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RESEARCH ARTICLE

Drought impact on Pb/Cd toxicity remediated by biochar in *Brassica campestris*

K. Fiaz[1], S. A, Malik[1], U.Younis[1*], S. Danish[2], M. H. Raza Shah[1], S. Niaz[1]

[1] *Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.*
[2]*Department of Soil Science, Faulty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan. *Corresponding author: uzma.botany@hotmail.com*

Abstract

Heavy metals toxicity in the human being is creating an alarming condition in the world. Not only are these metals largely effecting the growth of many plants but also the consumer's health. In sector of agriculture for many years Pakistan is facing the reduction in availability of edible oil. The demand is fulfilled by importation by spending huge cost. *Brassica campestris* L. is very important oil seed crop of Pakistan that is providing 31% of total oil seed production in Pakistan. But due to toxicity of metals like Cd and Pb its growth and yield is decreasing. Keeping in view the importance of *Brassica campestris L.* in Pakistan an experiment was conducted using Pollutary manure biochar as a reclaiming agent for heavy metals and an amendment as drought stress reducer to check the improvement in yield of *Brassica campestris* L. It is found that biochar application not only gave positive correlation in immobilization of Pb and Cd but also improves the plants growth and yield. Enhancements in the Photosynthetic and Accessory pigments are observed regarding good vegetative growth. Thus biochar can be utilized in reducing the metals uptake as well as growth promoter.

Keywords: Biochar, heavy metals, drought, photosynthetic pigments.

1. Introduction

Regular intake of such metal contaminated food can cause many diseases e.g cancer. Also these metals have tendency to do mutagenesis in the body of humans (Radwan and Salama, 2006). Mobilization of metals in soil and bioavailability of heavy metals in crops cultivated at contaminated sites is major contributor of these toxins (Uchimiya *et al.,* 2010). The fate of metals at contaminated sites is decided by the process of sorption and its efficiency (Vidal *et al.,* 2009). This term is used to illustrate the loss of solute from phase of liquid solution to the connecting solid phase (Sparks *et al.,* 1999). Metals accumulation especially Cd and Pb in various crops of Pakistan is one of major threat and burning issue for scientists. Soils in Pakistan contain Pb and Cd at sufficient levels (Ahmed *et al.,* 1994). In commonly found heavy metals which can cause drastic effects on health of animals is lead. In case of lead (Pb) the main source of its emission is burning of fossil fuels including sulfide ores melting (Bodek *et al.,* 1998). Accumulation of lead in the living bodies causes deficiency of many other trace elements. Although Pb, like many other heavy metals, has no prominent role in the body, its presence in the body can ultimately lead to deficiencies of other trace metals. Due to high solubility of Cd in water its toxicity to plants is becoming a great threat (Pinto *et al.,* 2004). Cd is a non-essential nutrient for growth of plants but in-spite of that reality it become accumulated in many crops via roots nutrients uptake channel. This high intake and accumulation of Cd reduced the photosynthesis activity and transpiration (Bazzaz *et al.,* 1974; Mahmood *et al.,* 2014). It also tremendously increases the respiration rate in many plants (Lamoreaux and Chaney, 1978; Lee *et al.,* 1976). Accumulation of Cd in soil also badly effect the germination rate (Larbi *et al.,* 2002) which reduced the effectiveness of photosynthesis and other process like respiration, transpiration (Krupa *et al.,* 1993; Larbi *et al.,* 2002). Mostly imbalance of nutrition is observed especially in case of iron (Fe) that becomes deficient in many crops due to Cd intake (Wallace *et al.,* 1992; Larbi *et al.,* 2002) while uptake of Mn, K, Mg and Ca is also disturbed when Cd is up taken by plants at toxic level beyond threshold level (Greger *et al.,* 1991; Larbi *et al.,* 2002; Dong *et al.,* 2006; Khanmirzaei *et al.,* 2013). At cellular level the activation and inactivation of many enzymes with various interactions of electron transport chain with nucleic acid become disturbed due to oxidative stress which is imposed by Cd (Chaoui *et al.,* 1997; Chen *et al.,* 2003a). In such conditions Cd basically replace the Zn, Fe and Mn to create that stress that badly effect the plants growth (Smeets *et al.,* 2005; Lin *et al.,* 2007). Also the deficiency of water in upcoming time is a headache for farmers to cultivate crops because drought stress is also very important factor that reduce the yields of crops up to many folds. To overcome the problem of metals toxicity and drought stress due to shortage of water usually organic amendments are recommended. Biochar as soil amendment is one of such organic nature reclaiming agent that can be effectively used to minimize the intake amount of heavy metals by crops in contaminated sites by process of immobilization (Verheijen *et al.,* 2010). Biochar is actually a black carbon organic compound (Verheijen *et al.,* 2010) which can be utilized as fertilizer. It is a fine-grained and porous substance that is considered as a soil conditioner which that can enhance the pH, CEC, water holding capacity of soil (Gundale and DeLuca, 2007; Amonette and Joseph, 2009). It remains in soil for very long time (Downie *et al.,* 2011) as it is very resistant to decomposition (Thies and Rillig, 2009) as compared to organic matter. It is produce in the limited supply of oxygen by creating almost anaerobic conditions through following the process referred as pyrolysis (Schnitzer *et al.,* 2007; Tagoe *et al.,* 2008). In pyrolysis heating volatile carbon, methane hydrogen and carbon mono oxide gases are released from C containing waste biomass used for biochar manufacturing. These volatile C compounds can be collected as bio oil by condensation of vapors in the form of alcohols, oils, tars and acids (Antal and Grønli, 2003). The rest of prepared biochar material contains C, H, O, Ca, N, K and ash. As a result the emission of greenhouse gases become condensed and their release in environment is decreased (Lehmann *et al.,* 2006). The temperature range can be varied from 450-650^{0}C (Sohi *et al.,* 2009) in conversion of carbon containing biomass into biochar during pyrolysis. Addition of biochar also retain the nutrients in soil especially nitrogen that improve the vegetative growth of most crops and increase the efficiency of fertilizer usage (Steiner *et al.,* 2008). Nutrients like P, Ca and K that accumulates in the biochar and other waste materials during pyrolysis improve the growth of many plants by providing these nutrional elements in supplement amount (Glaser *et al.,* 2002). Biochar has capability to immobile the bioavailable metals that are readily available to those plants which are cultivated in metals contaminated soils. Biochar can reduce the availability of heavy metals due to their metals sorption ability (Beesley *et al.,* 2010, Uchimiya *et al.,* 2010) which is provided to it by its structural modification done during pyrolysis.

2. Materials and Methods

2.1. Soil and Biochar

Sand was collected from the local area of Multan (Punjab, Pakistan). The texture of soil was sandy. In chemical properties soil has pH = 7.3. ECe of soil was = 0.210 dS/m when there was no fertilizer or biochar added. Minutes fractions of organic matter was found in sand having value of 0.27%. Pollutary waste for the production of biochar was collected from the local market. This waste material was spread on the plastic sheet and air dried. After air drying of waste material it was filled in the inner drum of pyrolyzer. The pyrolyzer was also covered with an outer drum to reduce the escape of heat as well as for safety measures. After filling the inner drum it was made air tight with cap of drum so as we could create anaerobic conditions. The burner was then burnet under the drum and temperature was increased up to 425^{0}C. After that it was kept at that temperature for half an hour. The burner was then closed and material drum was left in air for cooling. When drum become cool enough which could be touched by hand easily then all the biochar was removed from inner drum. The biochar was further grinded on grinder at 5mm size. After that it was packed in air tight plastic bags for further usage. The physiochemical properties of Pollutary waste biochar in provided in Table 1.

Table 1. Chemical properties of Biochar.

Chemical properties of Biochar

	Biochar	
pH	-	8.51
EC	dS/m	1.32
TSS	meq./L	13.2
Ash Content	%	44.7
Volatile Matter	%	32.1
Fixed Carbon	%	23.2

2.2. Plants Growth and Soil analysis

There were 2 rates of Pollutary waste biochar application (0% and 1%) were used in presence and absence of artificially introduced heavy metals Cd and Pb in 5kg sandy soils. Conduction of experiment was done as complete randomized design (CRD). Purchasing of *Brassica campestris* was done from local market and 6 seeds were sown in each pot that was later on thinned and 2 healthy plants were maintained. When plants become matured then harvesting was done with determination of parameters like stem length, root length, no, of flower and leaves, stem & roots fresh weight were measured. For further chemical analysis samples of roots, leaves, flower and stem were oven dried at 65^{0}C. Oven dried mass of stem, root, flower and leaves were measured on electronic balance. After that root, leaves and stem samples were crushed in grinder and saved in air tight plastic bags. During harvesting soil samples were also collected that were oven dried for further chemical; analysis at 105^{0}C. At the time of harvesting the soil samples that were collected from the pots were also oven dried at 105 ^{0}C. By passing from 2mm sieve the soil samples were also packed in the plastic bags that were air tight for future use. The organic matter of soil was calculated by using the methodology of Walkly-Black (Jackson, 1962). Soil pH was determined according to Schofield and Taylor (1955) while Total Dissolved Salts (TDS) in soils were calculated according to the USDA Handbook No. 60 page 12. Chemical properties of soil are given in Table 2.

Table 2. Chemical properties of Soil.

Soil		
pH_s	-	7.63
EC_e	µS/cm	926
TSS	meq./L	9.26
OM	%	0.45

2.3. Biochemical Attributes Analysis in Plants

Digestion of plant samples was done with HNO_3-$HClO_4$ acids for the determination of Cd and Pb on atomic absorption spectrophotometer, phosphorous on spectrophotometer (by Ammonium Vanadate-Ammonium Molybdate yellow color method) and potassium on flame photometer while for nitrogen determination H_2SO_4 digestion following kjeldahl distillation methodologies was adopted. For the Photosynthetic and Accessory pigments analysis in *Brassica campestris* methodology of Arnon (1949) and Ravelo-Pérez *et al.* (2008) was used.

2.4. Biochar Analysis

EC and pH of biochar was calculated according to Schollenberger and Simon (1945). McLaughlin (2010) methodology was used for the determination of volatile matter and ash content in biochar. The fixed carbon content was calculated using equation given by Noor *et al.*,. FC (%) = 100 – (%VM + %AC).

2.5. Statistical analysis

Statistical analyses were done for growth parameter of plants *Pisum sativum* separately using SPSS version 18.0 statistical software program in which treatments were compared by following Tukey-HSD test.

3. Results and Discussions

3.1. Plant growth parameters and metals intake

Results indicate that application of Pollutary waste biochar at 1% rate in metals contaminated soils significantly ($P < 0.05$) improve the plants growth. Data shows that Pollutary waste biochar enhance the plants stem length, root length (Figure 1 B), roots fresh weight (Figure 1 A), leaves number (Figure 1 C), as compared to non biochar amended *Brassica campestris*. The half irrigated plants also show improvement in growth due to biochar addition but maximum values were recorded in full irrigated biochar containing pots plants. Ouzounidou *et al.* (1995) reported that the uptake of heavy metals not only causes the chromosomal aberration but also division of cell become abnormal that significantly decreased the plants growth. Abiotic stresses like heavy metals induced stress can be demonstrated in term of dry biomass because dry biomass is the total outcome of different characters. Biochar application improves that dry biomass that indicates reduction in metals induced stress which is achieved by biochar that immobilized the metals in soil. According to Beesley and Marmiroli (2011) sorption is the mechanism through which we can immobilize these toxic metals by biochar addition. Bilgic and Caliskan (2001) suggested that π-electrons play a vital role in the immobilization when biochar is applied. These π-electrons are part of aromatic functional groups like –OH,–COOH and C=N. Uchimiya *et al.* (2010b) suggested the semi sorption of surface by d electrons in assistance with π-electrons that is major cause of heavy metals mobility reduction due to biochar addition. Similar trend of metals intake was observed by Park *et al.* (2011) where he successfully immobilized the metals using sludge biochar as that in our experiment where Pollutary waste biochar significantly decrease the Pb and Cd intake in *Brassica campestris.* While in non biochar plants the decrease in growth may be correlated with destruction of chlorophyll structure that reduce the photosynthesis and respiration. Also less water availability in non biochar pots may be one of basic reason of plants growth restriction as compared to biochar grown plants where biochar improves the water holding capacity. Biochar usage as soil amendment enhances the nutrients bioavailability that is shown in (Figure 2).

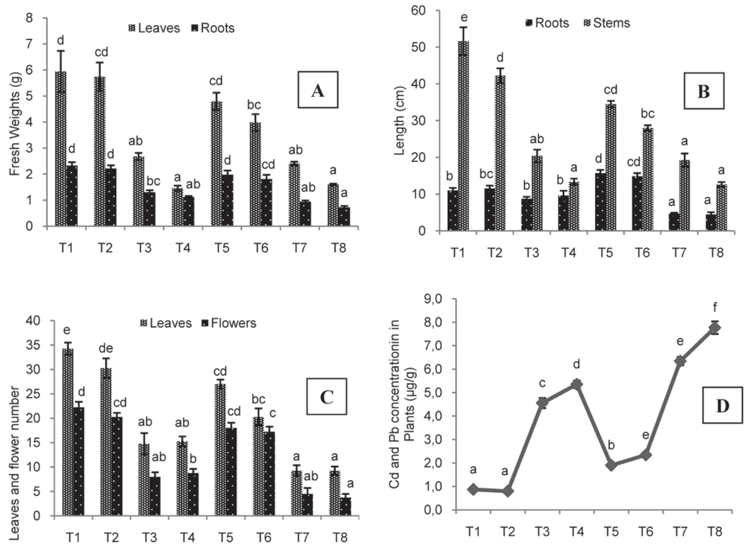

Figure 1. Impact of cadmium and lead treatments on various attributes of *Brassica compestris* under biochar, A) Fresh weights of leaf and root; B) Root and stem length; C) Leaves and flower number; D) Lead and cadmium concentrations in *Brassica compestris*. Note: Values having different letters significantly different from one another.

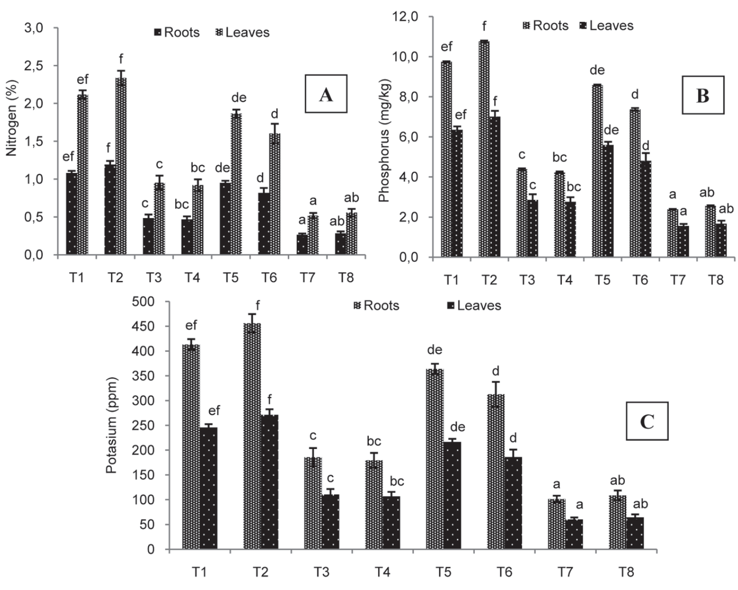

Figure 2. biochar application effect on concentrations of nitrogen (A), phosphorus (B) and potassium (C) in roots and shoots of Brassica compestris grown in lead and cadmium contaminated soils. Significant ($p \leq 5\%$) differences between treatments within each paramenter are indicated with different letters.

3.2. Photosynthetic and Accessory pigments

A significantly positive correlation ($P < 0.05$) is also observed in production of photosynthetic and accessory pigments by Pollutary waste biochar addition (Table 3). Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanin production was less in those *Brassica campestris* that were grown in non biochar amended soils. More availability of nutrients especially nitrogen in biochar containing soils enhances these pigments production as well as vegetative growth (Lehmann *et al.,* 2006) except the lycopene that is an anti-oxidant. Lycopene concentration was high in those plants where biochar was not added and metals induced the stress. According to De Filippis and Pallaghy (1994) reduction in protochlorophyllidereductase enzyme activity due to heavy metals toxicity is basic reason of less production of Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanin. Cd actually inhibits the photo activation of photosystem-II. This inhibition is the major cause of less pigments production in plants when they are grown in Cd contaminated soils.

Table 3. Production of photosynthetic and Accessory pigments in Brassica campestris under Cd and Pb treated soils with biochar. Note: Values sharing same letters are same, whereas, values sharing different letters are significantly different from one another.

		Treatments							
Parameters	**Units**	**T1 B+Cd+Fi**	**T2 B+Pd+Fi**	**T3 Cd+Fi**	**T4 Pd+Fi**	**T5 B+Cd+Hi**	**T6 B+Pd+Hi**	**T7 Cd+Hi**	**T8 Pd+Hi**
Chlorophyll a	mg/g	2.79 f	2.58 e	1.16 b	1.16 b	2.37 d	2.14 c	0.95 a	1.01 ab
Chlorophyll b	mg/g	7.28 d	7.23 d	4.77 b	3.02 a	5.43 bc	6.08 c	2.48 a	2.64 a
Total Chlorophyll	mg/g	10.75 e	10.29 de	5.06 b	4.91 ab	9.53 cd	8.94 c	4.41 ab	3.88 a
Carotenoids	mg/g	0.27 d	0.26 d	0.17 b	0.15 b	0.24 d	0.214 c	0.12 a	0.12 a
Anthocyanin	µmol/ml	0.11 e	0.09 d	0.04 ab	0.04 b	0.08 cd	0.07 c	0.03 ab	0.03 a
Lycopene	µg/g	0.30 a	0.30 a	0.62 cd	0.64 cd	0.50 b	0.52 bc	0.72 d	0.73 d

Note: Fi = Full irrigation and Hi = Half irrigation, B= Biochar, Cd = Cadmium, Pb = Lead.

4. Conclusions

Study indicates that the application of Pollutary manure biochar significantly decrease the metals uptake in *Brassica campestris* with significant improvement in growth. Biochar not only improve the soil physical and chemical conditions but also enhance the availability of nutrients especially nitrogen that usually loss due to leaching or volatilization in sandy soils. Photosynthetic and accessory pigments production is increased in biochar treatment due to more availability of nitrogen and less metals intake. Less production of antioxidant Lycopene in the plants indicates the reduction in metals induced stress.

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