INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 4, p. 85-95, 2024

RESEARCH PAPER OPEN ACCESS

Evaluation of Ameliorative Role of Biochar on Chromium Toxicity in Bottle Gourd

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Key words: Chromium, Bottle Gourd, Germination, Biochar, Wastewater-irrigation

http://dx.doi.org/10.12692/ijb/25.4.85-95 Article published on October 06, 2024

Abstract

The present attempt was made to elucidate the ameliorative role of Biochar (BC) in response to chromium toxicity on bottle gourd seed germination. In this study, seeds were grown in pot experiment with six treatments (Control, Low Cr-20mg/kg, High Cr-100mg/kg, BC-2%, BC-2%+Low Cr-20mg/kg, BC-2%+High Cr-100mg/kg). Harvesting was done after 40 days of development. Growth parameters, including physiological and biochemical variables, were observed to delimit the response in term of biochar exposure under chromium stress. Results showed that the application of Biochar (2%) significantly changed soil pH, improved crop growth and as well as reduced the antioxidant response of bottle gourd in Cr contaminated soil. Results revealed that in presence of Cr (20mg/kg soil and 100mg/kg soil) stress, a continuously reduction was observed in growth and physiological attributes and increased antioxidant enzyme activities accordingly in bottle gourd plant. In these parameters, more deterioration effect was observed when Cr (100mg/kg) was added in the soil. Biochar application (2%) played a crucial role in reducing the Cr toxicity (20 mg/kg and 100 mg/kg) level, resulting in significant increase in plant vine length (20% and 15%), biomass (fresh and dry) (7% and 24%), no. of leaves/plant (53% and 25%), chlorophyll a content (18%, 64%), chlorophyll b content (41%, 73%), relative water content (13% and 26%) and MSI (14%, 11%). Biochar's potential was seen to be slightly lower, when applied to high Cr-100 mg/kg concentration as compared to low Cr-20 mg/kg concentration. In conclusion, adding biochar to soils may be an effective environment friendly way to reduce Cr toxicity and improve plant health and growth.

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Introduction

Deterioration of terrestrial ecosystem with heavy metals is an increasingly noticeable challenge in the whole world and is enhancing due to the use of heavy metal-loaded materials in industries (Bashir *et al.*, 2021). The untreated industrial wastewater, composed of diverse toxic heavy metals including lead, cadmium, arsenic, and chromium, is being discharged into the environment and causing environmental health risks (Athar *et al.*, 2002). The leather tanneries are the chief source of soil contamination with chromium in Pakistan. About 1000 tanning units are established in Multan and neighboring cities, Pakistan, due to the availability of low-cost labor and extensive water in the Indus and Chanab rivers. Unfortunately inappropriate waste management of this leather industries effluent and un-treated discharge is a prime source of soil contamination. It is obvious that during tanning processes in the leather industries, Chromium is frequently utilized heavy metal (Yasin and Faisal, 2013) and is released continuously with wastewater in river and canals (Wionczyk, 2006).

Plants as well as animals are susceptible to this toxic heavy metal, even at low concentrations (2011). Presence of chromium in soil induces considerable stress symptoms in plants and retarded the seeds germination, suppresses photosynthesis, and also creates physiological disorder in term of nutrients uptake, consequently which influence the plant growth, carbon fixation and crop yield (Nigussie *et al.*, 2012; Singh *et al.*, 2013). Chromium has harmful effects of metabolic processes and osmotic balance by damaging the cell membrane of the plants cells. Beside of these, Chromium toxicity induces oxidative stress in plants by producing reactive oxygen species, which impaired the cell molecules and handicapped antioxidant system along with photosynthesis enzymes (Shanker,2005; Ghani,2010).

In underdeveloped countries, farmer use heavy metals contaminated wastewater for irrigation of food crops due to scarcity of underground water and energy crises (Tansar *et al.*, 2023). By using the Cr contaminated vegetables and food crops can causes severe health problems in humans including: stomach, respiratory, cardiovascular, immunological, neurological and reproductive disorders (Pan *et al.*, 2013; Tansar *et al.*, 2023; Atta *et al.*, 2023). Furthermore, Chromium can also induce the various types of cancers in humans consisting of bladder, skin, kidney and lungs (Mandal and Suzuki, 2002).

However, the magnitude of toxicity of Chromium depends upon its valence state. Chromium occurs naturally in multiple oxidation states (Kota and Stasicka, 2000), but Chromium-VI and Chromium-III frequently in the environment (Adriano, 2001). Chromium-III is comparatively harmless, less soluble and naturally found in stable state. Similarly, Chromium-VI is supposed to be more toxic, soluble and several health risks are associated with it (Razic *et al.*, 2011). Chromium-VI hardly found in nature and is commonly produced by anthropogenic activities (Costa and Klein, 2006). Considerable amount of Chromium-III is released through wastewater effluent from leather tannery. Toxicity caused by the presence of large amount of Cr in tannery waste polluted soils eliminates the vegetation cover (Beesley *et al.*, 2014). Thus, organic amendments (manure, biosolids, compost, biochar, etc.) are commonly used to increase soil conditions and crops production.

Biochar is a recalcitrant fine-grained carbon-rich substance synthesized from organic raw materials including agricultural waste and municipal solid waste, under controlled heat conditions with low oxygen (Lehman, 2009). Because of its high porosity, surface area, and cation exchange capacity, it is extremely effective in removing pollutants from soil and water (Yuan *et al.*, 2011). It improves soil water holding capacity, organic matter contents, reduces the nutrient loss, contribute significant role to stimulate the microbial activity of soil (Beesley *et al.*, 2014). In addition to enhancing the soils physical and chemical qualities, Biochar also increases the amount of available nutrients, resulting in increased soil fertility and plant growth (Shanker, 2005). Much of the research has been conducted previously by numerous investigators for delimiting the biochar potential to improve the soil physiognomies and nutrient uptake by plants, especially under pollutant stress conditions (Naveed *et al.*, 2020). Nigussie, *et al.*, (2012) made studies to determine effect of biochar application on soil attributes and nutrient uptake of lettuces grown in chromium polluted soils. Schulz *et al.*, (2013) conducted research work to evaluate the Positive effects of composted biochar on plant growth and soil fertility. Nagarajan, (2014) evaluated the effect of chromium on growth, biochemicals and nutrient accumulation of paddy. Bian *et al.*, (2014) also conducted studies to examine the continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. Younis *et al.*, (2016) made studies to examine the influence of biochar on spinach growing under cadmium stress. Choppala *et al.*, (2016) established experiment to explore the Differential effect of biochar upon reduction-induced mobility and bioavailability of arsenate and chromate. Behera *et al.*, (2020) conducted research work to elucidate the remediation effect of biochar and poultry manure against chromium stress in rice crops. Dadasoglu *et al.*, (2022) established experiment to investigate the ameliorative effects of biochar for cadmium stress on bean growth. No information is available on physiological as well as biochemical responses of Bottle gourd to biochar applied in soil polluted with indigenous tannery waste. Hence, the hypothesis of the present attempt was that biochar may decreased the toxic effect of Chromium by limiting it into its less toxic form, i.e., Cr (III) and decrease its accumulation in plants. Keeping in view the impact of biochar application in promoting soil characteristics along with the removal of toxic pollutants, a pot study was designed to assess the Cr availability and potential transfer to Bottle gourd seeds germination.

Material and methods

Experimental setup

The experiment was conducted at Bahauddin Zakariya University Multan, Pakistan in 2023 under greenhouse conditions, with average photosynthetically available radiation measured at noon ranging from 1098 to 1490 µ mol m-2 S-1. A total of six treatments with their four replicates were prepared. In all the pots containing a dose of biochar or chromium, the concerned treatment is thoroughly mixed with the sandy loam soil prior to filling. For the control treatment T1, no biochar and no chromium were added to the pots. In T2, pots were filled with biochar (2% w/w) along with soil. Similarly, T3 is composed of low chromium, having Cr 20 mg/kg, and T4 is composed of high chromium, having Cr 100 mg/kg. Treatment T5 comprises Low Chromium + Biochar, Chromium 20 mg/kg + Biochar 2% w/w, and Treatment T6, High Chromium + Biochar with measurement of Chromium 100 mg/kg + Biochar 2% w/w. After stabilization, the Bottle gourd seeds were sown into the soil separately. Equal amount of water was given to all pots after every three days. First sowing of Bottle gourd seeds was done. After 14 days of growth, three plants in each pot were selected for further growing by thinning them physically. Plants of vegetable Bottle gourd with amuleration of Biochar under Cr stress were ready to harvest after 40 days.

Chemical analyses

Harvesting of Bottle gourd seedlings were done after 40 days of sowing. In this study, shoot length, Root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and biochemical parameters (chlorophyll content, carotenoids, protein content, SPAD values) were explored. During harvest, plants were cut from the soil level, and above-ground biomass (shoot) and roots were separated for measurements and analysis. The leaf area was determined by Portable Laser Leaf Area Meter (CI-202- USA). All the biochemical analysis was performed using Perkin-Elmer, spectrophotometer (Optima 2100- USA) according to Sarafi *et al.*, (2018). For the determination of mineral nutrition content, the fruit of the bottle gourd were dried at 68 C for 48 h and grounded (Helrich, 1990).

Measurement of oxidative enzymes

Malondi-aldehyde was determined by following the standard procedure described by Lotus *et al*, (1996).

Hydrogen peroxide content was determined by following the standard method described by Jana and Chaudhry, (1981). Proline content was determined by following Betes *et al*, (1973). Similarly Membrane stability index was determined by following Sairam *et al*., (1994). Water Relations of Plants was determined by the equation documented by Mayak *et al*, (2004).

Measurement of antioxidant enzymes

POX assay and APX values were determined by standard procedure described by Nakano and Asada, (1981). SOD and Assay of catalase (CAT) was estimated by following standard procedure described by Aebi, (1983).

Statistical analyses

Analysis of variance was performed to evaluate the influence chromium stress in the presence of biochar on the growth parameters of bottle gourds and the accumulation of metals in plant tissues. Analysis of variance method used and each treatment was with four replications. For statistical analysis of data, Minitab (Version 17) computer program was used.

The differences among the means were compared using the Duncan multiple range test (DMRT) ($p < 0.05$).

Results

Morphometric attributes

In the present research work, the results described that Chromium imposes adverse influences on growth and development parameters of Bottle gourd. Chromium in soil notably reduced growth rate of plants by reducing leaf area, no. of leaves/plant, fresh and dry weight and vine length of Bottle gourd plant. Application of Biochar (2%) significantly also increased the morphological growth of Bottle gourd plant over their particular treatment. Biochar absorbs diverse charged species (Aluminum, Potassium, and Sodium) because of static attraction. Additionally, Biochar can support the growth and development, by giving different supplements because of its rich enhancement properties. In Cr contaminated soil, vine length, fresh and dry weight of Bottle, no. Of leaves/plant and leaves area of Bottle gourd plants gradually decreased with increase in Cr (0, 20mg/kg, 100mg/kg). After addition of Biochar (2%) vine length (1.75%, 20.37%, 14.91%; Table 1), fresh plant weight (2.3%, 5.3%, 15.41%; Table 2), plant dry weight (5.48%, 8.64%, 24.61%; Table 3; Fig. 3), no. of leaves/plant (3.90%, 53.13%, 25%; Table 4), and leaves area of plant(1%, 10.74%, 14.91%,Table 5) increased respectively as compared to their respective treatments(control, low Cr, high Cr).

Table 1. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of vine length of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr Control	BC vs no BC
No Cr	266.25	271.00		1.75
Low Cr	190.25	229.00	-28.55	20.37
High Cr	127.25	146.25	-52.21	14.91

Table 2. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of fresh weight of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	952.25	975.00		2.33
Low Cr	886.25	933.50	-6.93	5.33
High Cr	730.00	842.50	-23.34	15.41

Physiological/biochemical attributes

In the current review, Cr harmfulness showed adverse consequences on physiological parameter of Bottle gourd. Cr toxicity in soil altogether diminished plant development execution. The positive outcomes of Biochar were likewise seen regarding further developed plant physiological interaction. Photosynthetic pigments, i.e., SPAD chlorophyll, chlorophyll "a" and "b" as well as total carotenoid, are particles required to catch light energy of unequivocal frequency fundamental for photosynthesis and were impacted by Cr stress. As compared to control treatment decrease in SPAD value (15%, 39.81%; Table 6), chlorophyll 'a' (13.53%,32.69%; Table 7), chlorophyll b(37.26%, 56.41%; Table 8), carotenoid contents(29.40%, 44.82%) was observed under Cr (low Cr, high Cr). Chlorophyll a, chlorophyll b and carotenoid contents increased after addition of Biochar as compared to their respective Cr treatments.

Table 3. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of dry weight of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	104.50	110.56		5.48
Low Cr	95.15	103.37	-8.95	8.64
High Cr	75.47	94.05	-27.78	24.61

Table 4. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of No. of leaves/plant of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	49.25	51.25		3.90
Low Cr	24.00	36.75	-51.27	53.13
High Cr	18.00	22.50	-63.45	25.00

In treatment Biochar (2%) and low Cr (20 mg/kg), increase in SPAD value, chlorophyll a, chlorophyll b and carotenoid contents was about 14.46%, 18.13%, 64.09% and 3.1% (Table 9) as compared to only low Cr treatment while in treatment with Biochar (2%) and high Cr (100mg/kg.

Table 5. Morphological characteristics of Bottle gourd as affected by Chromium.

	Vine Length (cm)	No. of leaves/ plant	No. of branches/ plant	Plant FW (g)	Plant DW (g)	Leaf Area (cm²)
Control	266.25±5.40 a	49.25±3.03 a	4.50±0.50 a	952.25±11.45 ab	104.50±3.35 ab	254.25±5.80 a
BC	271.00±8.77 a	51.25±4.21 a	4.50±0.50 a	975.00±12.75 a	110.56±6.31 a	252.25±10.30 a
Low Cr	190.25±12.66 c	24.00±2.45 c	3.75±0.43 ab	886.25±13.88 c	95.15±3.73 c	216.50±4.72 c
High Cr	127.25±12.48 e	18.00±2.24 d	3.25±0.43 b	730.00±31.83 e	75.47±3.63 d	172.75±5.26 e
Low Cr+BC	229.00±14.09 b	36.75±2.38 b	3.75±0.43 ab	933.50±7.37 b	103.37±2.74 b	239.75±3.56 b
High Cr+BC	146.25±8.32 d	22.50±1.80 cd	3.50±0.50 b	842.50±13.07 d	94.05±3.16 c	198.50±7.37 d

*The treatment means (n=4) ± standard deviations are displayed in individual columns. Treatment means that do not share similar letters within the same column are statistically distinct from one another (p≤0.05).

Stress related metabolites

Application of Biochar (2%) enhanced MSI and protein levels in soils and markedly reduced stress-related metabolites activities. Cr stress decreased relative water content and membrane stability index of Bottle gourd while proline content and malondi-aldehyde(MDA) increased with increase in Cr concentration. Under Cr(low, high) treatments, decrease in relative water content(18.66%, 44.28%; Table 10) and MSI(19.50%, 34.85%; Table 11) was observed. Proline contents and MDA, (94.84%, 145%; Table 12) and (161.92%, 246.62%; Table 13) increased under Cr (low, high) treatments. After addition of Biochar (2%), increased in relative water contents (13.21%, 24.14%; Table 13) and MSI (13.31%, 10.85%) was observed then in only Cr (low, high) while proline content (27.49%, 7.59%; Table 12) and MDA (41.15%, 9.24%) decreased respectively under treatment (Biochar+low Cr, Biochar(2%)+high Cr) then in Cr(low Cr, high Cr).

Table 6. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of SPAD value of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	44.15	44.70		1.23
Low Cr	37.53	42.95	-15.01	14.46
High Cr	26.58	31.73	-39.81	19.38

Table 7. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Chlorophyl a content in Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	1.64	1.68		2.26
Low Cr	1.42	1.67	-13.53	18.13
High Cr	1.10	1.56	-32.69	41.78

Antioxidant enzymes

The results showed that presence chromium contents in the soil changed the activities of antioxidant enzyme in the Bottle gourd's. The Bottle gourd plant showed the greatest rise in APX, GPX, SOD, and CAT activity. Application of Biochar (2%) substantially reduced the actions of antioxidant enzymes. The guaiacol peroxidase (POD; Fig.14) activities were increased with the increasing Cr toxicity in the soil relative to absolute control. Moreover, Cd stress increased the superoxide dismutase (SOD; Fig.13) (29.10%, and 42.65%; Table 14) and ascorbate peroxidase (APX) (33% and 56%; Fig. 16) activities at 20 and 100 mg kg−1 Cr concentrations. Biochar amendment (2%) either alone or under Cr stress, significantly improved the activities of SOD, POD, CAT and APX when compared with respective Cr treatments with no Biochar.

Table 8. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Chlorophyl b in Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs No BC
No Cr	0.90	0.87		-0.5
Low Cr	0.56	0.92	-37.26	64.09
High Cr	0.39	0.68	-56.41	72.96

Table 9. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Carotenoid contents in Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	0.65	0.59		-10.26
Low Cr	0.46	0.47	-29.40	3.10
High Cr	0.36	0.35	-44.82	-1.06

Analysis of Chromium in plant samples

Exposure of Cr is largest in roots of the plants than any other section of plant as Cr content are carried out to other parts of plants by their roots.

In treatment, when alone low Cr added in soil, 963 percent increase was observed in Cr content in roots of Bottle gourd over control treatment. With increase in Cr Concentration (High Cr), Cr content in roots of Bottle gourd also increased up to 2134.96 % (Table 18) over control treatment. When Biochar was added in soil with Low Cr (20mg/kg), Cr content in roots decreased up to 6% over their respective treatment (Low Cr). In Treatment (High Cr+BC), Cr content in roots of Bottle gourd decreased 29% then their respective treatment (High Cr). When low Cr

(20mg/kg) was added in soil, 3148.05% (Table 19) increase in Cr contents was observed in shoots of Bottle gourd. Cr contents in shoots increased gradually with increase of Cr concentration. High Concentration of Cr (100mg/kg) in spiked soil resulted in 4292.86% (Table 19) increase of Cr contents in shoots of Bottle gourd over control treatment. With application of Biochar (2%) and low Cr (20mg/kg) in soil, 35% Cr content decrease was observed in shoots of Bottle gourd over their respective treatment (low Cr). When high Cr (100mg/kg) concentration and Biochar treatment were taken, 9% increase in Cr contents in shoots of Bottle gourd was recorded.

Table 10. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of RWC in Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	80.81	79.95		-1.07
Low Cr	65.73	74.41	-18.66	13.21
High Cr	45.03	56.80	-44.28	26.14

Table 11. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of MSI in Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	90.55	88.35		-2.48
Low Cr	72.89	82.59	-19.50	13.31
High Cr	59.00	65.40	-34.84	10.85

Discussion

In this research, we studied the toxic effects of Cr stress in B. rapa and ameliorative role of Biochar under Cr toxicity. It was examined that Cr stress had deleterious effects on morphological parameter of *B. rapa* plant. Low humus and toxic nature of Cr in the soil can lead to poor soil composition, which can inhibit growth and development of plants (Gregory *et al.* 2015; Ding *et al.* 2016). Our research showed similarity with research (Ranieri*et al.* 2013) that application of Biochar reduced the Cr state in the soil; it converts Cr (VI) to Cr (III), which is less mobile as compared with Cr(VI). The decline in development parameters percentage may be caused by a decrease in amylase action in the parameters of Bottle gourd. Less sugar is transferred as a result of lower amylase activity, which also slows down growth and development.

Table 12. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of proline contents in Bottle gourd.

	No BC	BC	% increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	0.63	0.69		9.03
Low Cr	1.23	0.89	94.84	-27.49
High Cr	1.55	1.43	145.63	-7.59

Table 13. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of MDA in Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	7.67	8.44		9.061
Low Cr	20.10	11.83	161.92	-41.15
High Cr	26.60	24.14	246.62	-9.24

A decline in growth and other parameters may also be caused by the powerful action of the enzyme (protease), which rises with an increase in Cr meditation as a result (Zeid, 2001). But there was a maximum reduction in root length, which could be due to a number of factors (Malick *et al*, 2010). Root development abnormalities can be brought on by a halt in root cell division and a decrease in cell accretion in the root zone of growth when exposed to high levels of Cr (Adrees *et al*, 2015).

Table 14. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of SOD value of Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	184.42	195.08		5.47
Low Cr	238.09	274.65	29.10	15.35
High Cr	263.08	315.28	42.65	19.84

Table 15. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of peroxidase in Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	38.88	37.98		-2.38
Low Cr	56.07	87.47	44.23	55.99
High Cr	64.81	65.36	66.71	0.84

The Biochar retained these toxins on its surface and reduced the harmfulness of metals in plants (Rizwan *et al.* 2016d). The addition of Biochar greatly reduced the effects of Cr, with an increase in shoot and root length of up to 10% and 30%, respectively. In Cr treatments, the roots of the Bottle gourd had a greater Cr assemblage than the leaves did. PO43- and Fe, the supplementary nutrients for proper plant development are retained with the greatest amount possible (Chan *et al*, 2008; Xu and Chan, 2009). Due to Cr's toxicity, leaves lost their mesophyll layer, light parenchyma cells, and palisades, which had an impact on the general growth of the leaves (Pandey *et al*, 2009; Oruko *et al*, 2019). High Cr (100 mg/kg) caused the concentration of Chlorophyll to be modulated up to 40%.

Table 16. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Catalase in Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	0.23	0.26		11.82
Low Cr	0.57	0.91	148.45	58.92
High Cr	0.63	0.97	174.23	54.14

Table 17. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of APX in Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	5.13	5.39		4.85
Low Cr	6.86	8.57	33.80	24.91
High Cr	6.18	10.09	20.37	63.46

The decrease in Chlorophyll contents may be caused by enzyme activity being inhibited, which plays a significant role in the synthesis of these pigments. Plants' synthesis of pigments and protein decreased when exposed to Cr stress (Shankar *et al.*, 2005; Dey *et al.*, 2009). When applied to Bottle gourd plants in conditions of Cr stress, Biochar caused an increase in chlorophyll and chlorophyll b of up to 20 and 64, respectively. Same results were also reported by Ali *et al.* (2013), Gill *et al.* (2015), and Arshad *et al.* (2017).

Restricting Cr's accessibility to plants and making it less available to roots directly, Biochar continues to advance the Chl a and Chl b contents with an increase of 9.5 percent. Similar results were reported by (Ipolito *et al*, 2011; Park *et al*, 2011) that mechanism of cation exchange and adsorption BC can stable HMs. When Biochar was added to Cr-spiked soil, the MSI and relative water content increased by about 15%. Under the influence of Cr (100mg/kg) stress, Bottle gourd plants' metrics including MSI and RWC were improved by BC. Peroxidation (Lipid) and oxidation (protein) are two of the main cellular repairs carried out by ROS. (Palma *et al*, 2002) explained in his review that MDA is thought to be a biological marker for damage caused by ROS. With an increase in the median concentration of Cr, the lipid peroxidation ratio in Bottle gourd plants fundamentally increased. Our research supported the main ideas of (Sinha *et al*, 2009; Pandey *et al.*, 2005).

Table 18. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Cr content in roots of Bottle gourd.

	No BC	BC	%increase/decrease	
			Cr vs Control	BC vs no BC
No Cr	4.75	4.08		-16.33
Low Cr	50.50	47.57	963.53	-5.80
High Cr	106.12	75.26	2134.96	-29.08

Plants have antioxidant enzymatic and non-enzymatic (SOX, POX, ascorbate, and non-protein thiols) mechanisms that protect plant cells. A similar finding was documented by Hossain *et al.* (2010); this reduction in antioxidant enzymes activities might be due to excessive electrolyte leakage (EL) or overproduction of ROS (Chen and Murata 2011). Since POX activity is correlated with reactive oxygen species development, detrimental HMs could reason arouse in development of POX (Panda and S.k, 2007). Plants have developed several antioxidant mechanisms to control various stress conditions, but when exposed to high concentrations of pollutants, such as Cr, they are unable to maintain cellular homeostasis (Wang *et al*, 2013; 2014). Super oxidase activity decreased, which caused the enzymes (production of proteins) to become vitiated and less active. However, results showed that the deadly effect of Cr was significantly reduced with the addition of Biochar (2%), as metals fluctuating from the soil and reaching the plants could be restricted (Choppala *et al.*, 2012; Li *et al.*, 2015). It was reported by Sharma and Dietz (2009) that non enzymatic antioxidant play vital role in plant life under stress.

Table 19. Average mean values of different treatments (Control, Low Cr, High Cr, BC, Low Cr+BC, High Cr+BC) with increase/decrease percentage over their respective treatment of Cr content in shoots of Bottle gourd.

	No BC	BC	% Increase/Decrease	
			Cr vs Control	BC vs no BC
No Cr	0.92	0.96		4.35
Low Cr	29.76	19.27	3148.05	-35.25
High Cr	40.25	36.59	4292.86	-9.09

The application of biochar enhanced ascorbic acid content under mild and severs Cr stress.. The application of biochar reduced the MDA and H2O2 content in stressed plants. Similar results reported by Rizwan *et al.* (2018). The use of Biochar decreased the Cr accumulation in all parts of the plant, similar results reported by Arshad *et al.* (2017) and Rizwan *et al.* (2018).

Conclusion

This research work provides clear evidence of the significant demands of biochar for enhancing plant growth under heavy metal stress. In Pakistan, due to the scarcity of underground water, the demand for irrigation of crops with wastewater is very high. A total of about 80 million rupees is paid internationally per year for fertilizers, electricity and fuel charges for obtaining the underground water, which affects the economy of the country. However, the findings of the present research work will be significantly useful in terms of balancing the country's payments and creating an opportunity for the farmers in Pakistan if the biochar could be commercialized on a local scale. According to our findings, bottle gourd plant growth attributes and chlorophyll synthesis clearly showed that an increase in Cr concentration had a negative effect. Additionally, the increase in MDA generation caused by Cr showed oxidative damage. Biochar boosted plant biomass and photosynthesis under Cr stress in plants. MDA concentrations in plants decreased, indicating that biochar lowered oxidative stress in plants. Under Cr stress, biochar application enhanced the metabolic characteristics of the plant. Overall, the biochar treatment is successful in lowering the toxicity and absorption of Cr in bottle gourd. The present investigation suggests further plant physiological research work for the improvement of the growth and yield of commercial crops irrigated with heavy metal-contaminated wastewater, particularly chromium.

Recommendation

By introducing biochar application at local as well as at commercial scale, we would improve the growth of bottle gourd in toxic Cr soil.

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