe NPs were foliar sprayed (0, 10, 20, and 30 mg/L) at different time intervals for all treatments either with biochar or without biochar. The selected solution levels of Fe NPs were made by adding the measured quantity of NPs in separate flasks containing half litter of distilled water and then ultra-sonication was done for about 30 min and final volumes were made by using distilled water before application and Tween 20 (0.1%) was used as a sticking agent. The 1st foliar spray was done with hand-held sprayer bottle after 14 days of transplanting by covering the soil mainly to restrict the entry of NPs to the soil. After this, the remaining three foliar sprays of NPs were done after 3rd, 4th, and 5th weeks of nursery transplantation in the pots. The fresh solution of NPs was prepared for each spray. One litter of NP solution per treatment with all four replicates was used in four foliar sprays. The only distilled water was used for the control treatments simultaneously and pots were rotated randomly during the entire experimental duration.

2.3 Plant harvesting and analysis

The rice was harvested after 55 days of transplanting the nursery in the pots. Before harvesting, height of the plants was recorded by using meter rod. After harvesting, shoots were washed with distilled water, while dilute acid was employed for the washing of roots and then final washing of roots was done with distilled water. Thereafter, the samples were dried at 65 ± 5 °C for about 4 days in an oven and then dry weight was measured. The samples were crushed by using stainless steel mill and stored for Cd and Fe analysis.

2.4 Chlorophyll determination and gas exchange parameters

Fresh leaf samples were taken at 55 days of nursery transplantation in pots and were used to extract chlorophyll contents by using acetone (85% *v*/*v*) solution by putting the tubes containing leaves and extractant solution in dark at about 4 °C until all the chlorophyll contents were extracted from the leaves. Absorbance of the samples was measured by using spectrophotometer (Halo DB-20/DB-20S, Dynamica Company, London, UK) at specified wavelengths, and coefficient was used to calculate chlorophyll contents (Lichtenthaler 1987). Infra-Red Gas Analyzer (Analytical Development Company, Hoddesdon, England) was used to record stomata conductance, photosynthetic rate, and the transpiration rate.

2.5 Cadmium and Fe concentrations in plants and soil analysis

About 0.5 g shoot or root sample of each treatment and replicate was digested 1:3 ratio of $HClO_4$-HNO_3 on a hot plate as detailed in Rehman et al. (2015). Iron and Cd concentrations in the digestate were meaured by using atomic absorption spectrophotometer (Analytik Jena novAA 350). The total plant uptake of Cd was calculated by multiplying the biomass obtained from each pot to the Cd concentrations and the obtained results were reported as μg/pot. The soil collected after harvesting the rice was analyzed for pH and AB-DTPA extractable Cd after post-harvest soil sampling and extraction as described in the section 'experimental materials'.

2.6 Statistical analysis

Two-way analysis of variance was employed, with 5% probability level, by using IBM SPSS Statistical software, version 21.0. Significant differences among the treatments were determined by Tukey's HSD post hoc test by considering $P < .05$ as significant.

3 Results

3.1 Effect of amendments on plant growth and photosynthetic efficiency

Results related to shoot length, shoot and root dry weights in different treatments are given in Fig. 1. The height of plants slightly increased with the increase of NP doses and biochar supply further increased the plant height relative to the control (Fig. 1a). There was a significant impact of NP doses on plant height, while the effects of biochar and biochar × NPs were non-significant. The dry weight of shoot increased with the NPs treatment and the combined use of NPs and biochar further increased the shoot dry weight relative to the control and only NP treatments (Fig. 1b). The shoot dry weight enhanced by 15, 31, and 38% in 10, 20, and 30 mg/L NP treatments, respectively, over the control. There was a highly significant effect of biochar and NP treatments when applied separately, whereas their combined effect was non-significant. Root dry weight significantly increased with NP treatments either applied alone or combined with biochar (Fig. 1c). The application of 10, 20, and 30 mg/L NPs improved the root dry weight by 86, 113, and 147% without biochar treatment and by 68, 69, 130% with biochar treatment, respectively, relative to the respective controls. The root dry weight was negatively correlated with root Cd concentration and soil AB-DTPA extractable Cd (Table 3).

In the current study, the chlorophylls and photosynthetic efficiency of the plants grown under Cd stress significantly increased after the application of treatments relative to the control, and the impact was more prominent in the NPs + biochar treatments (Fig. 2). At 30 mg/L NPs, the chlorophyll

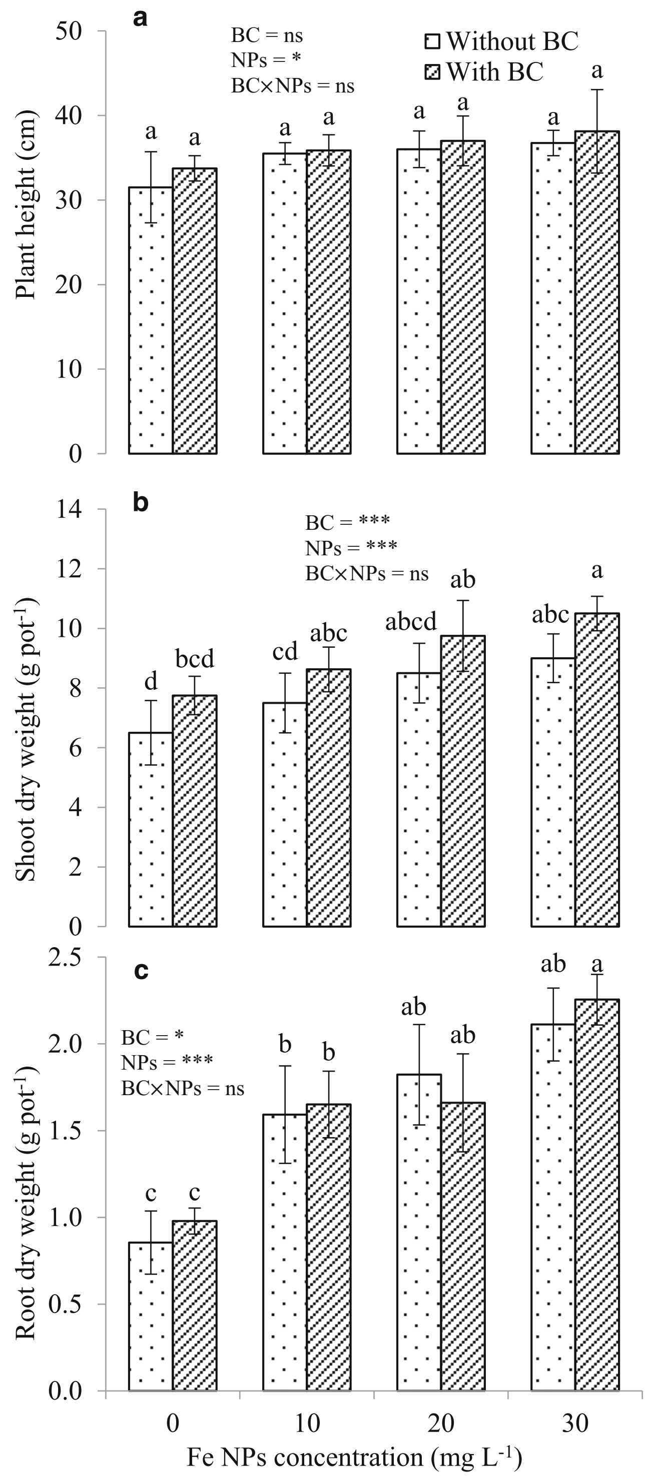

Fig. 1 Effect of iron nanoparticles (Fe NPs) alone and combined with biochar on the height of rice plants (**a**) and dry weight of shoots (**b**) and roots (**c**) grown in Cd-contaminated soil. Values reported are means of four replicates with standard deviation. Different letters on the bars represent the significant differences between treatments at $p \leq 0.05$. In figures, ns = non-significant; * = significant at 0.05, ** = significant at 0.01, and *** = significant at 0.001 levels

a concentration enhanced by 68 and 79% without and with biochar treatments, respectively, relative to the respective controls. At 30-mg/L NP treatment without biochar, the chlorophyll *b* concentration was increased by 58%, photosynthetic rate increased by 44%, stomatal conductance increased by 61%, and transpiration rate increased by 26% relative to the control. The highest concentrations of these parameters were observed in 30-mg/L NPs + biochar treatment, whereas lowest concentrations were observed in the control without biochar. The statistical analysis demonstrated that the impacts of biochar and NPs were significant on chlorophylls and photosynthetic attributes, whereas the effects biochar × NPs were only

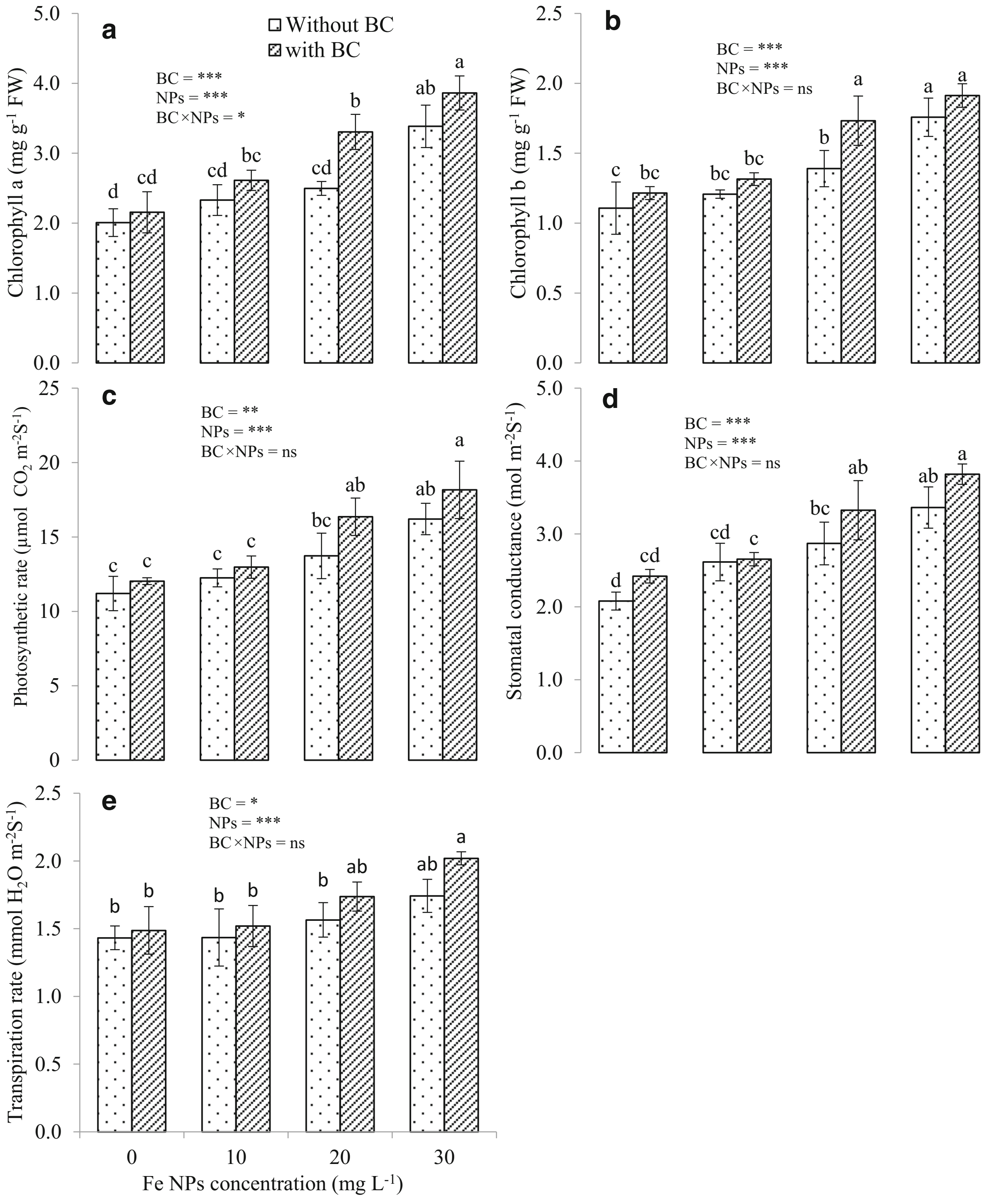

Fig. 2 Effect of iron nanoparticles (Fe NPs) alone and combined with biochar on the concentrations of chlorophyll a (**a**), chlorophyll b (**b**), photosynthetic rate (**c**), stomatal conductance (**d**), and transpiration rate (**e**) of rice grown in Cd-contaminated soil. Values reported are means of four replicates with standard deviation. Different letters on the bars represent the significant differences between treatments at $p \leq 0.05$. In figures, ns = non-significant; * = significant at 0.05, ** = significant at 0.01, and *** = significant at 0.001 levels

significant in chlorophyll *a* concentration (Fig. 2). There was a negative correlation among Cd concentration in rice tissues to the chlorophyll and gas exchange attributes (Table 3).

3.2 Cadmium and Fe concentrations in plants

The purpose of this study is to diminish the Cd accumulation by using NPs and biochar treatments. To verify the efficiency of NPs and biochar on Cd uptake in rice, the Cd concentration in rice tissues was measured. Cadmium concentration in rice tissues from different treatments have been given in Fig. 3a, b. Exposure of Fe NPs significantly decreased the Cd concentration in roots and shoot relative to control and the decreasing tendency was more prominent with higher NP treatments. Co-exposure of NPs and biochar further reduced the Cd concentrations relative to the respective NP treatments without biochar. The shoot Cd concentration decreased by 15, 20, and 31% in 10, 20, and 30 mg/L NPs, whereas the shoot Cd concentration decreased by 17, 34, and 42% in the same NPs + biochar doses, respectively, relative to the respective controls. At the highest NP treatment, the root Cd concentrations decreased by 26 and 39% without and with biochar, respectively, with compared to the respective controls. A significant impact of biochar and NPs was observed in reducing the Cd concentrations in rice tissues, whereas NPs × biochar was not significant for both shoots and root Cd concentrations.

Total Cd uptake (dry weights per pot × concentrations) by shoots and roots was calculated mainly to differentiate between the Cd dilution effect of yield or decrease in soil bioavailable Cd. Overall, total Cd uptake by shoot was lower

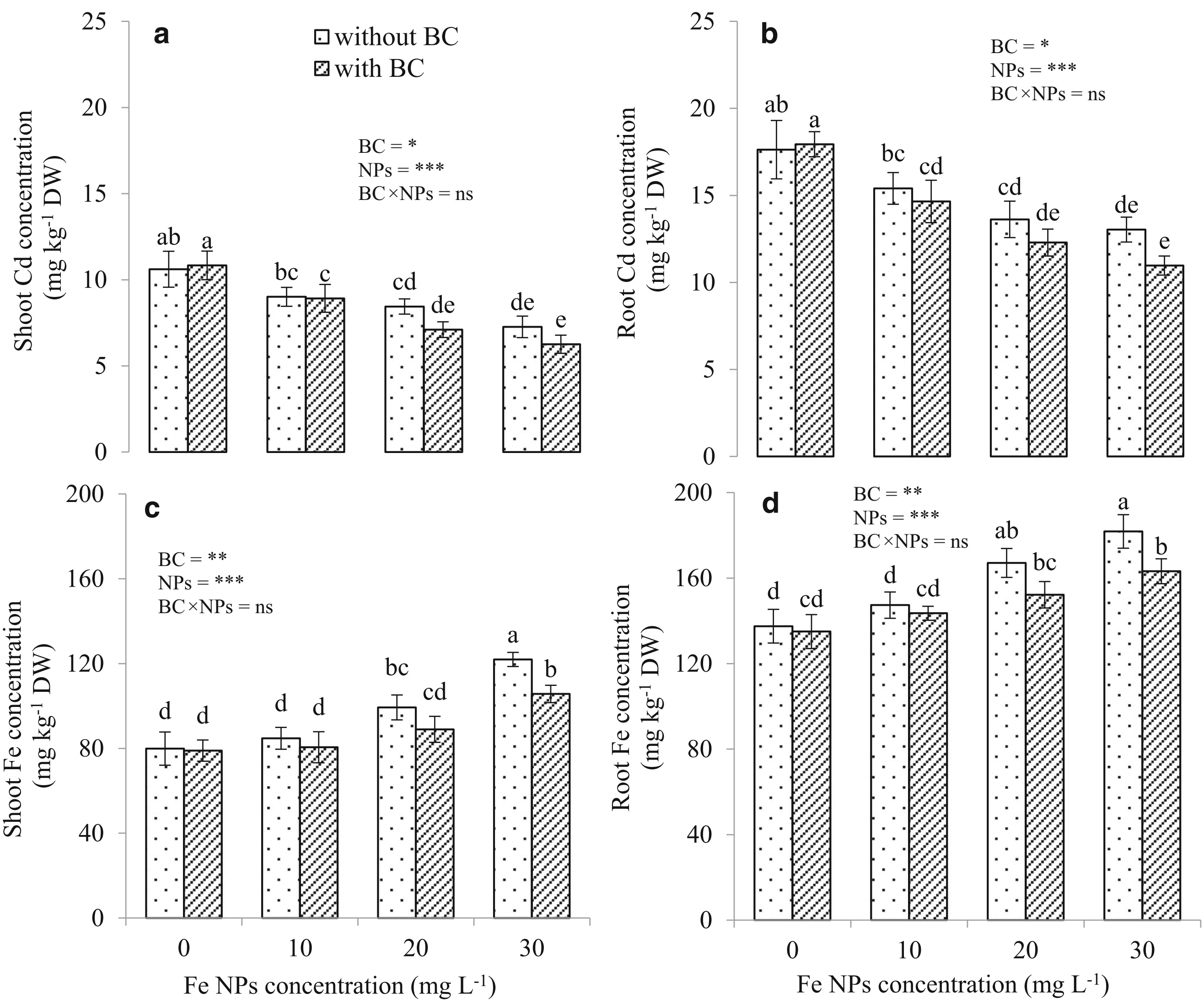

Fig. 3 Effect of iron nanoparticles (Fe NPs) alone and combined with biochar on the concentrations of Cd in shoots (**a**), roots (**b**), and Fe concentrations in shoots (C), and roots (**d**) of rice grown in Cd-contaminated soil. Values reported are means of four replicates with standard deviation. Different letters on the bars represent the significant differences between treatments at $p \leq 0.05$. In figures, ns = non-significant; * = significant at 0.05, ** = significant at 0.01, and *** = significant at 0.001 levels

when compared with the roots (Table 1). Compared with the control, total Cd uptake by shoot non-significantly decreased in NP treatments without biochar. There was a significant decrease in the total Cd accumulation by shoot with the highest NPs (30 mg/L) + biochar treatment over the respective control (Table 1). The uptake of total Cd by root increased with the NPs treatments either alone or in combination with biochar being significant at the highest dose of NPs compared to the control (Table 1). Total Cd accumulation was correlated negatively with Cd concentrations in rice aboveground and lower ground parts, whereas shoot Cd uptake was correlated positively with Cd concentrations in aboveground and lower ground parts (Table 3).

Iron concentrations in shoot and root increased in NP treatments either alone or combined with biochar treatment (Fig. 3c, d). There was a significant impact of biochar and NP treatments on shoot and root Fe concentrations, whereas the impact of NPs × biochar was not significant. It is noteworthy that plants grown under only foliar NP supplementation accumulated more Fe whereas less Fe was accumulated in the respective treatments with biochar application (Fig. 3c, d). At 30 mg/L NP treatment, shoot and root Fe concentrations increased by 52,and 32% without biochar application and increased by 33 and 21% with biochar treatment, respectively, relative to the control.

3.3 Soil pH and bioavailable Cd

The pH and estimated soil available Cd concentration after harvesting the plants are given in Table 2. Soil pH increased with exposure of different treatments in the experiment (Table 2). There was a significant enhancement in soil pH at the highest concentration of NPs + biochar over the control. The bioavailable Cd concentrations significantly decreased with the different treatments in comparison with the control. The supply of 10, 20, and 30 mg/L NPs diminished the soil available Cd concentrations by 12, 19, and 23% in comparison with the control, whereas the same NPs decreased the Cd concentrations by 15, 31, and 38% when applied with biochar, respectively, when compared with the control.

4 Discussion

The major aim of the study was to explore the impact of Fe NPs foliar spray in combination with biochar in the minimization of Cd toxicity and its accumulation in rice. It is well documented that plant biomass and photosynthetic pigments are very important indicators of trace element toxicity in plants. The results depicted that rice biomass and chlorophyll contents increased with treatments (Figs. 1 and 2). The lower biomass obtained in the control treatment may be due to the toxicity of Cd in rice as Cd is known to reduce the biomass and photosynthesis due to impairment in photosynthetic machinery (Xie et al. 2015; Rehman et al. 2017; Abbas et al. 2018; Chiao et al. 2019). The lower biomass and chlorophyll contents in control treatment might also be due to the lower concentrations of Fe in shoots (Fig. 3) as plant biomass and photosynthesis are vulnerable to the deficiency of Fe in the plants (Sebastian et al. 2017). This is due to the fact that Fe is critical for the synthesis of chlorophyll, phytohormones, as well as electron transfer in redox reactions (Lux et al. 2011; Rizwan et al. 2016a). Overall, it can be concluded that the increase of photosynthetic activity may result higher biomass production in the course of Fe NPs either alone or in combination with biochar treatments under Cd stress.

The main aim of Cd-contaminated soil remediation is to decrease the Cd concentration in plants and its translocation to aerial tissues. To verify this, the Cd concentrations in rice were measured. In comparison with the control, Cd decreased in shoots and roots, whereas Fe concentrations increased in these parts of rice (Fig. 3). The higher Cd concentrations in the untreated plants showed the strong ability of rice to accumulate Cd from the soil. Several studies demonstrated that biochar application diminished the Cd intake in many plant species including rice (Suksabye et al. 2016; Abbas et al. 2018; Rehman et al. 2017; Rizwan et al. 2018). The biochar application in acidic soil (pH 4.43) minimized the Cd and Cu concentrations in rice shoots and grains and stabilized the metals in the soil for longer period but the response varied with the type and aging of biochar (Li et al. 2016). Wheat straw biochar application in the acidic soil (pH 4.97) reduced the Cd contents in rice shoots and grains under field conditions but the response varied with the type of cultivars studied (Chen et al. 2016). Iron-NPs spiked sand decreased the Cd in rice and enhanced the Fe concentrations in plants (Sebastian et al. 2018). The NPs of Fe_3O_4 and Fe were more efficient in decreasing As concentrations in rice tissues than other NPs used in the experiment and the response varied with the rice cultivars (Huang et al. 2018b). The single or combined use of biochar and ZVI amendments decreased Cd and As concentrations in rice (Qiao et al. 2018). Similarly, iron phosphate supported with biochar decreased Cd concentration in cabbage (Qiao et al. 2017). The decreased Cd concentrations with amendments might be due to several factors occurring at the soil and within the plants with the amendments. The biochar and NPs application changed the Cd speciation in the soil and reduced the exchangeable Cd in the soil, whereas increased the Cd in less bioavailable forms (Qiao et al. 2017). In the soil under rice cultivation, Fe(II) is oxidized to Fe plaque at the surface of roots which may partially limit the Cd intake by rice due to adsorption of Cd. It is also believed that Fe and Cd share the same pathways during the uptake in plants and the higher Fe contents in plants may diminish Cd accumulation by crops due to the inactivation of these transporters as Fe transporters are being activated under Fe deficiency (Lux et al. 2011).

Table 1 Total Cd uptake (μg/pot) by shoots and roots of rice under biochar and NPs treatments

NP treatments	0	10	20	30
Shoot				
Without biochar	69.47 ± 16.15 ^{a}	67.41 ± 8.35 ^{a}	71.84 ± 9.57 ^{a}	65.69 ± 11.10 ^{a}
With biochar	83.96 ± 9.38 ^{a}	76.59 ± 4.58 ^{a}	68.86 ± 4.89 ^{a}	65.82 ± 7.95 ^{a}
Root				
Without biochar	59.18 ± 18.59 ^{c}	106.79 ± 20.19 ^{ab}	131.94 ± 32.77 ^{ab}	138.61 ± 28.00 ^{a}
With biochar	81.96 ± 8.37 ^{bc}	126.60 ± 17.82 ^{ab}	113.52 ± 13.99 ^{ab}	148.84 ± 23.46 ^{a}

For shoot Cd uptake, there were non-significant effects for BC, NPs, and BC + NPs and for root Cd uptake only significant effect was observed in NP treatments (NPs = ***) at 0.001 level

The higher Fe concentrations in rice under NP treatments (Fig. 3c, d) depicted that Fe NPs may act as a source of Fe which prevented the deficiency of Fe in rice. The higher concentration of Fe in plants is known to reduce the concentration of heavy metals such as Cd in rice due to competitive absorption (Bashir et al. 2018). The increased adsorption of heavy metals with magnetite NPs enhanced the metal tolerance in rice (Sebastian et al. 2017). Therefore, it can be concluded that increased Cd immobilization in the soil with biochar as well as increased Fe concentrations in plants with Fe NPs (Fig. 3c, d) resulted in the reduced Cd concentrations in rice (Fig. 3a, b) which increased the rice biomass and photosynthesis (Figs. 1 and 2). These results highlighted that Fe NPs combined with biochar in the study help to decrease Cd in rice. To demonstrate the differences in Cd intake by rice due to dilution effects or decreased in Cd bioavailability, the total Cd uptake in shoot and roots was calculated. In comparison with control, total Cd concentrations decreased in shoots especially at the highest treatment but total Cd uptake increased in roots (Table 1). This suggests that decrease in shoot Cd was not caused by dilution effect, whereas decrease in root Cd is due to dilution effect in plants. The reduction in shoot Cd concentrations may decrease the Cd concentrations in grains as was reported previously (Hussain et al. 2018; Rizwan et al. 2019). The addition of biochar decreased the Fe concentration in plant tissues than respective NPs treatments without biochar (Fig. 3c, d). This indicated that biochar could immobilize soil Fe as reported previously (Qiao et al. 2017). Overall, single use of Fe NPs could significantly increase the Fe concentrations in plant tissues, whereas combined application of NPs and biochar inhibit the absorption of Fe by plants which might be due to the adsorption of soil Fe with biochar amendment.

Soil plant-available Cd concentration decreased with the treatments (Table 2). These results agreed with the studies those reported a lower plant-available Cd in soils treated with biochar (Abbas et al. 2017; Qiao et al. 2017). Furthermore, the application of nZVI decreased the availability of heavy metals in calcareous or acidic soils (Gil-Diaz et al. 2017). Soil pH increased with the biochar + NPs especially when the highest level of NPs was used (Tables 2 and 3). The biochar application significantly increased the pH of acidic soil after rice cultivation for four seasons (Chen et al. 2016). The increase in pH may diminish the mobility of Cd in the soil by markedly changing the Cd speciation and bioavailability in the soil. Under various conditions, the high pH may form numerous oxidates, carbonate, or phosphate in the soil (Lu et al. 2012). Furthermore, Cd bioavailability may decrease in the soil under flooded conditions due to the precipitation of Cd with the reduced sulfur under such conditions (Rizwan et al. 2016a). Overall, NPs + biochar treatments were more effective in the immobilization of soil Cd which could weaken the Cd uptake in the soil-plant system. It still remains controversial regarding the application of NPs especially Fe NPs in agriculture aiming to decrease the concentration of Cd and probably other toxic trace elements in rice tissues because the application of NPs in agriculture has not been deeply studied. In future, the agricultural application of Fe NPs only be possible if Fe NPs are produced in a cost-effective process with minimum toxic effects to plants.

Table 2 Post-harvest soil pH and bioavailable Cd. Values are means of four replicates

NP treatments	0	10	20	30
pH				
Without biochar	8.01 ± 0.04 ^{c}	8.02 ± 0.03 ^{bc}	8.10 ± 0.06 ^{ab}	8.17 ± 0.06 ^{ab}
With biochar	8.00 ± 0.06 ^{c}	8.12 ± 0.07 ^{bc}	8.24 ± 0.09 ^{ab}	8.30 ± 0.05 ^{a}
AB-DTPA extractable Cd (mg/kg)				
Without biochar	1.10 ± 0.05 ^{a}	0.97 ± 0.03 ^{bc}	0.89 ± 0.07 ^{cd}	0.84 ± 0.02 ^{de}
With biochar	1.07 ± 0.02 ^{ab}	0.90 ± 0.06 ^{cd}	0.74 ± 0.03 ^{ef}	0.66 ± 0.04 ^{f}

For pH, there were non-significant effects for BC, NPs, and BC + NPs and for AB-DTPA extractable Cd; BC = ***; NPs = ***; BC × NPs = * where * = significant at 0.05 and *** = significant at 0.001 levels

Table 3 Pearson correlation co-efficient among the study parameters

Parameters	Shoot DW	Root DW	Shoot length	Chl a	Chl b	Pr	SC	Tr	Cd shoot	Cd root	Cd shoot uptake	Cd root uptake	Fe shoot	Fe root	Cd soil	Soil pH
Shoot DW	1															
Root DW	.728**	1														
Shoot length	.771**	.641**	1													
Chl a	.859**	.821**	.697**	1												
Chl b	.875**	.803**	.706**	.939**	1											
Pr	.843**	.796**	.704**	.931**	.955**	1										
SC	.869**	.841**	.733**	.923**	.967**	.938**	1									
Tr	.831**	.679**	.826**	.846**	.871**	.877**	.849**	1								
Cd shoot	.031	− .264	.326	− .193	− .135	− .111	− .108	.119	1							
Cd root	.099	− .198	.352*	− .117	− .038	− .020	− .030	.179	.961**	1						
Cd shoot uptake	.592**	.131	.556**	.260	.303	.281	.315	.429*	.748**	.739**	1					
Cd root uptake	.781**	.919**	.583**	.749**	.720**	.686**	.758**	.585**	− .161	− .129	.357*	1				
Fe shoot	.708**	.758**	.750**	.783**	.812**	.794**	.820**	.813**	.125	.182	.368*	.652**	1			
Fe root	.788**	.726**	.829**	.757**	.794**	.784**	.798**	.808**	.260	.346*	.528**	.667**	.905**	1		
Cd soil	.079	− .204	.367*	− .141	− .052	− .037	− .039	.183	.943**	.972**	.697**	− .157	.219	.386*	1	
Soil pH	.700**	.476**	.858**	.575**	.628**	.658**	.646**	.779**	.607**	.663**	.715**	.433*	.726**	.858**	.669**	1

Pr photosynthetic rate, *SC* stotmatal conductance, *Tr* transpiration rate

**Correlation is significant at the 0.01 level (two-tailed)

*Correlation is significant at the 0.05 level (two-tailed)

5 Conclusions

In the current experiment, different concentrations of Fe NPs were foliar applied either with biochar or without biochar in the soil to investigate their potential impacts on Cd bioavailability and its uptake by rice. The results highlighted that NP-biochar treatments increased the rice growth, photosynthesis, and simultaneously decrease the soil bioavailable Cd and its accumulation by rice. In comparison with single NPs, NPs-biochar mixtures have synergistic impacts on the Cd reduction in rice. Iron concentrations increased in the shoots and roots with NPs while less impact on Fe concentrations were observed with NPs-biochar treatments. Taken together, for treating the Cd-contaminated sites, the use of biochar and foliar Fe NPs might be a good choice to reduce Cd in crops in the future.

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