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# Biochar affects growth and biochemical activities of fenugreek (*Trigonella corniculata*) in cadmium polluted soil

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## Summary

Cadmium (Cd) has no defined biological role and may enter the food chain from polluted soils. Biochar has been proposed as an organic amendment to minimize the toxic effects of Cd for plants grown on contaminated soils. In this study, biometric and biochemical attributes of fenugreek (*Trigonella corniculata*) grown on artificially cadmium (Cd) contaminated soil (0, 25, 50, 75 and 100 mg Cd/kg soil) at three levels of cotton-sticks derived biochar (CSB; 0, 3 and 5 %) were studied. Data show significant decline in the growth, photosynthetic pigments (chlorophyll *a, b* and *total, carotenoids, anthocyanin and lycopene)*, and physiological attributes (sub-stomatal $CO_2$ concentrations, photosynthetic and transpiration rate) in the presence of high Cd concentrations (50 and 100 mg Cd/kg soil). However, the decline was reduced in the presence of CSB. A steady amplification in lipid peroxidation (assessed via Malondialdihyde (MDA)) and ascorbic-acid assembly was noted with increasing Cd. The concentration of Cd in the root and shoot also decreased with increasing CSB application rates from 3 % - 5 %. Overall, the greater production of protein, amino acids and sugar contents in response to higher application rates of CSB seems to be due to alleviation in Cd toxicity. Thus, cotton-sticks can be safely utilized in the form of biochar as amendment with additional benefit of reducing Cd bioavailability and toxicity to crop plants.

## Introduction

Cadmium (Cd) is a toxic element for crop plants that enters into environmental cycle through different anthropogenic activities such as electroplating, waste materials of industries, manufacturing of Ni-Cd batteries, plastic, pigments, as well as cement and ceramic (NRIAGU, 1996). Entry of Cd to agricultural lands and consequently in plants is through use of sewage irrigation water and sewage sludge (QADIR et al., 2000). There are certain adverse effects of Cd on plants when taken up by the roots in large quantity and accumulated in the aerial parts (REEVES and BAKER, 2000) such as metabolic, physiological and restricted growth disorders (MARKERT et al., 2003). The photosynthetic pigments are very susceptible to Cd toxicity that results in disturbed photosynthetic activity (SANDALIO et al., 2001). Moreover, the structure and functions of various membranes are destroyed by Cd as it facilitates partial reduction of oxygen molecules by restricting the activity of photosynthetic electron transport which produces free radicals (DHIR et al., 2004). High levels of Cd also become one of major contributors in the oxidative damage as in higher plants it is engaged in lipid peroxidation.

The accumulation of Cd and other heavy metals in plants can be minimized by the addition of organic amendments such as biochar (a black carbon compound produced by pyrolysis of biomass). Biochar can immobilize heavy metals in soil through various stabilization mechanisms (VERHEIJEN et al., 2010). Biochar derived from various feedstocks can improve soil physicochemical properties (texture, structure, bulk density pH, electrical conductivity, and cation exchange capacity), biological as well as fertility status (AMONETTE and JOSEPH, 2009; GUNDALE and DELUCA, 2007). Besides, the most important use of biochar is the safe utilization of organic wastes and carbon-sequestration through reduction of greenhouse gases into atmosphere (KAMMANN et al., 2012). Due to high surface area and organic functional groups, biochar can immobilize heavy metals in soils through sorption (BEESLEY et al., 2010; LIU and ZHANG, 2009; UCHIMIYA et al., 2010; ZHANG et al., 2013). BUSS et al. (2012) reported better performance and reduced uptake of Cu by *Chenopodium quinoa* after biochar application at 4 %. They reported adsorption of Cu to biochar surfaces as possible mechanism of reduced Cu uptake.

In Pakistan, most of the vegetables are grown using sewage water irrigation that poses serious threat of heavy metals accumulation (HOSSAIN et al., 2010; QADIR et al., 2000). In this scenario, there is need of research to minimize the bioavailability of metals to crop plants especially vegetables. The objective of our research was to study the impact of Cd and biochar on biochemical activities of fenugreek (*Trigonella corniculata*). It was hypothesized that biochar can minimize the stress effects of Cd by minimizing its bioavailability to *T. corniculata*. Biochar was prepared using cotton-sticks because of their easy and cheap availability in the farming community. The changes in growth, chlorophyll, MDA, sugar, protein, amino contents and bioaccumulation of ions and Cd were investigated in tissues of fenugreek seedlings grown in metal-contaminated soil and amend with biochar.

## Materials and methods

The experiment was conducted at the botanical garden of Bahauddin Zakariya University Multan, using a completely randomized factorial design involving fenugreek (*Trigonella corniculata)* and two treatment factors: biochar (0, 3, and 5 % w/w) and cadmium additions (0, 25, 50, 75 and 100 mg Cd/kg air dry soil using $CdCl_2$). Each treatment was replicated four times. The biochar (CSB) was prepared using pyrolysis of cotton-sticks at 450 °C in vertical-silo kiln-type reactor. The physicochemical properties of the cotton-sticks derived biochar (CSB) are provided in Tab. 1. The soil was collected from the agricultural field of the Bahauddin Zakariya University and had following characteristics (texture, Sandy loam; pH, 6.5; AB-DTPA extractable Cd, 0.742 ppm). The plants were grown in clay pots filled with mixture of 5 kg soil and respective treatments (CSB and Cd). Twenty seeds of fenugreek (*T. corniculata)* were sown and later thinned to ten seedlings per pot. The plants were irrigated on regular basis to maintain 50 % of soil's water holding capacity.

At maturity, the plants were harvested and fresh and dry biomasses (shoots and leaves) were determined. The concentration of Cd in the plant samples was determined using di-acid digestion followed by measurement on Atomic Absorption Spectrophotometer (RASHID

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**Tab. 1:** Physiochemical properties of biochar used in the experiment

| $pH_{(1:10)}$ | $EC_{(1:10)}$ | Total Carbon | Total Nitrogen | Hydrogen | Phosphorus | Potassium | Ash content | Volatile matter |
|---|---|---|---|---|---|---|---|---|
| | dS/m | % (w/w) | | | | | | |
| 9.51 | 1.52 | 46.3 | 1.7 | 3.6 | 0.4 | 1.6 | 15 | 20 |

1986). The total soluble protein and amino acids were determined following BRADFORD (1976). For ascorbic acid determination, the formula of KELLER and SCHWAGER (1977) was used. Plant malondialdihyde contents were assayed as thiobarbituric acid (TBA) method and soluble sugar was determined with the anthrone reagent (CAKMAK and HORST, 1991). The gas exchange attributes were determined by an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). It was used for measurements of photosynthetic rate, transpiration rate ($E$) and sub-stomatal $CO_2$ concentration ($C_i$). The photosynthetic pigments were determined by following the method of ARNON (1949).

**Statistical analysis**
The data were analyzed for statistical differences by performing analysis of variance (ANOVA) test using statistical software package SPSS version 18.0. The Tukey-HSD Test was used to find significant differences between various treatments.

## Results

**Plant growth attributes**
The results show significant reduction in the fresh and dry biomass of fenugreek (*T. corniculata*) by increasing Cd application rates but this retardation was less pronounced in CSB treated soil (Fig. 1). In the absence of Cd, plants produced more fresh-biomass (32.35 g) than at 3 % CSB (23.3 g) and 0 % CSB (20.4 g). At all the levels of Cd (25, 50 and 100 mg Cd/kg soil) with CSB applications at 3 % and 5 %, the plants had highest values of fresh biomass than at sole 5 % CSB treatment. The plants which received 100 mg Cd/kg soil had lowest biomass (13.25 g).

**Photosynthetic pigments**
The effect of Cd with various application rates of CSB on photosynthetic pigments is depicted in Tab. 2. The increase in chlorophyll (a, b, total), carotenoids and anthocyanin content was observed by increasing the CSB application from 0 % to 5 %. The lycopene contents were not significantly affected by any of the CSB application rate. Similar results were observed at 50 and 100 mg Cd/kg soil + 5 % CSB for chlorophyll b, total chlorophyll, carotenoids and anthocyanin but significantly higher chlorophyll a and lycopene content were noticed at 100 mg Cd/kg soil.

**Physiological attributes**
The increasing application rates of Cd significantly decreased the physiological attributes of plants (Fig. 2). The lowest values of all the physiological attributes were observed at 100 mg Cd/kg soil. However, at different combinations of Cd (25, 50, and 100 mg Cd/kg soil) and CSB, the reduction in physiological parameters was very less which demonstrates the role of biochar in increasing photosynthetic pigments at various percentages. The maximum increase in photosynthetic rate, transpiration rate and sub-stomatal $CO_2$ concentration was observed at 5 % CSB.
The concentration of ascorbic acid was increased with an increase in Cd application from 0 to 100 mg Cd/kg soil which were significantly lower at 3 % and 5 % CSB amended soil at the same Cd levels. At 100 mg Cd/kg soil + 5 % CSB, the ascorbic acid concentration was not significantly different from plants treated with 100 mg Cd/kg soil. 3 % CSB + 0 mg Cd/kg soil treatment exhibited the lowest values of ascorbic acid.
The concentrations of MDA were also increased by increasing Cd level from 25-100 mg Cd/kg soil as compared to the control (0 mg Cd/kg soil). By applying CSB, the MDA concentration was signi-

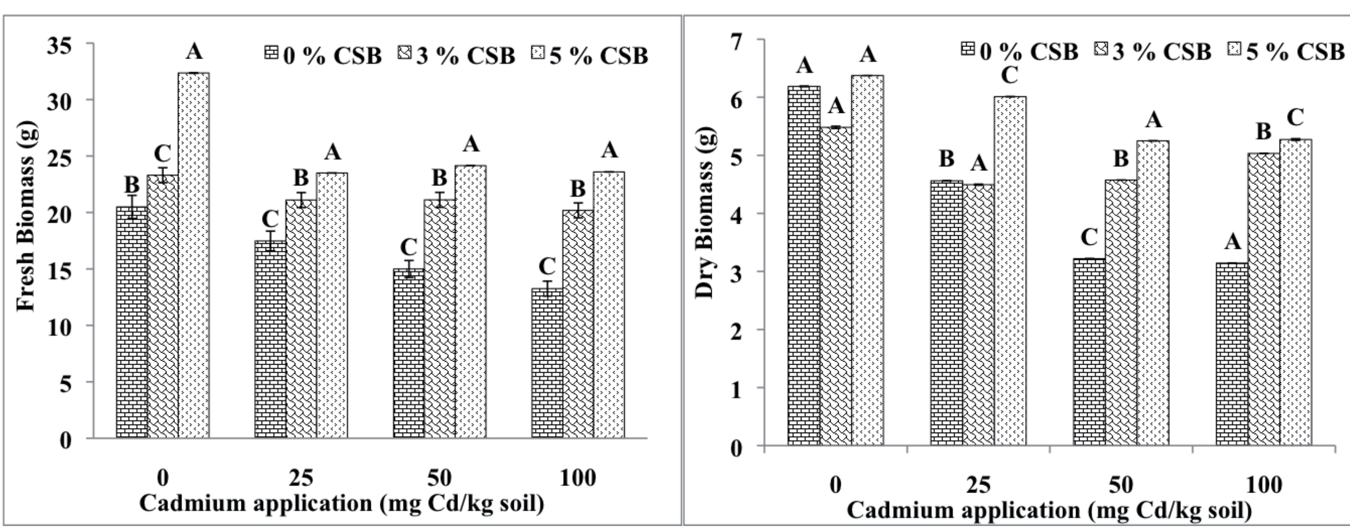


**Fig. 1:** Effect of various application levels of cadmium (0, 25, 50 and 100 mg Cd/kg soil) and cotton sticks biochar (CSB, 0 %, 3 % and 5 %) on fresh and dry biomass of fenugreek (*Trigonella corniculata*). The different letters on bars show significant differences (P ≤ 0.05) between biochar application levels within a cadmium application.

**Tab. 2:** Effect of various application levels of cadmium (0, 25, 50 and 100 mg Cd/kg soil) and cotton sticks biochar (CSB, 0 %, 3% and 5 %) on photosynthetic attributes of *T. corniculata*. The different letters within column show significant differences ($P \leq 0.05$) between biochar application levels within a cadmium application.

| Cd Treatment | Biochar application | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Carotenoids | Lycopene | Anthocyanin |
|---|---|---|---|---|---|---|---|
| mg Cd/kg soil | (%) | (mg /g fresh mass) | | | | | (umol/ml) |
| Control | 0 | 2.26 c | 0.71 c | 2.96 c | 0.51 c | 0.12 a | 0.12 c |
| | 3 | 2.47 b | 1.13 b | 3.60 b | 0.94 b | 0.12 a | 0.19 b |
| | 5 | 2.73 a | 1.37 a | 4.09 a | 1.19 a | 0.10 a | 0.23 a |
| 25 | 0 | 1.63 b | 0.99 c | 2.63 c | 0.69 b | 0.10 a | 0.14 b |
| | 3 | 1.09 c | 1.20 b | 3.08 b | 0.28 c | 0.06 b | 0.14 b |
| | 5 | 2.25 a | 1.21 a | 3.45 a | 1.09 a | 0.10 a | 0.20 a |
| 50 | 0 | 1.89 b | 0.70 c | 2.59 c | 0.53 c | 0.08 b | 0.11 c |
| | 3 | 1.88 c | 0.84 b | 2.71 b | 0.68 b | 0.07 b | 0.14 b |
| | 5 | 2.08 a | 1.18 a | 3.25 a | 0.89 a | 0.09 a | 0.18 a |
| 100 | 0 | 1.74 a | 0.44 a | 2.18 b | 0.46 b | 0.09 a | 0.10 b |
| | 3 | 1.67 c | 0.52 a | 2.19 b | 0.11 c | 0.08 c | 0.10 b |
| | 5 | 1.73 b | 0.88 a | 2.61 a | 0.62 a | 0.09 b | 0.13 a |

ficantly decreased at various levels of Cd. A similar trend was also observed in the case of sugar concentrations at various levels of Cd and biochar percentages (Fig. 3).

The protein concentration was increased with an increase CSB application from 3 % to 5 %. However, at different concentrations of Cd, the protein concentrations were decreased by increasing the Cd application. The CSB + Cd treatments showed significant differences from all other treatments. Similarly, soluble amino acids concentrations were also decreased by Cd stress but this decrease was less pronounced in biochar treated soil (Fig. 3).

### Cadmium concentration in plants

The application of 5 % CSB significantly reduced the Cd concentration in *T. corniculata*. Whereas, in the absence of CSB, Cd uptake was increased from 2.55 to 3.82 mg/kg DM by increasing the Cd level from 25 to 100 mg Cd/kg soil (Tab. 3).

## Discussion

The results of our study clearly depict the toxic effects of Cd on growth of fenugreek (*T. corniculata*) in terms of fresh and dry biomass of plants. However, the decline in growth due to Cd-toxicity was very less in plants grown in biochar-amended soil. JIANG et al. (2000) also reported high reduction in growth parameters such as biomass and leaf area after application of Cd to the garlic. The decline in growth in our study may be attributed due to toxic effects of Cd on various biochemical attributes of the plants (Fig. 2 and 3). PARK et al. (2011) noted an increase in dry biomass of mustard plants by 1 % application of chicken manure-derived biochar. Moreover, they found a significant decrease in Cd and Pb uptake by plants in biochar amended soils. Our results show no significant effect of higher application rates (3 % and 5 %) of CSB on the dry biomass of shoots and roots in the control (Cd free) treatment. However, when CSB was applied along with Cd-stress, the growth parameters were significantly enhanced. In our study, the photosynthetic rate of plants grown in CSB treatments along with Cd stress was not reduced. Chlorophyll contents were comparatively higher at 3 % and 5 % CSB applications with Cd. Decrease in pigment production by high Cd could be attributed to decrease in chlorophyll biosynthesis

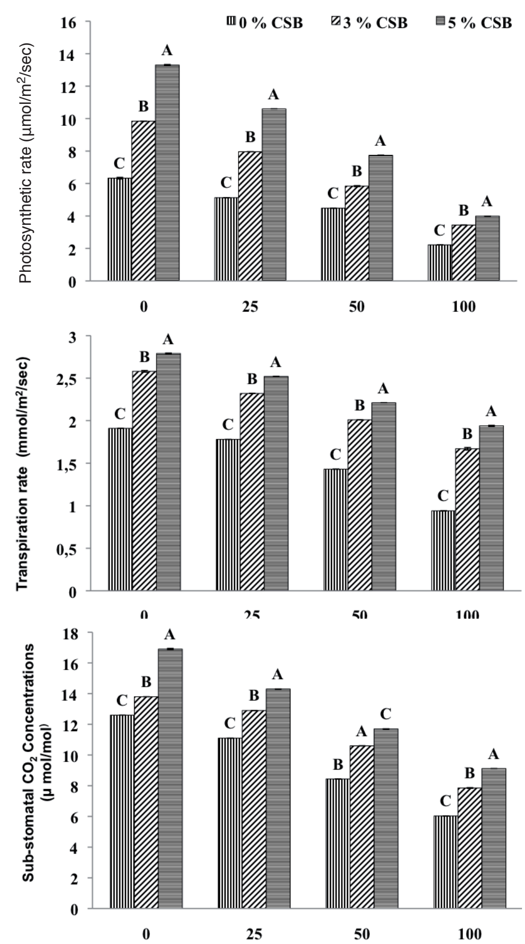


**Fig. 2:** Effect of various application levels of cadmium (0, 25, 50 and 100 mg Cd/kg soil) and cotton sticks biochar (CSB, 0 %, 3 % and 5 %) on photosynthetic rate, transpiration rate and sub-stomatal $CO_2$ concentrations of fenugreek (*Trigonella corniculata*). The different letters on bars show significant differences ($P \leq 0.05$) between biochar application levels within a cadmium application.

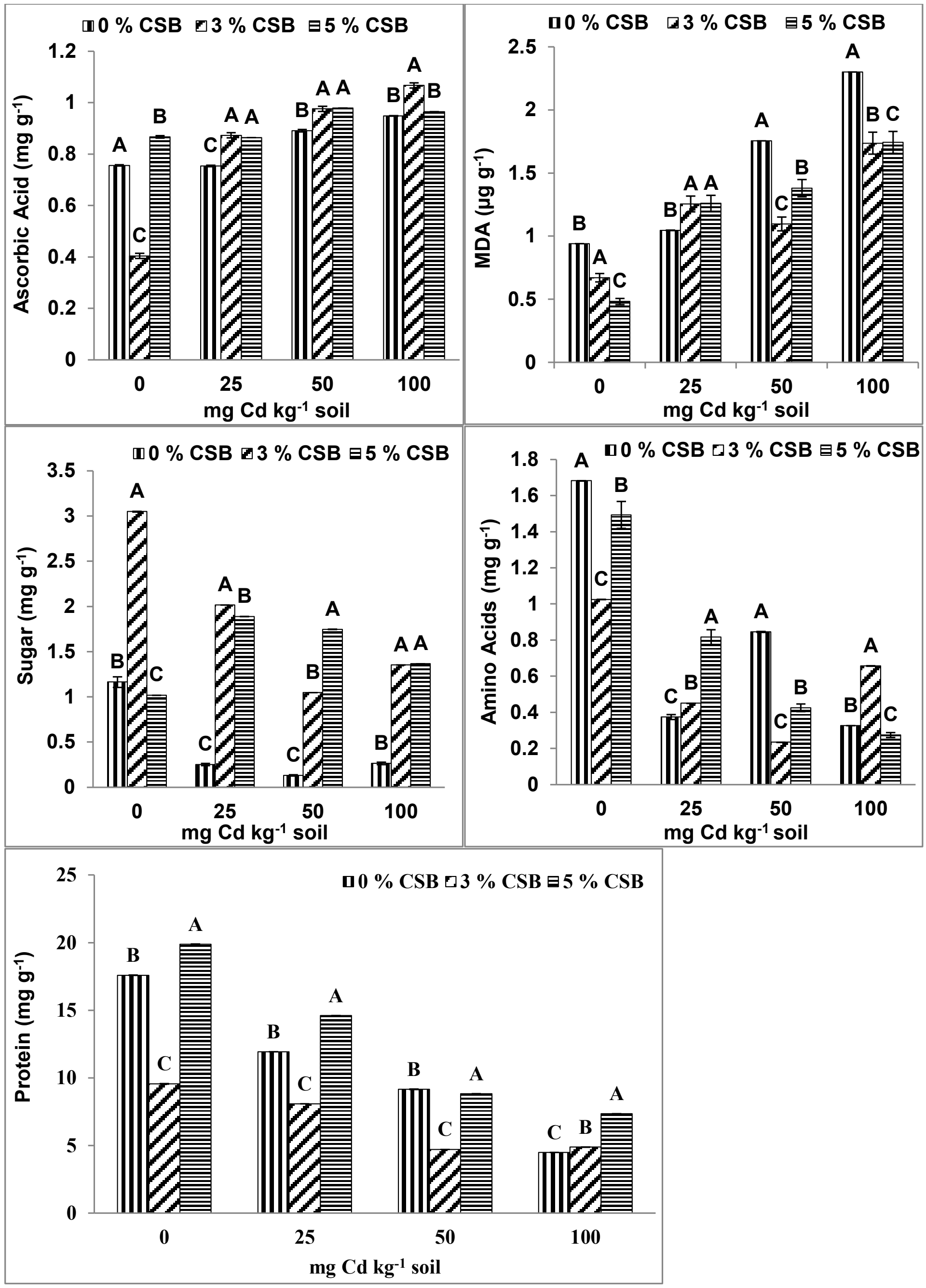


**Fig. 3:** Effect of various application levels of cadmium (0, 25, 50 and 100 mg Cd/kg soil) and cotton sticks biochar (CSB, 0 %, 3% and 5%) on biochemical attributes (Ascorbic acid, malondialdihyde, sugar, amino acids and protein) of fenugreek (*Trigonella corniculata*). The different letters on bars show significant differences ($P \leq 0.05$) between biochar application levels within a cadmium application.

by restriction of important enzymes (δ aminolevulinic acid dehydratase and protochlorophyllide reductase) involved in the production of chlorophyll. Similarly, Cd destructs the supply of Mg and Fe that are essential for chlorophyll manufacturing (KUPPER et al., 1996) and that would decrease photosynthetic rate, transpiration and sub-stomatal $CO_2$ concentrations.

The transport and bioaccumulation of metals in the plants could be affected by many factors such as light, temperature, transport mechanism, ionic strength and anoxic conditions (JOHN et al., 2009). High production of MDA is an indication of oxidative stress by heavy metals. An increased level of lipid peroxidation (high MDA production) at high concentrations of Cd can be an expression of metal toxicity due to the generation of alkoxy and peroxy radicals that cause oxidative damage to biological membranes through destruction of polyunsaturated fatty acids (DHIR et al., 2004). In our study, high production of amino acids and proteins was promoted by CSB application under toxic levels of Cd in tissues that indicates the activation of defensive mechanism against oxidative damage. Production of such compounds is an evolutionary comeback of plants to stress which has been observed in diverse groups of organisms (CHEN et al., 2001; WU et al., 1995).

The observed variations in the analyzed parameters clearly depict that increasing levels of Cd affect the growth and chlorophyll production in fenugreek (*T. corniculata*). Moreover, the enhanced production of MDA showed oxidative damage as a result of Cd toxicity. Biochar derived from cotton sticks has shown the capability to

**Tab. 3:** Effect of various application levels of cadmium (0, 25, 50 and 100 mg Cd/kg soil) and cotton sticks biochar (CSB, 0 %, 3 % and 5 %) on concentrations of cadmium in *T. corniculata*. The different letters within column show significant differences ($P \leq 0.05$) between biochar application levels within a cadmium application.

| Cd Treatment mg Cd/kg soil | Biochar %age | Cadmium concentration (mg Cd/kg dry mass) | |
|---|---|---|---|
| | | Root | Shoot |
| Control | 0 | 0.199 a | 0.166 b |
| | 3 | 0.133 c | 0.173 a |
| | 5 | 0.143 b | 0.129 c |
| 25 | 0 | 2.553 a | 2.613 b |
| | 3 | 1.679 c | 2.697 a |
| | 5 | 1.743 b | 0.455 c |
| 50 | 0 | 2.521 b | 2.031 b |
| | 3 | 2.546 a | 2.885 a |
| | 5 | 1.777 c | 1.480 c |
| 100 | 0 | 3.823 a | 3.032 a |
| | 3 | 3.029 b | 2.964 b |
| | 5 | 1.698 c | 1.299 c |

sequester metals in the rhizosphere and to inhibit its transfer to the aerial parts of plants. Protein and amino acids appeared to play a key role in the defense against cadmium toxicity under biochar application. Therefore, biochar can be a choice in situations where irrigation water contains appreciable amounts of cadmium as well as for utilization in abandoned soils contaminated with Cd.

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