Biochar influences growth performance and heavy metal accumulation in spinach under wastewater irrigation

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Abstract: This pot-based study investigated the influence of cow-manure-derived slow pyrolyzed biochar on the growth performance and accumulation of Nickel (Ni), Zinc (Zn), Copper (Cu) and Iron (Fe) in the aboveground plant biomass of spinach (Spinacia oleracea L.) grown under wastewater and groundwater irrigation. Biochar was applied in soil with or without manure (mixture of dung of cow and sheep/goat) at 3, 5 and 10% rates. Biochar application at 5% and when co-applied with manure at all application rates increased aboveground plant biomass under wastewater and groundwater irrigation. Application of biochar at 5 and 10% rates and when co-amended with manure at all application rates increased the leaf area index under wastewater irrigation but caused no influence under groundwater irrigation. Biochar amendment at 5% rate reduced while at 10% rate and when co-applied with manure at 3 and 5% application rates increased root biomass under wastewater irrigation.

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irrigation. Under groundwater irrigation, amendment of biochar at 10% and when co-applied with manure at 3 and 10% increased root biomass. Under wastewater irrigation, application of biochar tended to reduce the concentration of Ni when co-amended with manure while increased the concentration of Cu in aboveground plant tissues at 3 and 5% rates and when co-applied with manure at 10% rate. In conclusion, manure-derived biochar increased crop growth performance and influenced accumulation of heavy metals in aboveground plant tissues under wastewater and groundwater irrigation. Application of manure with biochar reduced concentration of Ni under wastewater irrigation and enhanced crop growth performance under groundwater irrigation.

Subjects: Agriculture & Environmental Sciences; Agriculture; Environmental Sciences; Agriculture and Food

Keywords: Spinach; manure-derived biochar; municipal wastewater irrigation; heavy metal accumulation; plant growth performance

1. Introduction
Irrigation of agricultural lands with municipal wastewater is a common practice in developing countries and the treated wastewater irrigation has also been practiced in advance countries such as France, USA and Spain (Murtaza et al., 2010; literature review by Becerra-Castro et al., 2015). Wastewater irrigation is not only an option to save surface and groundwater for human consumption, it is also a means to utilize wastewater as a renewable resource than its improper disposal, which can cause serious environmental and human health issues (Almuktar & Scholz, 2015; Christou, Karaoli, Hapeshi, Michael, & Fatta-Kassinos, 2017; Gatta et al., 2016; Kiziloglu, Turan, Sahin, Kuslu, & Dursun, 2008). Besides this, wastewater contains nutrients in high concentration and therefore is a cost-efficient alternative to inorganic fertilizers (Murtaza et al., 2010). In particular, the agricultural lands of arid and semi-arid regions, where groundwater does not fulfill both the human consumption and high agronomic demand, wastewater irrigation is commonly practiced to overcome this problem (Becerra-Castro et al., 2015; Gatta et al., 2016).

Despite the potential regarding cost-efficient benefits to agronomic sector especially in arid regions, wastewater irrigation influences negatively the plant growth performance and causes high accumulation of heavy metals in the edible parts of crops, thus poses a threat to human health through food chain (Elgallal, Fletcher, & Evans, 2016; Murtaza et al., 2010). The growth performance of plants can be improved and high concentration of heavy metals in plants due to wastewater irrigation can be reduced via amendment of organic fertilizers such as manure and compost or biochar in agricultural soil (Khan et al., 2015; Murtaza et al., 2010; Xu et al., 2016; Zhang et al., 2016). Manure and biochar have high adsorption capacity; moreover, manure forms insoluble metal complexes, which reduces the bioavailability of heavy metals in soil solution thus reduces their accumulation in plant tissues (Murtaza et al., 2010; Xu et al., 2016).

Biochar is a porous pyrogenous material that is produced by burning biomass under oxygen-deficient conditions (Gul, Whalen, Thomas, Sachdeva, & Deng, 2015). The use of biochar in agricultural lands is frequently reported to have a positive influence on crop growth performance and soil quality (Gul & Whalen, 2016; Gul et al., 2015). Due to its high porosity, it reduces soil bulk density and increases its water holding capacity (Gul et al., 2015). Its amendment in soil improves the water use efficiency (WUE) of plants, which can improve plant growth performance especially in arid and semi-arid regions (Uzoma et al., 2011).

Pakistan is an agrarian country where 66% population depends on agriculture for livelihood and agriculture accounts for 22% GDP of the country (Government of Pakistan, [GOP], 2015). In warm regions, most of the rainfall occurs in spring and summer seasons, which reduce the need of
irrigation of agricultural lands during that period of the year. However, cold regions in the west of Pakistan are arid and semi-arid and have Mediterranean-type climate that receive most of the rain-fall in winter and early spring seasons while summer and autumn seasons usually go dry. Therefore, irrigation with ground or surface water is a limiting factor in agricultural sector. To combat with this factor, municipal wastewater irrigation is a cost-efficient option in these regions while the risk of high accumulation of heavy metals in crops through wastewater irrigation can be reduced by applying manure and biochar in soil (Murtaza et al., 2010).

Balochistan is the province of Pakistan where arid to semi-arid climate prevails. Agricultural lands depend on ground and surface water irrigation. Agriculture in urban areas such as Quetta city depend on municipal wastewater irrigation. Vegetables such as cabbage, tomato, spinach are the most-grown in the agricultural lands of Quetta city. Leafy vegetables such as spinach grow well and accumulate more heavy metals than other vegetables under wastewater irrigation (Al-Jassir, Shaker, & Khaliq, 2005; references in Ahmad, Bakhsh, & Hassan, 2006). Research about evaluating the influence of manure, biochar and co-application of biochar and manure on growth performance and accumulation of heavy metals in the edible parts of leafy vegetables under wastewater irrigation is important for future agronomic purpose. This study aims to investigate the influence of cow manure-derived biochar, manure (mixture of dung of cow and sheep/goat) and the mixture of manure and biochar on the growth performance (i.e. biomass production, leaf area index and water use efficiency) and heavy metal accumulation in the leaves of Spinach irrigated with municipal wastewater.

2. Materials and methods

2.1. Manure and biochar

Fresh (not composted) cow manure was collected from various dairy farms in Quetta city while fresh sheep/goat manure was obtained from local market of Quetta. Manure was air-dried and mixed as ~70% cow-manure and ~30% sheep/goat manure. The biochar of cow manure was made by its burning in anoxic conditions in traditional kilns, followed by extinguish of fire with water to prevent complete combustion. Since the manure-derived biochar was made in kiln, where the temperature ranges between 350–550 °C, this type of biochar is known as slow-pyrolyzed biochar (Deal, Brewer, Brown, Okure, & Amoding, 2012; Mia et al., 2015; Spokas et al., 2011). The chemical characteristics of biochar are presented in Table 1.

| Element | Groundwater (ppm) | Wastewater (ppm) | Biochar (mg g⁻¹) |
|---------|-------------------|------------------|-----------------|
| pH      | -                 | -                | 9.72            |
| Cu      | 0                  | 0                | 0.057           |
| Fe      | 0                  | 3.582            | 8.005           |
| Mn      | 2.105              | 3.779            | 0.041           |
| Ni      | 0.487              | 0.331            | 0.039           |
| Zn      | 0.730              | 4.88             | 0.323           |
| Cr      | 0                  | 0.138            | 0.024           |
| Mg      | 87.5               | 111.7            | 7.756           |
2.2. Experimental design and growth of plants

Sandy-textured soil was air-dried, crushed with hands and passed through 2 mm mesh sieve. Plastic pots of 10 cm diameter, 12 cm height with holes in the bottom were used in this study. Pots were prepared in a completely randomized design where there were three factors; type of irrigation, organic amendment (biochar, manure or their mixture) and application rates of organic amendments. The treatments were control (without biochar but with 2% w/w manure to prevent nutrient limitation to plants), amendment of biochar at 3, 5 and 10% applications rates in soil and co-amendment of biochar at 3, 5 and 10% rates in soil with manure as 2% amendment. The amendment of manure as 2% in other treatments along with control, was carried out to prevent nutrient limitation; moreover, biochar influence more positively when it is co-amended with organic fertilizers such as compost and manure (Gul & Whalen, 2016). All the treatments were replicated six times and the total experimental units were 84. Six replications were used for crop growth performance parameters and three replications (out of six) were used for the analysis of heavy metals. The experimental design and abbreviations of treatments is provided in Table 2.

Pots were filled to saturation prior to sowing of seeds to provide a soft and moist bed for seeds to germinate and establish. Seeds of spinach were obtained from local market of Quetta city. Seeds were broadcast on the top of soil in pots. After ten days of germination, pots were thinned to five plants per pot and were irrigated with a given water type (groundwater or wastewater depending on treatment) to ~58–63% water filled pore space (WFPS) at alternate days. Pots were weighed once in a week to adjust the water contents of pots to desirable WFPS (~60%). The water required to get desired WFPS was calculated based on equation described in Gul and Whalen (2013) as;

\[ \text{Soil porosity} = 1 - \frac{\text{BD}}{\text{PD}} \]  

(1)

where BD is bulk density and PD is particle density, and WFPS was calculated as;

\[ \text{WFPS} = \text{Soil porosity} \times P \times V \]  

(2)

where \( P \) is desired WFPS and \( V \) is the volume of soil in pot.

The municipal wastewater was obtained from Spini road, Quetta city. This wastewater is from sewage sludge from houses and hospitals whereas there is no industrial zone in that area.

| Irrigation treatment | Biochar type | Biochar application rate (%) | Abbreviation of treatment |
|----------------------|--------------|------------------------------|---------------------------|
| Swage water          | Control      | 0                            | Control SW                |
|                      | Biochar without manure amendment | 3 | B3SW |
|                      |              | 5 | B5SW |
|                      |              | 10 | B10SW |
|                      | Biochar with manure amendment | 3 | B3MSW |
|                      |              | 5 | B5MSW |
|                      |              | 10 | B10MSW |
| Groundwater          | Control      | 0                            | Control GW                |
|                      | Biochar without manure amendment | 3 | B3GW |
|                      |              | 5 | B5GW |
|                      |              | 10 | B10GW |
|                      | Biochar with manure amendment | 3 | B3MGW |
|                      |              | 5 | B5MGW |
|                      |              | 10 | B10MGW |
Groundwater was obtained from Brewery Road, Quetta city. The chemical properties of wastewater and groundwater are presented in Table 1.

2.3. Assessment of water use efficiency (WUE)
The WUE was calculated as described in Uzoma et al. (2011);

\[
WUE = \frac{\text{plant biomass}}{\text{Total water added into pot}}
\]  

(3)

2.4. Assessment of leaf area index
After two months of plant growth, three plants from each pot and three leaves of each selected plant were subjected to leaf area index assessment. The apparently largest leaf was selected first followed by the two nearest leaves in clockwise direction.

2.5. Assessment of plant biomass and rhizosphere soil
After two months of growth, aboveground plant biomass was harvested and oven-dried at 40°C for 48 h. Pots were cut in two opposite sides and the soil was carefully removed. Roots were carefully isolated from bulk soil. Roots were shaken to remove bulk soil and the soil remained on roots was considered as rhizosphere soil (Gul & Whalen, 2013). Roots with adhered soil were placed in 10 ml water containing petri plates soil was isolated into petri plates. Petri plates and the roots were allowed to air dry followed by oven-drying at 40°C for 48 h. Aboveground plant biomass, rhizosphere soil and root biomass were weighed after oven drying.

2.6. Assessment of heavy metals in leaves
Three randomly selected pots of each treatment were subjected for this purpose. A 0.5 g of powered plant tissue was dissolved in 5 ml of 60% concentrated Nitric Acid (HNO₃) for 6 h followed by the addition of 10 ml di-acid (mixture of 60% concentrated HNO₃ and 37% concentrated HClO₄ in 9:4 ratio), and heated on hot plate till the formation of transparent remnant at the base of flask. Digested sample was diluted with 10 ml of distil water, filtered and an analyzed under atomic absorption spectrometer, AA-700 (shimaszu). Range of dilutions prepare from the standard stock solution. Digested and diluted sample compared against standard’s stock dilutions.

2.7. Statistical analysis
All data were subjected to normal distribution assessment by D’Agostino-Pearson K². The normally distributed data was subjected to the analysis of variance (ANOVA) in a one way completely randomised design followed by least significance difference (LSD) test. The non-parametric data (data of heavy metal accumulation) were subjected to Kruskal-Wallis test. Data was analyzed with CoStat software.

3. Results and discussion

3.1. Water use efficiency
The plants grown under wastewater irrigation showed greater WUE than groundwater irrigation \((P \leq 0.05; \text{Figure 1})\). Application of biochar at 5% amendment when applied without manure under wastewater irrigation and when applied with manure at 3% amendment rate under groundwater irrigation significantly enhanced the WUE of plants than control treatments \((P \leq 0.05; \text{Figure 1})\).

Wastewater irrigation significantly improved WUE by 63%, leaf area index by 80% and aboveground plant biomass (crop yield) by 44.5% as compared to groundwater irrigation, indicating that wastewater is an attractive cost-efficient alternative to inorganic fertilizer and groundwater for the growth of spinach. Our findings are in agreement with other empirical evidences in this regard (Gatta et al., 2016; Guidi Nissim, Jerbi, Lafleur, Fluet, & Labrecque, 2015; Mojid, Biswas, & Wyseure, 2012).
3.2. Leaf area index

Plants grown under wastewater irrigation showed higher leaf area index than the plants under groundwater irrigation ($P \leq 0.05$; Figure 1). Application of biochar without manure at 5% and 10% and with manure at all amendment rates significantly increased leaf area index than control treatment under wastewater irrigation. Under groundwater irrigation, application of biochar without...
manure at only 3% amendment rate significantly increased leaf area index than control treatment ($P \leq 0.05$; Figure 1).

Under wastewater irrigation, biochar amendment at higher application rates (i.e. 5 and 10%) and at all application rates, when co-amended with manure, enhanced leaf area index by 30.4 to 47%. Spinach is a leafy vegetable. Positive influence of biochar especially when co-applied with manure in increasing the leaf area index under wastewater irrigation provide an insight to the local farmer about the potential of this organic resource in increasing the market value of spinach.

3.3. Aboveground plant biomass

Plants grown under wastewater irrigation showed higher aboveground plant biomass than the plants grown under groundwater irrigation ($P \leq 0.05$; Figure 1). Under wastewater irrigation, application of biochar without manure increased significantly the aboveground plant biomass at 5% amendment rate while when applied with manure increased the aboveground plant biomass at all amendment rates than control treatment ($P \leq 0.05$; Figure 1). Under groundwater irrigation, application of biochar without manure increased aboveground plant biomass at 3% and 5% amendment rates while when applied with manure, increased aboveground plant biomass at all application rates than control treatment ($P \leq 0.05$; Figure 1).

Under wastewater irrigation, at 5% amendment rate, under groundwater irrigation at 3 and 5% amendment rates and at all amendment rates when co-applied with manure under both irrigation treatments, enhanced significantly the aboveground plant biomass by 19–39%. It indicates that co-application of biochar with manure enhances plant growth performance at both irrigation treatments. Our findings are consistent with other reports, demonstrating that biochar perform better when it is co-applied with organic fertilizers in soil (Alburquerque et al., 2013; Doan, Henry-des-Tureaux, Rumpel, Janeau, & Jouquet, 2015; Schulz, Dunst, & Glaser, 2013; Schulz & Glaser, 2012).

3.4. Root biomass

Under wastewater irrigation, the application of biochar without manure at 10% rate significantly increased root biomass than control ($P \leq 0.05$; Figure 2). Under groundwater irrigation, application of biochar without manure at 5% amendment reduced while at 10% amendment increased root biomass than control ($P \leq 0.05$; Figure 2). When applied with manure, application of biochar increased root biomass at 3% and 10% amendment rates than control treatment under groundwater irrigation ($P \leq 0.05$; Figure 2).

Biochar also influenced positively the root growth of plants. Under wastewater irrigation, amendment of biochar 10% application rate rate significantly increased root biomass by 13%. Under groundwater irrigation, biochar at 10% application rate and when co-applied with manure at 3 and 10% rates increased root biomass by 20–28%. This indicates that under surface or groundwater irrigation the co-amendment of biochar with other organic fertilizers have greater positive influence on plant growth performance than when it is amended without organic fertilizers (Gul & Whalen, 2016).

3.5. Rhizosphere soil accumulation

Soil was significantly higher in the rhizosphere of roots of plants of wastewater control treatment than groundwater control treatment ($P \leq 0.05$). Biochar did not increase accumulation of soil in the rhizosphere of roots under wastewater irrigation (Figure 2). Under groundwater irrigation, application of biochar with manure at all application rates significantly increased soil accumulation in the rhizosphere of roots ($P \leq 0.05$).

Under groundwater irrigation the co-amendment of biochar with manure at all application rates increased significantly the accumulation of soil in rhizosphere by 48–57%. Moreover the root:rhizosphere soil ratio was 64% lower for the plants of wastewater control treatment as compared to groundwater control treatment, indicating higher rhizodeposits in the roots of plants grown
under wastewater irrigation as compared to groundwater irrigation. Rhizosphere is the zone under influence of roots (Kuzyakov, 2010). The amount of soil accumulated per unit weight of roots indicate rhizodeposits of roots and microorganisms (Haichar, Santaella, Heulin, & Achouak, 2014). Rhizodeposits determine plant defence, nutrient cycling, microbial-mediated reduction in toxicity of heavy metals to plants (Dessaux, Grandclément, & Faure, 2016) and help increase soil quality in terms of soil aggregation and nutrient retention (Haichar et al., 2014). The role of wastewater in influencing rhizodeposits and associated soil quality improvement, crop growth performance and microbial-mediated attenuation of heavy metal toxicity to crops merit further research.
3.6. Concentration of heavy metals in leaves

The concentration of Ni was significantly higher in the aboveground plant tissues of wastewater control treatment as compared to groundwater control treatment ($P \leq 0.05$; Figure 3). Application of biochar with manure significantly reduced the concentration of Ni in plant tissues under wastewater irrigation ($P \leq 0.05$). Concentration of Zn and Fe was not significantly different across treatments (Figure 3). Concentration of Cu was not different in the plant tissues of wastewater control treatment than groundwater control treatment while amendment of biochar without manure at 3 and 5% and with manure at 10% application rates increased concentration significantly than control under wastewater irrigation ($P \leq 0.05$; Figure 3).

Although wastewater irrigation reduces the need of freshwater irrigation; however, wastewater tend to increase the concentration of heavy metals in the edible parts of crops (Khan et al., 2015; Murtaza et al., 2010), which in return pose serious health concerns in humans by causing high accumulation of these heavy metals in the human body through food chain (Murtaza et al. (2010). Organic amendments reduces the bioavailability of heavy metals in soil solution thus help reduce heavy metal accumulation in plant tissues under wastewater irrigation (Khan et al., 2015; Murtaza et al., 2010; Xu et al., 2016). Our results show that amendment of biochar tended to reduce the concentration of Ni especially when co-amended with manure under wastewater irrigation. Our findings are in agreement with published reports that amendment of biochar tend to reduce concentration of heavy metals (Xu et al., 2016). The reduction in accumulation of heavy metals in response to biochar amendment with or without co-amendment of manure was observed only for Ni, while concentration of Zn and Fe was not influenced. There are number of published reports that reveal no influence of biochar amendment on heavy metal accumulation in the edible parts of crops (Lucchini, Quilliam, DeLuca, Vamerali, & Jones, 2014; Nigussie, Kissi, Misganaw, & Ambaw, 2012; Wagner & Kaupenjohann, 2013). For example, applying Concarpus-derived biochar in soil as 1, 3 and 5%, did not reduced the concentration of Fe and cadmium (Cd) while reduced the concentration of Zn, manganese (Mn) and Cu in the maize shoots (Al-Wabel et al., 2015). In our study, biochar amendment at 3% and 5% application rates and when co-amended with manure at 10% application rate significantly increased accumulation of Cu in leaves. Song, Xue, Chen, He, and Dai (2014) also found that concentration of Zn was 33% higher in the tomato fruits grown in soil amended with sewage sludge-derived biochar as compared to control. It merits further research under field conditions, to evaluate such an effect of biochar and biochar-manure mixture in vegetables under wastewater irrigation.

Figure 3. Influence of amendment of biochar and biochar + manure on the accumulation of Ni, Zn, Cu and Fe in the aboveground plant tissues of spinach grown under wastewater and groundwater irrigation. Kruskal-Wallis test revealed significant difference between treatments for Ni and Cu.

Note: Bars with different letters represent significant differences at $p < 0.05$ ($n = 3$).
4. Conclusions

Wastewater irrigation had positive influence of the growth performance of spinach while application of cow manure-derived biochar especially when co-applied with manure further enhanced the growth performance of this leafy vegetable regarding biomass production. Biochar also increased plant biomass and accumulation of soil in the rhizosphere (an indication of more rhizodeposits) under groundwater irrigation and the positive influence was more profound when biochar was co-applied with manure. Co-application of biochar with manure also reduced significantly the concentration of Ni in leaves of spinach under wastewater irrigation. Our findings suggest that the positive influence of biochar on growth performance of spinach and reduction in the concentration of Ni in leaves can be enhanced when it is co-applied with manure in soil. Our results provide an avenue to the local farmers of arid and semi-arid regions, where wastewater irrigation is a common practice, to use biochar-manure mixture for improving the growth performance of leafy vegetables. The use of manure for agronomic purpose as organic fertilizer and biochar production can help use manure as a renewable resource than its waste. Further research is required to assess under wastewater irrigation, the influence of co-amendment of cow-manure-derived biochar with organic fertilizer on the yield and heavy metal accumulation in other vegetables under field conditions in arid environment.

Funding
The funding for this research was from Sardar Bahadur Khan Women’s University and Government of Balochistan under Balochistan Educational Endowment Fund program.

Competing interest
The authors declare no competing interest.

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Citation information
Cite this article as: Biochar influences growth performance and heavy metal accumulation in spinach under wastewater irrigation, Saniya Tahir, Shamim Gul, Sadaf Aslam Ghoir, Meenah Sahai, Saira Bato, Nelofer Jamal, Muhammad Naeem Shahwani & Mujeeb ur Rehman Butt, Cogent Food & Agriculture (2018), 4: 1467253.

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