Research Article

Influence of Biochar based Organic Fertilizers on Growth and Concentration of Heavy Metals in Tomato and Lettuce in Chromite Mine Tailings Contaminated Soil

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Abstract | Chromite mining activities in Balochistan are causing serious heavy metal contamination in agricultural soils. Due to high adsorption capacity of biochar, its amendment in heavy metal-contaminated soils is known for reducing soil toxicity to crops. This pot-based study investigated influences of wood-derived biochar and farmyard manure-derived (cow dung-derived) biochar as mixtures with air-dried poultry manure on the growth and concentration of heavy metals in edible tissues of tomato and lettuce. These biochars were separately mixed with poultry manure as 1:1 ratio on air-dry weight bases. Soil was contaminated with chromite mine tailing debris at 2% amendment rate (980 g soil + 20 g debris). Biochar-poultry manure mixtures were applied in soil at 10% and 20% amendment rates (respectively, 900 g soil + 100 g mixture and 800 g soil and 200 g mixture of poultry manure and biochar in 1:1 mixture ratio). After harvest of tomato, lettuce was grown in the same soil. As compared to control, amendments of biochar-poultry manure mixtures had a significantly positive influence on growth of both crops. There was ~ 254.49% - 871.9% (~3.5 – 9 fold) increase in aboveground biomass of tomato and 25.67% – 94.59% increase in aboveground plant biomass of lettuce. These fertilizers also increased significantly water use efficiency by ~3.5 – 12.5 times (364.8% - 1166.6%) of tomato plants (as only tomato plants were analyzed for WUE). Furthermore, these amendments reduced concentration of heavy metals in plants of both crops. As compared to contaminated control treatment, amendment of biochar-poultry manure mixtures increased the concentration of soluble mineral phosphorus by 288% - 914% in soil. Our study suggests that amendment of wood-derived biochar and cow manure-derived biochar as mixtures with poultry manure, significantly improved growth of both crops and increased concentration of bioavailable phosphorus in soil.

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Introduction

Mining activities are hazardous to human health and environment because of production of radon gas and particulate dispersion of metal (oids) in air, soil and water (Gil-Loaiza *et al*., 2016; Ahmad *et al*., 2019). High concentrations of heavy metals in soil also become part of food chain via crops and livestock (Gregori *et al*., 2003; Limie *et al*., 2008; Gramss and Voigt, 2014). These activities cause contamination of air, soils and water from high distances. Limie *et al*. (2008) reported that agricultural lands in Chenzhou City, Southern China, at distances from mining activities as long as 23km to 63km, had concentrations of cadmium many times higher than its critical concentration in soil, vegetables and rice. The province Balochistan, Pakistan is well-known for various mineral reserves such as chromite, fluoride, copper and coal. Chromite has been extensively excavated from Zhob valley and Muslim Bagh since 1903 (Atiq *et al*., 2005; Kakar *et al*., 2013). Due to mining activities, water and soils of agricultural lands of this region have been heavily contaminated with trace metals (Geological Survey of Pakistan unpublished data; Umer *et al*., 2012).

Biochar and other biowastes (e.g. compost, saw dust and paper mill wastes etc.) as soil amendments are found to be useful in phytostabilization of mine tailings (Fellet *et al*., 2011; Paktunc, 2013; Anawar *et al*., 2015; Wijesekara *et al*., 2016) and have a positive influence on soil quality, plant growth and reduction of heavy metals in plant tissues (Fellet *et al*., 2011; Anawar *et al*., 2015; Wijesekara *et al*., 2016). Biochar is a pyrogenous biomass and is characterized by high pH, high porosity and high surface area (Gul *et al*., 2015). When amended in contaminated soils, biochar absorbs contaminants and reduces their toxicity to plants (Anawar *et al*., 2015). The positive influence of biochar on soil quality and plant growth can be enhanced via its co-amendment with other organic wastes such as manure and compost (Gul *et al*., 2015; Gul and Whalen, 2016). This is because biochar adsorbs nutrients from organic wastes and acts in soil as a slow-release fertilizer; whereas, organic wastes promote soil aggregation, which further helps in retaining nutrients and water in soil (Kammann *et al*., 2015; Schmidt *et al*., 2015).

Organic wastes such as manures and woods from pruned trees can be utilized as fertilizers of biochars or as fertilizers of mixtures of biochars and manures in mining-affected agricultural lands. Tomato is the major summer vegetable crop of Muslim Bagh. Most of agricultural lands are also cultivated with winter and spring crops such as lettuce and spinach after harvest of summer vegetable crops. Present study investigated influences of mixtures of farmyard (FY) manure (as cow manure)-derived biochar with poultry manure or wood-derived biochar with poultry manure on (1) growth of tomato and lettuce, (2) concentration of heavy metals in the edible parts of these crops and (3) quality of the soil that was contaminated with chromite mine tailing-debris. As pot-based crop rotation experiment, lettuce was grown in the same soil after harvest of tomato. Furthermore, this study also assessed the influence of application of organic fertilizers in the root zone of crops. This type of placement of fertilizers are found to have significantly positive influence on crop growth as compared to when such fertilizers are mixed thoroughly in soil (Schmidt *et al*., 2015). In present study, inorganic fertilizer was not used for two main reasons; (1) organic fertilizers act as binding agents with mine-tailing debris and heavy metals, thus have potentially more beneficial influence on crops than inorganic fertilizers (Anawar *et al*., 2015; Wijesekara *et al*., 2016) and (2) organic fertilizers are many-times inexpensive than inorganic fertilizers (poultry manure does not cost if is required in few kgs) and are easily available (Hameeda *et al*., 2019).

Materials and Methods

Biochars and manure

Slow-pyrolyzed (produced in kiln) wood-derived biochar was purchased from a timber market from Quetta city (the only timber market at Joint road) as leftover broken pieces in low price (40 PKR per kg or ~0.25 US $). The feedstock of wood-derived biochar was *Acacia* and citrus trees from Sindh province, Pakistan. The FM biochar was produced from cow dung in a kiln as described in Tahir *et al*. (2018). A parcel of 15 kg air-dried cow dung costs 300 PKR (20 PKR per kg and approximately 0.125 US $). Poultry manure was obtained from poultry farm of Quetta city free of cost. Manures were further air-dried to constant weight (as was done to other manures used in our other studies Tahir *et al*., 2018; Ghori *et al*., 2019; Hameeda *et al*., 2019) before use. Poultry manure was mixed with wood-derived or FM biochar with 1:1 ratio on air-dry weight bases. The properties of these fertilizer sources are provided in Table 1.

Table 1: *Chemical properties of organic fertilizers.*

Treatment	Wood biochar + poultry manure	FM biochar + poultry manure
pH	5.49	4.93
EC (µs cm^{-1})	164.3	125.9
Cu (mg g^{-1})	0.16	0.13
Fe (mg g^{-1})	14.06	40.7
Mn (mg g^{-1})	3.65	2.83
Cr (mg g^{-1})	0.195	0.072

Experimental setup

The sandy loam soil (575 g kg^{-1} sand and 325 g kg^{-1} silt) used in this study was aridisol according to USDA soil classification system. Further properties of this soil are given in Hameeda *et al.* (2019). Soil was air-dried, stones were removed and was passed through 2 mm mesh sieve. The experiment was designed as completely randomized. The experimental setup and abbreviations of different treatments are provided in Table 2. Two control treatments were added in this experiment; (1) control 1 with no amendment, (2) control 2 with 2% amendment of chromite (Cr) mine tailing debris (980 g soil, 20 g debris). The picture of chromite mine tailing debris is given in Supplementary Figure S1. Soil for other treatments was amended with 2% Cr mine tailing debris prior to the amendment of biochar-based organic fertilizers. Two biochar-poultry manure mixtures were produced i.e. wood-derived biochar + poultry manure and FM biochar+poultry manure. Biochar-poultry manure mixture was applied as (1) thoroughly mixed in soil and (2) in the root zone of crops at ~5 cm below soil surface as a mass of fertilizer spread over soil surface, followed by filling the pots with soil up to 5 cm above fertilizer (Siddiqui *et al.*, 2020 accepted manuscript; see also Schmidt *et al.*, 2015). The fertilizers were amended at two application rates (1) 10% (900 g soil mixed with 100 g biochar-poultry manure mixture) and (2) 20% (800 g soil mixed with 200 g biochar-poultry manure mixture) in soil. The 10% and 20% amendment rates are equivalent to 60 t ha^{-1} and 120 t ha^{-1}, respectively. The conversion of % amendment to t ha^{-1} is based on calculations made in Watzinger *et al.* (2014), Xu *et al.* (2015) and Ghori *et al.* (2019). The selection of these application rates are based on previous reports (Ameloot *et al.*, 2014; Watzinger *et al.*, 2014; Xu *et al.*, 2015; Ghori *et al.*, 2019). Each treatment had three replications.

Tomato was grown first and after its harvest, soils of all treatments except for root-zone treatment were used to grow lettuce. The root-zone fertilizer treatment for lettuce was performed also as fresh treatment because tomato-grown soil could not be reused as root-zone fertilizer for lettuce. Total number of pots for each crop was 30. Pots used in this study were 35cm in diameter and 15cm in height (Figures 1 and 2) with a hole in the bottom for drainage of excess water. The pots were placed in open air (Figures 1 and 2). The cumulative rainfall during growing period of tomato and lettuce was 12.8 mm and 1.8 mm, respectively. The average daytime temperature during growing period of tomato and lettuce was 29.8°C and 16.6°C, respectively. The average nighttime temperature during growing period of tomato and lettuce was 20.15°C and 7.3°C, respectively.

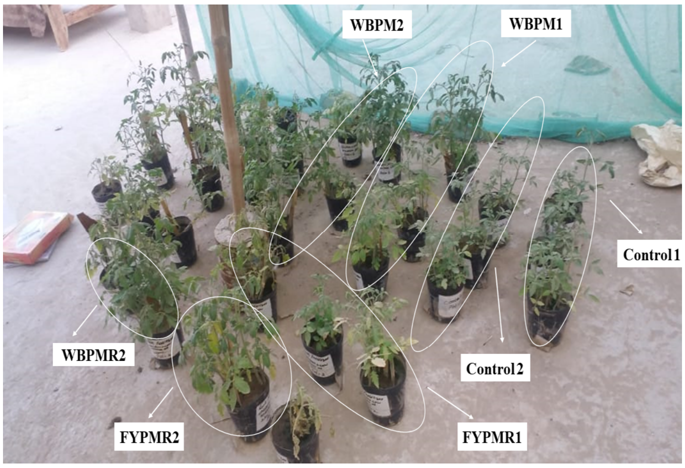

Figure 1: *Tomato plants grown under various treatments, control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.*

Sowing, harvesting and processing of plant tissues

Seeds of Roma tomato (Supplementary Figure S2) were broad-casted on wet soil surface in April (April 20, 2018). Before that, tomato seedlings purchased from nursery were grown, but they did not survive in pots; therefore, seeds of tomato were used for direct germination and growth. After approximately one week of germination, seedlings were thinned to one per pot. In October (October 26, 2018), seeds of lettuce were broad casted on wet soil surface and approximately after one week of germination, three seedlings were left in each pot. Water was provided twice or three times per week. Water contents of pots were maintained to 400 ml per pot (40% of soil weight). For sandy loam soil, this amount of water is approximately equivalent to 60% water filled pore space (Gul and Whalen 2013). Tomato was harvested in August (August 03, 2018) while lettuce was harvested in January (January 05, 2019). Only three plants of different treatments produced only one tomato fruit per plant (Supplementary Figure S1), although plants produced flowers but those flowers did not reach to the stage of fruit development. Same results for this variety of tomato were observed in our previous pot-based study, in which no plant produced any fruit in pots (Batool *et al*., 2019), whereas the same variety produced high number of fruits when was grown in field (Hameeda *et al*., 2019). Aboveground plant biomass of tomato and lettuce and tomato fruits were air-dried followed by 24 h placement in oven at 40°C for complete drying. Their oven-dry weight was recorded for biomass estimation. After harvest, pots were teared, soil was carefully removed and roots were carefully removed, washed, dried as described above and their oven-dried weight was recorded for biomass estimation.

Assessment of water use efficiency of plants

Water use efficiency (WUE) of only tomato crop was assessed as described in Aller *et al*. (2017) and Ghori *et al*. (2019) as;

$$WUE = \frac{Plant\ biomass\ (g)}{Amount\ of\ water\ (L)\ applied} \quad \ldots (1)$$

Water was added two - three times per week. Before addition of water in pots, pot weight was measured, the weight of pot was adjusted with water to 1440 g (1000 g soil + 40 g plastic pot weight + 400 ml water). At watering event, amount of water added in a pot was recorded. The total amount of water applied to a pot during entire growth period of plant was measured as the sum of water added per watering event. To calculate WUE, oven-dried aboveground plant biomass after harvest (at the end of experiment), was thereafter divided by the total amount of water applied to pot (in liter) during entire growth period of plant.

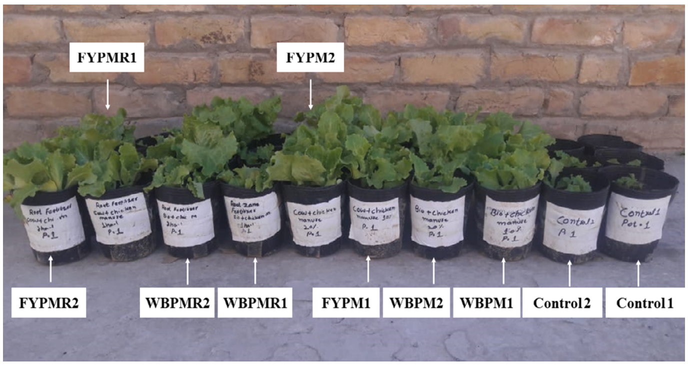

Figure 2: *Lettuce plants grown under various treatments, control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPM1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPM2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.*

Table 2: *Treatments, organic manure application rates and abbreviations of treatments.*

		Amendment rate (%) in soil		
Treatment		Biochar	Manure	Abbreviation
Control without any amendment		0	0	Control 1
Control with Cr mine tailing debris-amendment		0	0	Control 2
Non root zone fertilizer treatments	Wood Biochar+ poultry manure	5% (w/w)	5% (w/w)	WBPM1
	Wood Biochar + poultry manure	10% (w/w)	10% (w/w)	WBPM2
	FM biochar + poultry manure	5% (w/w)	5% (w/w)	FYPM1
	FM biochar + poultry manure	10% (w/w)	10% (w/w)	FYPM2
Root zone fertilizer treatments	Wood Biochar+ poultry manure	5% (w/w)	5% (w/w)	WBPMR1
	Wood Biochar + poultry manure	10% (w/w)	10% (w/w)	WBPMR2
	FM biochar + poultry manure	5% (w/w)	5% (w/w)	FYPMR1
	FM biochar + poultry manure	10% (w/w)	10% (w/w)	FYPMR2

Assessment of heavy metals in organic fertilizers and plant tissues

Biochar-poultry manure mixture and aboveground plant tissues of lettuce and tomato fruits were ash-digested following protocol of Rechcigl and Payne (1989) as described in Ghori *et al*. (2019). Analyses of heavy metals i.e. chromium (Cr), manganese (Mn), copper (Cu), iron (Fe) were performed on Atomic Absorption spectrophotometer (Thermo SOLAAR S series AA (Thermo Scientific) following the protocol of Khan *et al*. (2019).

Soil chemical analysis

After removal of roots, bulk soil samples were thoroughly mixed, air–dried and stored at 4°C until chemical analysis. Soil pH and electrical conductivity were analyzed following protocol of Estefan *et al*. (2013). Soil samples were extracted with 2M KCl solution as 1:5 soil: KCl ratio following protocol of Estefan *et al*. (2013). Mineral nitrogen (N) of soil extracts was analyzed as described in Gul and Whalen (2013). Soluble mineral phosphorus was analyzed following protocol of D'Angelo *et al*. (2001). Mineral N and soluble mineral P of soil extracts were analyzed using UV-visible spectrophotometer (Shimadzu UV-700). Soil pH and electrical conductivity were analyzed following protocol of Estefan *et al*. (2013).

Statistical analysis

Data of aboveground and belowground plant biomass, WUE and soil nutrients were subjected to analysis of variance (ANOVA). The differences between treatment means were assessed by least significant difference (LSD) test. Statistical analyses were carried out using CoSTAT software version 6.311 and Microsoft Excel.

Results and Discussion

Plant biomass of tomato and lettuce plant and water use efficiency

Amendments of biochar-poultry manure mixtures at both application rates and for both application procedures (thoroughly mixed or applied at root zone) positively influenced aboveground plant biomass and WUE of tomato and growth of lettuce (for both aboveground and belowground plant biomass) as compared to control 1 (Figures 1, 2, 3 and 4; $P<0.05$).

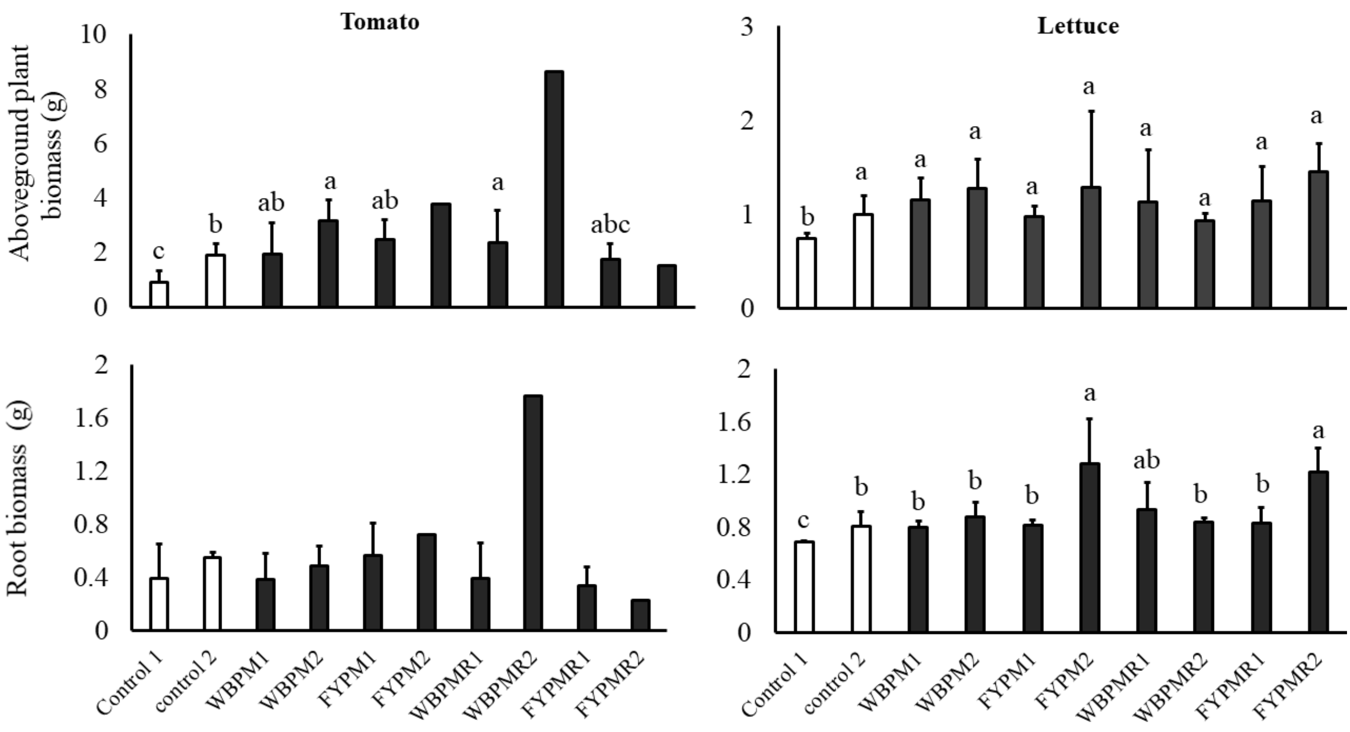

Figure 3: *Average (±SD) of aboveground plant biomass and root biomass of tomato and lettuce (n= 3, FYPM2 and FYPMR2 for tomato had only one replication). Bars with different letters show significant difference at P<0.05. control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPM1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPM2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.*

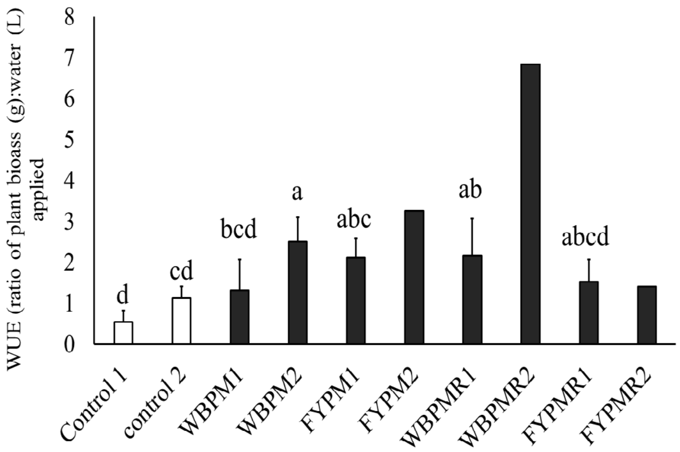

Figure 4: *Average (± SD) of water use efficiency (WUE) of tomato (n= 3, FYPM2 and FYPMR2 for tomato had only one replication). Bars with different letters indicate significant difference at P<0.05. control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPM1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPM2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.*

No differences between treatments for root biomass of tomato plants were observed (Figure 2). Amendment of biochar-poultry manure mixtures had significantly positive influence ($P<0.05$) on growth of both crops. These treatments caused ~254.49% - 871.9% (~3.5 – 9 fold) increase in aboveground biomass of tomato, 25.67%-94.59% increase in aboveground plant biomass of lettuce and 16% - 87% increase in root biomass of lettuce. These treatments also caused ~3.5-12.5 fold (364.8% - 1166.6%) increase in the water use efficiency (WUE) of tomato ($P<0.05$). Although in Figure 2, height of lettuce of control 2 treatment was substantially lower, biomass was not significantly lower than biochar-poultry mixture treatments (Figure 3). This may be because concentration of heavy metals was very high in the plants of control 2 treatment. Under stress conditions, plants deposit more lignin in their secondary cell walls (Bouazizi *et al*., 2011; Gomes *et al*., 2011), thus high concentration of heavy metals and probably high deposition of lignin in plants of control 2 treatment, might had resulted in non-significant differences between control 2 and other organic fertilizer treatments for plant biomass. The positive influence of amendment of biochar-poultry manure mixtures on plant growth performance in Cr mine tailing debris-contaminated soil may be due to the absorbance capacity of these organic fertilizers, which might had reduced toxicity of heavy metals to plants (Hameeda *et al*., 2019; Gul and Whalen, 2016). Our results demonstrate that amendment of organic fertilizers, which are made of biochar-poultry manure mixtures, can promote growth of vegetable crops of agricultural lands of those regions, where mining activities are going on and agricultural soils are contaminated with heavy metals due to mining activities. Field-based studies are required to be carried

Table 3: *Concentration of chromium (Cr), Manganese (Mn), copper (Cu) and iron (Fe) in leaves of lettuce and fruit of tomato.*

Crop	Treatment	Cr ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)
Lettuce	Control 1	0.26	39.84	0.55	13.7
	Control 2	0.23	27.74	0.46	9.46
	WBPM1	0.044	13.16	0.11	6.65
	WBPM2	0.042	10.8	0.17	37.0
	FYPM1	0.102	14.9	0.32	16.4
	FYPM2	0.038	4.12	0.10	4.09
	WBPMR1	0.038	6.23	0.05	6.66
	WBPMR2	0.062	2.03	0.08	1.69
	FYPMR1	0.032	1.33	0.10	78.1
	FYPMR2	0.011	0.91	0.04	30.6
Tomato	Control 2	0.056	9.96	0.21	140.9
	WBPM2	0.059	4.41	0.18	7.75
	WBPMR2	0.018	6.23	0.26	25.4

Values are based on pooled digested samples of lettuce and only one-grown tomato per plant of three pots of control, WBPM2 and WBPMR2 treatments control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPM1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPM2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.

Table 4: *Average (± SD) of pH, electrical conductivity, mineral nitrogen and soluble mineral phosphorus of soils grown with tomato.*

Treatment	pH	Electrical conductivity ($\mu s\ cm^{-1}$)	Mineral nitrogen ($mg\ kg^{-1}$ soil)	inorganic soluble phosphorus ($mg\ kg^{-1}$ soil)
Control 1	8.54 ± 0.07^{ab}	3.22 ± 4.57^{e}	87.96 ±28.69^{ab}	7.63 ± 1.24^{f}
Control 2	8.50 ± 0.18^{abc}	1.26 ± 1.70^{de}	87.641±7.64^{ab}	2.78 ± 6.83^{f}
WBPM1	8.59±0.03 ^{a}	5.04 ± 6.56^{de}	88.51±15.46^{a}	10.80 ± 2.26^{e}
WBPM2	8.29 ±0.07^{de}	21.11 ± 30.1^{a}	113.50 ± 37.76^{a}	22.2 ± 1.37^{b}
FYPM1	8.18±0.04^{e}	7.08 ± 9.22^{ab}	63.46 ± 6.19^{b}	21.1±1.19^{bc}
FYPM2	8.37 ± 0.08^{bcd}	4.26 ± 5.74^{ab}	63.69	28.24
WBPMR1	8.35 ± 0.23^{cde}	2.68 ± 3.53^{ab}	96.881±25.2^{a}	17.6 ± 1.71^{d}
WBPMR2	8.18 ± 0.11^{e}	15.8 ± 22.0^{cde}	84.19	22.4
FYPMR1	8.27±0.06^{de}	2.02 ± 2.66^{abc}	111.61±10.82^{ab}	21.1 ± 1.04^{bc}
FYPMR2	--	4.66 ± 6.29^{bcd}	121.35	17.9

Within column values followed by different letters indicate significant difference at P<0.05. control 1; soil without any amendment, control 2; soil with amendment of Cr mine tailing debris, WBPM1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPM2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPM1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPM2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, WBPMR1; wood-derived biochar + poultry manure amended at 10% rate in Cr mine tailing debris-contaminated soil, WBPMR2; wood-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil, FYPMR1; cow manure –derived biochar + poultry manure amended at 1-% rate in Cr mine tailing debris-contaminated soil, FYPMR2; cow manure-derived biochar + poultry manure amended at 20% rate in Cr mine tailing debris-contaminated soil.

out to confirm our hypotheses in this regard. There was no difference between root-zone and thoroughly-mixed treatments for biomass of both crops. This indicates that application of these organic fertilizers in the root-zone did not enhance growth performance as compared to when these fertilizers were thoroughly mixed. Our results are not in agreement with Schmidt *et al.* (2015), who found 4-fold increase in the yield of pumpkin when urine-enhanced biochar was placed in the root-zone of pumpkin as compared to when the same amount of biochar was mixed thoroughly in soil. We applied biochar-poultry manure mixture in soil as 60 t ha^{-1} and 120 t ha^{-1} and found positive influence on growth performance for both crops. High application rates are used in previous studies also and found to have a positive influence on crop yield (Watzinger *et al.*, 2014; Xu *et al.*, 2015; Ghori *et al.*, 2019). In Balochistan province of Pakistan, as stated above, cow manure and poultry manure are many times less expensive then inorganic fertilizer. Furthermore, wood produced from pruning of orchard trees of this province can be cost-efficient bio resource for biochar production. These amendment rates can be economic for local farmers.

Due to limited funding and resources, unfortunately data related to WUE and soil analysis of lettuce were not collected. However, as positive influences of these fertilizers on yield and reduction in heavy metals in lettuce were observed; furthermore, as proved from previous published data (Gul and Whalen, 2016; Ghori *et al.*, 2019), we hypothesize that biochar-poultry manure mixtures as soil amendments might had positively influenced WUE of lettuce and concentration of soluble inorganic phosphorus of soil grown with this crop.

Concentration of heavy metals in edible tissues of crops

As compared to control treatments, amendment of biochar-poultry manure mixtures tended to reduce concentration of heavy metals in these crops (Table 3). Amendment of biochar in contaminated soils with heavy metals has been frequently reported to reduce concentration of heavy metals in plants (Ghori *et al.*, 2019; Haider *et al.*, 2018).

Concentration of mineral nitrogen and soluble mineral phosphorus in soil

The amendment of biochar poultry manure mixtures in soil had profound positive influence on concentration of soluble mineral phosphorus (P<0.05; Table 4). Our results are consistent with previous reports that amendment of biochar-based fertilizers enhances concentration of this nutrient in soil (Gul and Whalen, 2016; Batool *et al.*, 2019; Hameeda *et al.*, 2019).

Phosphorus ties up heavy metals and makes them immobile in the soil, so plants cannot take them up. This may be one of the reason of improved growth of crops in fertilizer-treated soil that was contaminated with chromite mine tailing debris (Alghamdi *et al.,* 2018).

Conclusions and Recommendations

Our results show that mixture of wood-derived or FM biochars with poultry manure enhanced biomass of crops significantly and reduced concentration of heavy metals in edible parts of lettuce in chromite-contaminated soil. Our findings provide an avenue for the local farmers of Balochistan to use biochar-poultry manure as cost-efficient bio fertilizer in agricultural lands, which are in close vicinity of mining activities. Future research is required to carry out field-based experiment with different crops, using biochar-based organic fertilizers, in the agricultural lands of the regions where mining activities are under practice.

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Novelty Statement

The novel aspect of this study is to evaluate the influence of two biochar types, in chromite mine tailing debris-contaminated soil on two economically important crops with regard to their growth performance and concentration of heavy metals.

Author's Contribution

Faiza Altaf conducted research. Shamim Gul and Gul Bano Rehman supervised research. Main theme of research was provided by Tasawar Ali Chandio. Atiq-ur-Rehman Kakar, Sami Ullah, Naqeebullah Khan, Muhammad Naeem Shahwani and Muhammad Ajmal provided assistance and lab facilities for heavy metal analysis. Umbreen Shaheen assisted in statistical analysis and data presentation.

Supplementary Material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.sja/2021/37.1.315.324

Conflict of interest

The authors have declared no conflict of interest.

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