Research Article

Influence of Biochar on Yield and Heavy Metal Accumulation in Roots of *Brassica Rapa* under Groundwater and Wastewater Irrigation

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Abstract | The present study investigated the influence of wood-derived and cow manure-derived biochars on the crop growth performance and accumulation of chromium, iron, manganese and zinc in the edible parts (roots) of *Brassica rapa* (turnip) under groundwater and wastewater irrigation. Biochars were applied at 0.25, 0.5 and 1 kg m^{-2} to soil. Amendment of biochar increased significantly the aboveground plant biomass by 28% - 34.3% under groundwater and 18.3% - 30.4% under wastewater irrigation. Dry weight of root biomass increased by 16.1% - 20.2% under groundwater and by 21% - 31.2% under wastewater irrigation in response to biochar amendment. Wood-derived biochar at higher application rates and manure-derived biochar at all application rates showed significant influence on crop growth performance of *B. rapa*. Wood-derived biochar at all application rates and manure-derived biochar at higher application rate reduced significantly the concentration of iron and zinc in roots of *B. rapa*.

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Introduction

Geographically Balochistan covers the area of about 34 million hectare that account for the ~ 39% area of Pakistan (Ahmad and Islam, 2011). Despite covering larger area than the other three provinces, this province is the least populated that accounts for only 5% of the total population of Pakistan. Livestock rearing on rangelands and agriculture are the major sources of income for the local population of this province (Ahmad and Islam, 2012; Ashraf and Routray, 2013). Agriculture sector of this province depends largely on groundwater irrigation, which is continuously depleting due to drought (Steenbergen et al., 2015).

An alternative to groundwater irrigation and to overcome the threat to agricultural sector from water scarcity is the use of wastewater, which is a common practice in the developing countries (Murtaza et al., 2010). The utilization of wastewater for agricultural purpose is a means of its management as a waste

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and is also occasionally used to improve growth of plants in some part of Balochistan, Pakistan. However, wastewater irrigation of agricultural lands as a regular practice can cause accumulation of heavy metals in soil and in plant tissues (Murtaza et al., 2010). Furthermore, this kind of irrigation is also reported to have a negative influence on morphological traits of crops (Kakar et al., 2010; Achakzai et al., 2012).

Wastewater toxicity to crops can be reduced with the amendment of organic wastes such as compost and manure and pyrogenic biomass i.e. biochar (Uchimiya et al., 2010; Anwar et al., 2015; Khan et al., 2015). These amendments reduce the bioavailability of heavy metals to plants through adsorption, promoting soil aggregation and by forming their insoluble complexes in soil solution (Oleszczuk et al., 2012; Anwar et al., 2015; Khan et al., 2015). Biochar is produced by burning biomass under oxygen deficient conditions (Lehman et al., 2007; Gul et al., 2015). Due to the porous nature, biochar has high sorption capacity for nutrients, organic pollutants and heavy metals (Mohamed et al., 2015; Gul et al., 2015). Its beneficial influence on the soil physico-chemical properties and crop productivity in agricultural soils is frequently reported (Brewer and Brown, 2012; Lehman, 2015; Gul et al., 2016). This black carbonaceous material is also considered as an effective solution to the remediation of contaminated soils (Anwar et al., 2015). The amendment of biochar in agricultural soil can reduce the negative influence of wastewater effluent on crops regarding growth and accumulation of heavy metals in edible parts (Khan et al., 2015; see also Anwer et al., 2015).

The influence of biochar on soil quality, crop growth performance and reduction in the bioavailability of heavy metals to crops may depend on its properties, which largely depend on its production source (e.g. wood, manure, crop residues etc.) (Brewer and Brown, 2012; Gul et al., 2015; Gul and Whalen, 2016; Anwar et al., 2015). For example, the properties of biochars i.e. porosity, pH and concentrations of nutrients depends on their source of production (feedstock) and are generally in the order of manure > crop residue > wood (Gul et al., 2015). Therefore, influence of biochar on crop yield and concentration of heavy metals in crops under wastewater irrigation need to be evaluated with regard to the type of biochar with regard to its production source (e.g. wood, manure crop residue etc.).

The purpose of present study was to investigate the influence of wood-derived and cow-manure-derived biochars on growth performance and heavy metal accumulation in edible parts of *Brassica rapa*. Since *Brassica rapa* is a root vegetable, this study will have implications for the crop production of root vegetables, under wastewater irrigation in arid regions of Pakistan. Following hypotheses were tested for this study; 1) biochar improves crop growth performance under groundwater and wastewater irrigation, 2) biochar reduces heavy metal accumulation in edible parts (roots) of *B. rapa* irrigated with wastewater.

Materials and Methods

Study area and experimental layout

The experiment was carried out under natural light in a barren area of Mastung city Balochistan, Pakistan in 2015. Mastung is located in Quetta district and lies between 28°57′ - 30°8′ north and 66°17′ - 67°31′ east latitude (Anon, 1997). Around 50% of the local population depends on agriculture (Tahira et al., 2014). The altitude of plain area of Mastung is between 1760 - 1880 m from sea level (Masood et al., 2004). The climate of this arid region is of Mediterranean type, receiving most of the rainfall in winter and spring while summer season goes usually dry (Figure 2). Winter is cold and receives snow precipitation. The mean annual temperature and total annual precipitation in 2015 was 20.06 °C and 200.1 mm respectively (data obtained from World Weather Online https://www.worldweatheronline.com/). The soil of study area was of clay loam texture. Wastewater was obtained from Mastung city.

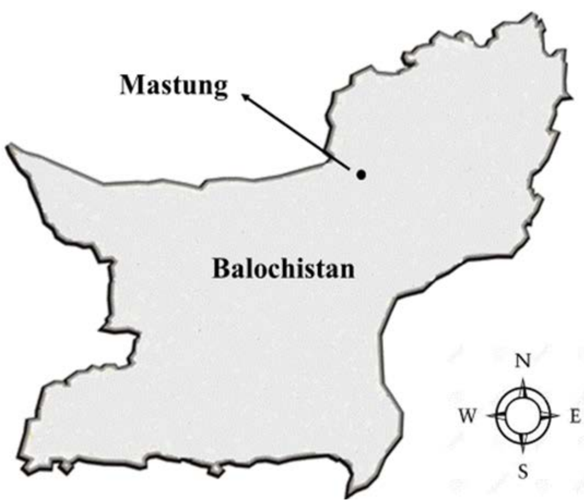

Figure 1: *Map of Balochistan indicating location of Mastung.*

Table 1: *Experiment layout and abbreviation of treatments.*

Irrigation treatment	Biochar type	Biochar application rate	Abbreviation of treatment
Groundwater	Control	0 kg m^{-2}	GW
	Wood-derived biochar	0.25 kg m^{-2} (0.5 t ha^{-1})	0.25WB GW
		0.5 kg m^{-2} (1 t ha^{-1})	0.5WB GW
		1 kg m^{-2} (2 t ha^{-1})	1WB GW
	Manure-derived biochar	0.25 kg m^{-2} (0.5 t ha^{-1})	0.25MB GW
		0.5 kg m^{-2} (1 t ha^{-1})	0.5MB GW
		1 kg m^{-2} (2 t ha^{-1})	1MB GW
Wastewater	Control	0 kg m^{-2}	WW
	Wood-derived biochar	0.25 kg m^{-2} (0.5 t ha^{-1})	0.25WB WW
		0.5 kg m^{-2} (1 t ha^{-1})	0.5WB WW
		1 kg m^{-2} (2 t ha^{-1})	1WB WW
	Manure-derived biochar	0.25 kg m^{-2} (0.5 t ha^{-1})	0.25MB WW
		0.5 kg m^{-2} (1 t ha^{-1})	0.5MB WW
		1 kg m^{-2} (2 t ha^{-1})	1MBWW

The experimental layout and abbreviations for treatments are presented in **Table 1**. The soil of study area was fine-textured. A total of 42 plots of 1m^2 size were established along parallel transect lines in open area in the first week of August. The plots were separated with 1 m buffer along transect lines. Twenty one plots were for groundwater and the rest of twenty one plots were for wastewater irrigation treatment. The plots for wastewater irrigation were established separately from the plots, which were irrigated with groundwater. A mixture of air-dried cow and sheep manure (50% each) was applied to every plot for each irrigation treatment as 0.5 kg (~1 t ha^{-1}) prior to the application of biochars. The treatments were control (no biochar added), wood-derived and cow-manure biochars each applied with 3 application rates i.e. 1 kg m^{-2}, 0.5 kg m^{-2}and 0.25 kgm^{-2}with three replications of each treatment. These application rates are equivalent to 2 t ha^{-1}, 1t ha^{-1} and 0.5t ha^{-1}respectively (Ameloot et al., 2014). The application rates were selected according to Kimetu et al. (2008) and Masto et al. (2013). After surface application of manure and biochars, they were subsequently mixed in soil up to 5 cm depth. Prior to the application of biochar, the plots of sewerage water treatments got three irrigations of sewerage water spread over three weeks. Wastewater was obtained from Mastung city. The chemical characteristics of groundwater and wastewater used in this study are provided in Table 2.

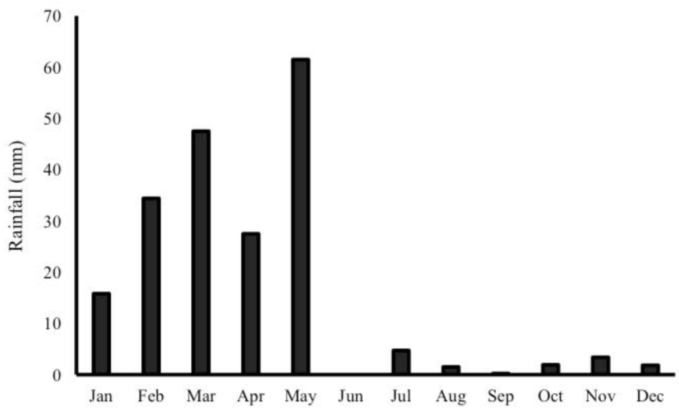

Figure 2: *Rainfall distribution during 2015.*

Table 2: *Chemical properties of groundwater, wastewater, wood-derived biochar and manure-derived biochar used in this study.*

Chemical properties	Groundwater (ppm)	Wastewater (ppm)	Wood-derived biochar mg g^{-1}	Manure-derived biochar mg g^{-1}
pH	--	--	8.73	9.92
Chromium (Cr)	0	0.139	0.002	0.040
Copper (Cu)	0	0	0	0.029
Iron (Fe)	0	9.299	1.345	15.26
Manganese (Mn)	2.261	3.666	0.107	0.771
Nickle (Ni)	0.227	0.401	0.008	0.059
Zinc (Zn)	0.875	1.209	0.030	0.171
Magnesium (Mg)	266.5	161.4	1.406	5.146

Sources and preparation of biochars

Biochars were produced from two feedstocks i.e. cow manure and wood. Cow manure was randomly selected from farms located in the Mastung City andwood biochar was prepared from the wood of apple trees. Cow manure and wood were separately heated in homemade kiln followed by extinguish of fire with water to prevent complete combustion. The biochar production temperature by this method is reported to be 350^{o}C-500^{o}C (Spokas et al., 2011; Deal et al., 2012; Mia et al., 2015).

Cultivation and harvesting of B. rapa

The *B. rapa* seeds were broadcasted in the last week

Table 3: *Concentration of heavy metals in aboveground plant biomass and in roots (mg g^{-1} plant tissue).*

Treatments	Chromium (Cr)		Iron (Fe)		Manganese (Mn)		Zinc (Zn)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	**Roots**
Control GW	0.018 ± 007	0.019 ± 0.005	1.487 ± 1.489	0.959 ± 0.227	0.772 ± 1.041	0.289 ± 0.161	0.310 ± 0.14	0.239 ± 0.055
0.25WB GW	0.017 ± 0.002	0.015 ± 0.002	1.079 ± 0.854	0.697 ± 0.077	0.917 ± 0.515	0.374 ± 0.182	0.221 ± 0.133	0.138 ± 0.055
0.5WB GW	0.013 ± 0.007	0.013 ± 0.007	1.212 ± 0.424	0.659 ± 0.776	0.689 ± 0.480	0.275 ± 0.057	0.227 ± 0.045	0.174 ± 0.031
1WB GW	0.011 ± 0.01	0.013 ± 0.003	0.321 ± 0.316	0.787 ± 0.217	0.588 ± 0.781	0.199 ± 0.157	0.094 ± 0.079*	0.138 ± 0.046
0.25MB GW	0.016 ± 0.004	0.013 ± 0.003	0.405 ± 0.128	1.041 ± 0.052	0.111 ± 0.124	0.559 ± 0.651	0.217 ± 0.061	0.164 ± 0.052
0.5MB GW	0.015 ± 0.002	0.010 ± 0.005*	0.623 ± 0.258	1.346 ± 0.186	1.007 ± 0.480	0.331 ± 0.155	0.300 ± 0.022	0.127 ± 0.047
1MB GW	0.010 ± 0.004	0.008 ± 0.008*	1.145 ± 0.493	0.626 ± 0.342	0.902 ± 0.668	0.362 ± 0.059	0.183 ± 0.093	0.091 ± 0.049
Control WW	0.020 ± 0.003	0.02 ± 0.006	2.029 ± 0.751	1.843 ± 0.434	1.553 ± 0.659	0.753 ± 0.399	0.301 ± 0.114	0.457 ± 0.475
0.25WB WW	0.016 ± 0.006	0.018 ± 0.005	2.289 ± 1.702	1.581 ± 0.458*	0.889 ± 0.264	0.854 ± 0.459	0.21 ± 0.124	0.175 ± 0.060*
0.5WB WW	0.014 ± 0.005	0.014 ± 0.002	2.747 ± 0.876	0.926 ± 0.066*	0.799 ± 0.399	0.281 ± 0.147	0.293 ± 0.155	0.157 ± 0.016*
1WB WW	0.015 ± 0.006	0.013 ± 0.005	0.987 ± 0.057	0.602 ± 0.209*	0.681 ± 0.442	0.261 ± 0.095	0.182 ± 0.033	0.108 ± 0.067*
0.25MB WW	0.016± 0.002	0.017 ± 0.008	2.138 ± 0.659	1.526 ± 0.587	0.949 ± 0.207	0.494 ± 0.267	0.359 ± 0.203	0.263 ± 0.141
0.5MB WW	0.015 ± 0.005	0.016 ± 0.005	1.639 ± 1.169	1.71 ± 0.326	0.87 ± 0.421	0.805 ± 0.421	0.262 ± 0.094	0.246 ± 0.151
1MB WW	0.013 ± 0.005	0.014 ± 0.002	2.217 ± 1.153	0.282 ± 0.053*	0.525 ± 0.253*	0.259 ± 0.181	0.233 ± 0.050	0.156 ± 0.034*

Values are averages ± SD (n:3; pool pf five plants per plot and there are three plots per treatment). Values in bold within column followed by asterisk are significantly different than control treatment at P≤0.05.

of August over all the plots. After two weeks of seed germination, seedlings were thinned to five seedlings per plot. Wastewater irrigation was carried out after two weeks of germination, and once in a week afterwards until harvest. Harvesting was carried out in the last week of October 2015. Aboveground biomass was harvested, roots were removed from soil with spade, washed and roots and aboveground biomass were oven-dried at 40°C for 48 hours, weighed and crushed to fine homogenous powder for chemical analysis.

Chemical analysis

Plant and manure-derived biochar extracts were prepared by kjeldahl method as described in Estifan et al. (2013). The digest for wood-derived biochar was obtained by dry ash digestion (e.g. Rechcigl, Payne 1989). The digests of plant tissues and biochars were analyzed for concentration of various elements by using flame atomic absorption spectrophotometer (AA 7000 Shimadzu). For assessment of pH of biochars, the finely-ground biochar was dissolved in water as 1:10 w/v ratio for 18 hours followed by pH measurement (Ameloot et al. 2013).

Statistical analysis

Data were subjected to normal distribution assessment by D'Agostino-Pearson K^2 test available in the statistical software Costat (version 6.45). Difference between treatments was analyzed by analysis of variance (ANOVA) test and the differences between treatment means were measured with Least Significance Difference (LSD) test. Analysis was carried out by using CoStat software (version 6.45).

Figure 3: *Two weeks old plants receiving groundwater irrigation (A), ten days old plants grown receiving wastewater irrigation (B), one month old plants receiving groundwater irrigation (C), one month old plants receiving wastewater irrigation (D), fresh turnip from soil amended with 1 kg manure derived-biochar under wastewater irrigation (E), fresh turnip from soil amended with 1 kg wood-derived biochar under wastewater irrigation (F).*

Results and Discussion

Aboveground plant biomass

Biochar application in soil positively influenced the aboveground biomass of plants under both wastewater and groundwater irrigation (Figure 3, Figure 4). Except for the treatment of 0.25 kg m^{-2} wood-derived biochar amended in groundwater treated soil, all the other treatments showed significant increase in aboveground plant biomass ($P<0.05$; n = 15).

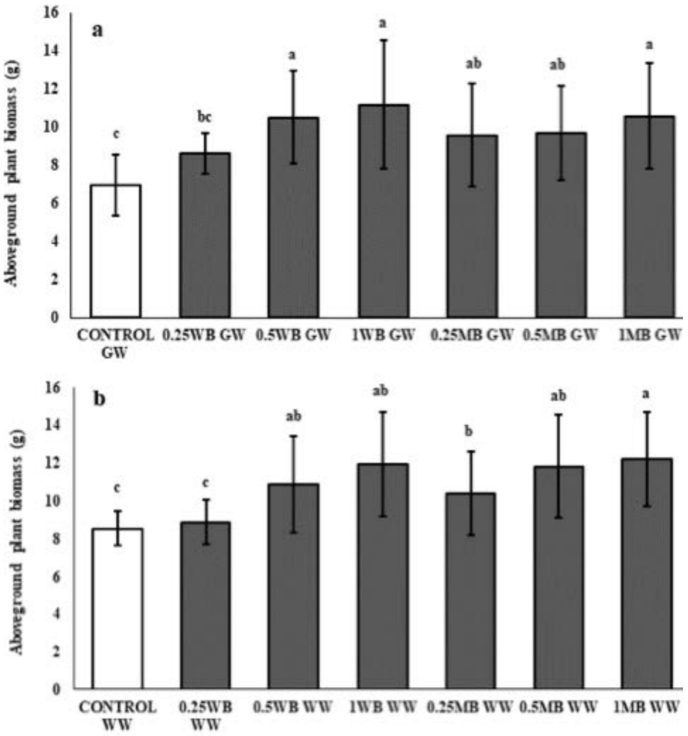

Figure 4: *Mean ± SD of aboveground plant biomass (n = 15; five plants per plot) of B. rapa grown under groundwater (a) and wastewater (b) treatments. Bars with different letters represent significant difference at P<0.05 (n = 15; 5 plants per plot). GW-groundwater, WW-wastewater, WB-wood-derived biochar, MB-manure-derived biochar, 0.25-application rate of biochar as 0.25 kg m^{-2}, 0.5-application rate of biochar as 0.5 kg m^{-2}, 1-application rate of biochar as 1 kg m^{-2}.*

Application of biochar positively influenced root biomass (Figure 3). Wood-derived biochar at lower application rate i.e. 0.25 kg m^{-2} and 0.5 kg m^{-2} did not increase the dry weight of *B. rapa* roots in both groundwater and wastewater treatments (Figure 4). Wood-derived biochar at higher application rate i.e. 1 kg m^{-2} increased root dry weight in both groundwater and wastewater treatments ($P<0.05$). Manure-derived biochar at lower application rate i.e. 0.25 kg m^{-2} did not increase the dry root weight in groundwater treatment soil while for wastewater treatment, manure-derived biochar significantly increased the dry weight of roots (Figure 5; $P<0.05$).

Heavy metal accumulation

Wood-derived biochar at higher application rates i.e. 1 kg m^{-2} and manure-derived biochar at all application rates reduced significantly the concentration of iron and zinc in roots of *B. rapa* grown in wastewater treatment soil (Table 2; $P<0.05$). Although not significantly, the concentration of heavy metals was higher in root and leaf tissues of *B. rapa* grown in wastewater as compared to groundwater treatments (Table 2).

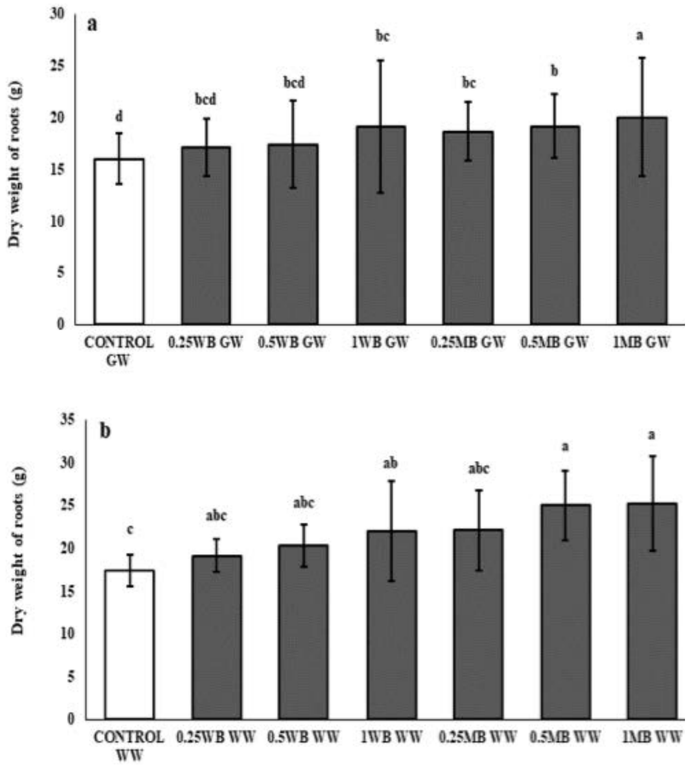

Figure 5: *Mean ± SD of dry weight of B. rapa roots (n = 15; five plants per plot) grown under groundwater (a) and wastewater (b) treatments. Bars with different letters represent significant difference at P<0.05 (n = 15; 5 plants per plot). GW-groundwater, WW-wastewater, WB-wood-derived biochar, MB-manure-derived biochar, 0.25-application rate of biochar as 0.25 kg m^{-2}, 0.5-application rate of biochar as 0.5 kg m^{-2}, 1-application rate of biochar as 1 kg m^{-2}.*

Under field conditions, both biochar types showed positive response for the growth performance of *B. rapa* in terms aboveground plant biomass and dry weight of root biomass under groundwater and wastewater irrigation treatments. The dry weight of roots improved from 16.1% - 31.2% by amendment of biochars. The effect was significant at higher application rates for wood-derived biochar (i.e. 0.5 kg m^{-2} and 1 kg m^{-2} amendment rate) and at all application rates for manure-derived biochar in both irrigation treatments. This finding indicates that the influence of manure-derived biochar on crop growth performance was more positive than wood-derived biochar. As manure-derived biochars have ≥ 5 times greater nutrient contents than wood-derived biochars, therefore, they cause more profound positive influence on crop growth performance than wood-derived biochars (Gul et al., 2015; Gul and Whalen, 2016).

Dry weight of root biomass

Wastewater did not increase crop growth performance than groundwater treatment. This finding is not consistent with the previous reports in this regard (Singh et al., 2012; Zema et al., 2012; Lal et al., 2015), indicating that this domestic wastewater does not improve crop growth performance under field conditions. However, the amendment of biochar in soil under wastewater irrigation improved crop growth performance greater than its amendment in soil irrigated with ground water. For instance, wood-derived biochar applied at 1kg m^{-2} and manure-derived biochar applied at 0.25 kg m-2, 0.5 kg m^{-2} and 1 kg m^{-2} rates increased dry weight of roots by 21%, 18.1%, 26% and 24% respectively than their amendment in soils irrigated with groundwater. This suggests that biochar application improves crop yield under wastewater treatment. Our findings are in agreement with the previous reports demonstrating the positive influence of biochar on crop growth performance irrigated with wastewater (Gwenzi et al., 2016).

Wastewater irrigation did not cause significant increase in heavy metal accumulation in root tissues of *B. rapa*. In general, wastewater contains high concentration of heavy metals than conventional water and cause high metal accumulation in the edible parts of crops (Murtaza et al., 2010; Singh et al., 2012; Lal et al., 2015). In this study, we applied the mixture of air-dried cow and sheep manure as 0.5 kg m-2 rate to soil prior to sowing of *B. rapa* seeds. Organic amendments insolubilize heavy metals and other pollutants and therefore reduce their bioavailability to plants (Murtaza et al., 2010; Gul et al., 2014; Khan et al., 2015). The amendment of air-dried manure to soil may cause the non-significant increase in heavy metal accumulation in roots of *B. rapa* suggesting that the toxicity of wastewater to food chain regarding heavy metal accumulation in edible parts of plants can be reduced by organic amendments (see also Murtaza et al., 2010; Khan et al., 2015). Amendment of wood-derived biochar at all application rates and manure biochar at high application rate (i.e. 1 kg m^{-2}) to soil irrigated with wastewater reduced significantly the concentration of Fe and Zn in roots than control but the concentration was not lower than the groundwater control treatment. This suggests that biochar greatly improved the quality of roots of *B. rapa* irrigated with wastewater. Our findings are consistent with previous reports that biochar reduces accumulation of heavy metal in edible parts of crops irrigated with wastewater and thus is a good agricultural management practice (Khan et al., 2015; see also Anwer et al., 2015).

Conclusions

In conclusion, application of biochar improved growth performance of *B. rapa* under both irrigation treatments and reduced the accumulation of Fe and Zn in root tissues under wastewater irrigation. The heavy metal accumulation in roots of *B. rapa* was not significantly higher under wastewater irrigation treatment. Our finding suggest that organic amendments i.e. manure and biochar to agricultural lands irrigated with wastewater have potential to improve crop growth performance and reduce heavy metal accumulation in the edible parts of plant tissues, making the use of wastewater appropriate for agricultural purpose. Using manure and wood as source of biochar for agronomic purpose in Pakistan can reduce the need for inorganic fertilizer and can be a viable means for waste management of manure.

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Author's Contribution

Feroza Haider: Conducted research in field and laboratory.
Shamim Gul: Supervised research work and data analysis and reviewed manuscript
Javaid Hussain, Sadaf Asalam Ghori, Muhammad Naeem Shahwani, Muhammad Murad and Abdul Manan Kakar: Provided laboratory facility and assistance in the chemical analysis of plant tissues, water and biochar samples.

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