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Effects of biochar applications on tomato plant under cadmium metal stress and on soil cadmium characteristics

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Abstract: A greenhouse experiment was carried out in order to determine the effects of biochar on soil Cd fractions, Cd mobility and bioavailability, Cd concentration and uptake of tomato plant, and plant stress indicators. In the factorial experiment design Cd metals and biochar were applied to the experimental soil. After incubation of 3 months, tomato plant was grown and experimental soil were analyzed. The bioavailable Cd content in the soil and the soluble and exchangeable amounts of Cd in the soil fractions, Cd content and Cd uptake in the plant were significantly increased by Cd applications to the soil. Cd metal applied to the soil increased the stress indicators in tomato plant and decreased the plant biomass values significantly. Biochar applications significantly reduced the soluble and exchangeable forms of Cd and metal mobility factor of Cd in the soil. Depending on biochar applications, biomass values of the tomato plant was increased, and Cd concentration and Cd uptake of plant were decreased.

Keywords: Biochar, Cadmium, Tomato

1 Introduction

Heavy metal pollution in soils is among the most important agricultural and ecological problems that threaten the sustainable fertility of soils, food safety, environment and public health. Metal pollution is becoming an increasingly important problem in the world's soils due to the participation of heavy metal emissions in agricultural soils from mostly non-point and wide sources, and their non-biodegradable properties in nature. There are many technical and

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economic limiting factors in the applicability of many proposed methods for removing metal pollution in soils. In addition to phytoremediation of metals, stabilization of the soil with various soil conditioners is among the leading alternative solutions in reducing metal pollution.

Biochar is a porous and high carbon product obtained by pyrolysis of various organic substances. Biochars have chemically high cation exchange capacity and alkaline chemical character. Among the most important features of biochars are their large surface areas and their ability to adsorb heavy metals (Lu et al., 2012). Biochar applied to the soil can significantly reduce the mobility of heavy metals in the soil (Park et al., 2011) and the amount of heavy metal transported to the plant (Namgay et al., 2010). With these features, biochar is gaining increasing interest in the reclamation of contaminated soils. Many soil factors such as pH value, cation exchange capacity, lime, organic matter, clay type and texture play a role on the bioavailability of metals in soil. It has been widely reported in the literature that biochar promotes plant growth and is effective on metal fractions in soil (Topcuoğlu, 2022). Due to the adsorptive effects of biochar on soil metals, it is thought that biochar has the potential to be used in the remediation of soils contaminated with heavy metals (Topcuoğlu, 2022).

Heavy metals are an important abiotic stress factor that affects the primary and secondary metabolites of plants through biochemical and physiological ways and limits the life cycle of the plant (Kisa et al., 2019). Plants have developed a wide variety of metal tolerance mechanisms to adapt themselves to existing conditions in order to survive under metal stress conditions (Gratão et al., 2019). Various aromatic phenolic compounds that plants produce in their tissues to adapt to adverse environmental conditions have an important function in reducing various environmental stresses they encountered. The highly accumulation of phenolic compounds was reported as a response to abiotic stress conditions (Piccolella et al., 2018). Proline is kind of amino acid that accumulates in plants in response to stress conditions (Jogawat, 2019). In this regard, phenolic compounds and proline can be used as a stress indicator in plants.

The aim of this study was to determine the effects of Biochar on Cd bioavailability, Cd fractions and mobility, growth and metal uptake in tomato plants and plant stress factors in Cd applied soil.

2 Materials and Methods

2. 1. Soil characterization and analysis

The uncontaminated soil used in this experiment was sampled from a red mediterranean soil, representative of the major agricultural areas of Turkey Antalya. Experimental soil was taken from a depth of 0-30 cm, air dried, sieved by 2 mm. The main analytical characteristics of the experimental soil are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation (C.E.C., 1986).

Table 1. The analytical characteristics of the experimental soil before treatments.

Parameters		**Parameters**	
Texture Grade	Clay Loam	Total N, %	0,112
Sand, %	24	P (ex), mg kg^{-1}	5,7
Silt, %	48	K (ex), mg kg^{-1}	139
Clay, %	27	Ca (ex), mg kg^{-1}	1356
pH- H_2O (1:5 w/v)	7.78	Mg (ex), mg kg^{-1}	148
$CaCO_3$, %	26,2	Total Zn, mg kg^{-1}	144 (150-300)*
Free CaCO3, %	2,3	Total Cu, mg kg^{-1}	18 (50-140)*
Organic matter, %	1,53	Total Ni, mg kg^{-1}	5 (30-75)*
CEC, cmol kg^{-1}	26,2	Total Pb, mg kg^{-1}	4 (50-300)*
EC, dS m^{-1} 25°C	0,96	Total Cd, mg kg^{-1}	< 0,001 (1-3)*

***: Metal limits in soil, mg kg-1 dry wt (C.E.C., 1986).**

Soil texture was determined by the hydrometer method, the soil pH was measured by the $CaCl_2$ method, organic matter content, as determined by the Walkley-Black method, $CaCO_3$ was determined by Scheibler Calcimeter, free $CaCO_3$ was determined by ammonium oxalate method, the total Zn, Cu, Ni, Pb and Cd contents of the soil were digested by the aqua regia method (1:3 HNO_3/HCl). Total soil metal concentrations were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

2. 2. Preparation of biochar for experiment

The biochar material used in the experiment was obtained from orchard pruning waste. Biochar production was obtained by pyrolysis of 0.5-1 cm ground waste biomass and then pyrolysis at 550 °C in an oxygen-free environment and then regrind. The C/N ratio of the biochar used in the research was determined as 185/1 and the pH value as 7.4. The particle size of the material was less than 1 mm.

2. 3. Experimental design

An experiment was conducted in randomized complete block design including 2 levels of Biochar (1 control and 1 treatment) and 2 levels (1 control and 1 treatments) of Cd metals with 5 replications. Ten kilograms of air-dried and sieved soil were filled into plastic pots. A pot-plate was placed under each pot to prevent leaching. Cadmium metal was added to experimental soil as metallic salt solutions ($Cd(NO_3)_2$). Cadmium concentrations were designed to maintain Cd polluted soil, 10 fold of maximum metal limits of European Union (C.E.C., 1986) as 0 and 30 mg kg^{-1}. Biochar were added to pot soil at 400 g kg^{-1} (4%) rate. A uniform application was obtained by homogenization of the soil. The soil was subsequently incubated in the greenhouse conditions for 3 months before experiment. During this period the soil was irrigated 1-2 times in a week with deionised water to maintain field capacity level of water. After incubation, soil samples were taken from each pot for the analyses.

In the experimental soil, available plant nutrients were not sufficient and there was a need for basic fertilization requirement at the agronomic level for the optimum growth of the Tomato plant. After incubation period, basic N-P-K fertilization was applied to experimental soil at the rate of 100, 500 and 100 mg kg^{-1} of N (as NH_4NO_3), P (as KH_2PO_4) and K (as K_2SO_4).

2. 4. Soil analysis after incubation

Bioavailable Cd: The bioavailable metal fractions were extracted from the soil with diethylenetriaminepentaacetic acid-$CaCl_2$-triethanolamine adjusted to the pH 7.3 (DTPA) procedure.

Sequential extraction: Sequential extraction method (Tessier et al., 1979) was applied to soil samples to identify metal fractions. The heavy metal sequential extraction procedure had the following steps:

F1: 1 M $MgCl_2$ (1:8 w/v, pH 7) for 1 h at room temperature; metals in soil solution and in exchangeable forms.

F2: 1 M NaOAc (1:8 w/v, pH 5) for 5 h at room temperature; metals mainly in the carbonate fraction.

F3: 0,04 M NH_2OH/HCl in 25% (v/v)HOAc (1:20 w/v) for 6 h at 96 °C ; metals associated with Fe and Mn oxides.

F4: 3 ml 0,02 M HNO_3 + 5 ml 30% H_2O_2 (pH 2) for 3 h at 85 °C; metals associated with organic matter.

F5: HNO_3-HCl digestion; residual fraction.

To determine the bioavailable and sequentially extracted metal concentrations, soil samples were digested in aqua regia (1:3 HNO_3/HCl) according to the international standard. Zn, Cu, Ni, Pb, and Cd concentrations of greenhouse soil samples were analyzed using ICP-MS under optimized measurement conditions and values adjusted for oven-dried material (at 105 °C for 12 h).

Soil Metal Mobility Factor: The mobility of the soil metal was calculated based on the theoretically mobile fractions in the sequential extraction procedure in which the metals were softly bonded to the solid phases. The relative metal mobility index was calculated as a "mobility factor" (MF) based on the following equation (McGrath, 1984):

$$MF: \frac{(F_1 + F_2 + F_3)}{(F_1 + F_2 + F_3 + F_4 + F_5)} x100$$

Heavy Metal Transfer Factor: Soil-to-plant transfer is one of the key components of human exposure to metals through food chain. Heavy metal transfer factor (TF) is a parameter used to describe the transfer of heavy metals from soil to plant body. The TF of metals from the soil to shoots and roots of the plants was defined as the ratio of the heavy metal concentration in the plants to that in the soil.

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows (Romkens et al., 2002):

$$TF = \frac{c\ plant}{c\ soil} \qquad (1)$$

where: C_{Plant} is heavy metal concentration in plant tissue, mg kg-1 dry weight; and C_{Soil} is heavy metal concentration in soil, mg kg-1 dry weight.

Metal uptake: Theoretical total metal uptake was calculated using Eq. 2, as follows (Stevenson, 1994):

$$Metal\ uptake\ (mg\ pot^{-1}) = C\ x\ W\ x\ n \quad (2)$$

where: C is heavy metal concentration in plant tissue, mg kg^{-1} dry weight; and W is plant dry weight kg $plant^{-1}$, and n is number of plant.

2. 5. Plant growth and analysis

Seeds of tomato plant (*Lycopersicon esculentum*) were disinfected by sodium hypochlorite solution of 5% during a few minutes and then rinsed in the distilled water before sowing to substrate medium. The Seeds were germinated in peat + perlite substrate mixture. Then, 1 seedling of each plant was transplanted into pot soil. All plants were grown under greenhouse environmental conditions. During the experiment, plants were irrigated regularly and treated according to common agrotechnical principles. After 60 days of growth all plants were harvested. Shoots and roots of plant samples were rinsed briefly in deionised water and were dried at 65 ºC in a air-forced oven, ground and then digested in aqua regia (1:3 HNO_3/HCl). Total plant and plant metal concentrations were analysed by using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

2. 6. Determination of plant stress parameters

Total polyphenol content in plant was determined according to the method of Folin-Ciocalteu phenol reagent and contents expressed as mg EAG g^{-1} DW (Singleton and Rossi, 1965). The flavonoid content in plant was determined by the aluminum trichloride method (Zhishen et al., 1999) and the flavonoid contents were expressed as mg EQ g^{-1} DW. Proline content in plant was determined according to the method of Bates et al. (Bates et al., 1973) and contents expressed as mg kg^{-1} DW.

2. 7. Statistical analysis

ANOVA test ($p \leq 0.05$) calculated using the statistical package $SPSS_{23}$ for Windows program were applied to compare the differences in parameters.

3 Results and Discussions

3. 1. Bioavailable (DTPA extractable) metal content of the soil

Before applications, the experimental soil generally has slightly alkaline reaction, moderate CEC, low EC values, high level of lime and low level of free $CaCO_3$ contents. The physical and chemical properties of the experimental soil are within the acceptable range of normal agronomic values, and the heavy metal concentrations are below the EU-specified levels (C.E.C., 1986). The bioavailability of metals was expressed in terms of DTPA extractable concentrations, meaning that the metals can be easily taken up by plants. Metal applications to the soil sharply increased the amount of bioavailable metals (Figure 1). Biochar applications resulted in significant reductions in DTPA extractable Cd concentrations in Cd-treated soil. The effect of biochar on DTPA extractable metals in metal-applied soil was higher than that of the control soil. Biochar applications significantly reduced the bioavailable form of metals in soil. In this regard, it has been reported that biochar applied to soil significantly reduces $CaCl_2$-extractable metals (Dai et al., 2018).

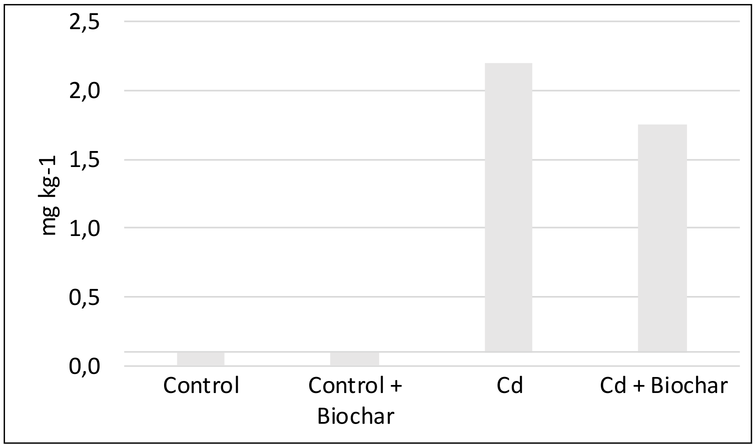

Figure 1: DTPA extractable metal concentration in control soil and biochar treated soil.

3. 2. Soil metal fractionation and metal mobility

The ratios of Cd in soil fractions are presented in Figure 2. The distribution of metals in the metal-free natural agricultural soil (control treatment) showed that the largest percentage of all metals was found in the residual fraction and a small percentage was found in F1, F2 and F3 fraction. The residual phase represents metals largely embedded in the crystal lattice of the soil fraction, showing that these metals cannot move under normal conditions (McGrath, 1984). In the control treatment, the distribution of Cd metal in the soil followed the following order for the metals studied: F1< F2< F3< F4< F5.

The metal mobility factor (MF) term describes the potential mobility of metals in the soil. Biochar applications to metal-free control soil decreased the mobility factor value of Cd metal (Figure 3). As Cd applications to the soil naturally give soluble metal salts, MF values of Cd sharply increased. Biochar applications significantly reduced MF values in Cd-treated soil. The decrease in the mobility of metals in biochar-treated soils can be explained by the adsorption of metals on the active surfaces of biochar. In this regard, it has been reported that biochar applications increase the metal adsorption in the soil and decrease the concentration of bioavailable metals (Awad et al., 2021; Soria et al., 2017).

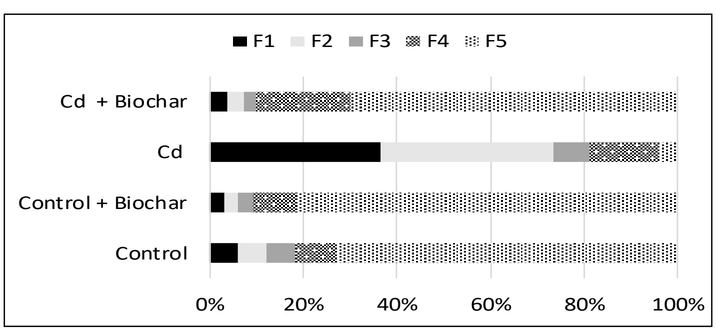

Figure 2: Relative distribution of soil Cd fractions in treatments.

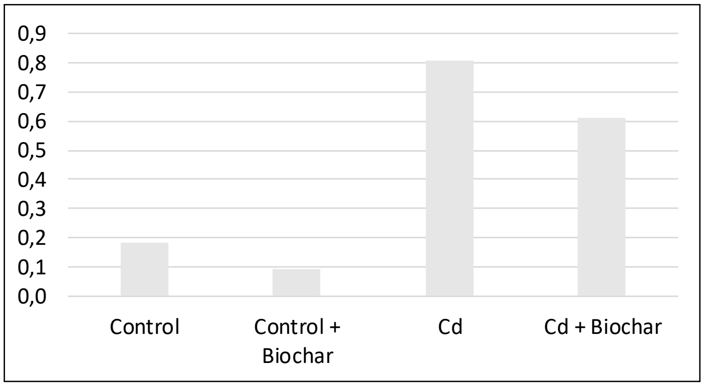

Figure 3: Mobility factor of Cd in treatments.

3. 3. Plant growth and heavy metal concentration of plants

Cd metal applications have statistically significant effect on dry matter (biomass) value of the tomato plant. Plant dry matter was decreased by Cd applications (Figure 4). Dry matter values of tomato plant were found to be higher in biochar applications. It is known that the tomato plant can contain heavy metal elements at high levels and adapt to levels that can be toxic to many plants. Total metal concentrations both in the shoots and roots of plant were increased by the increasing amounts of Cd treatments. Metal concentrations of tomato in the root tissues was found higher than that of shoots. Biochar applications to soil decreased Cd concentration both in shoot and root tissues of plant.

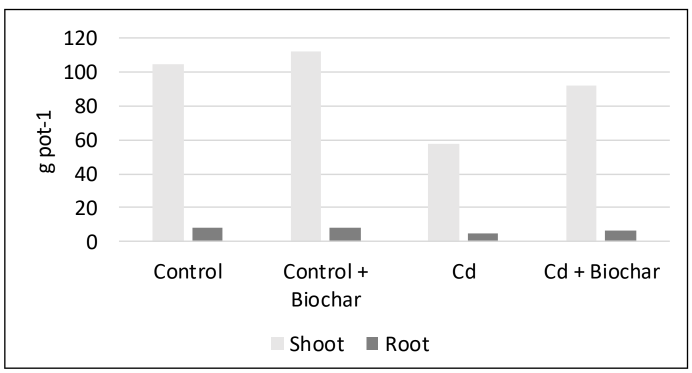

Figure 4: Shoot and root dry matter (DM) amount of tomato plant in treatments.

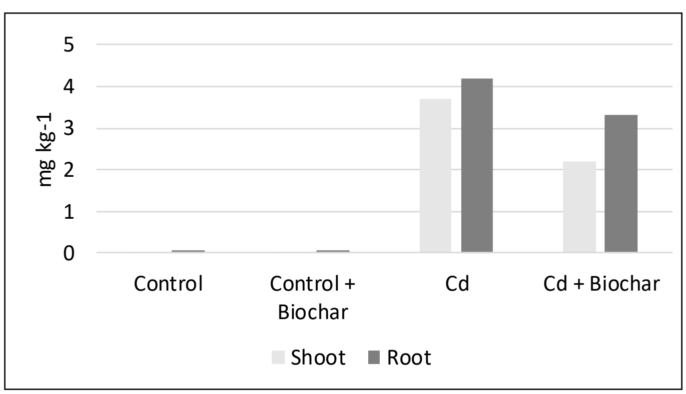

Figure 5: Cd contents of tomato plant in treatments.

3. 4. Metal transfer factor (TF) and metal uptake (MU) of plants

Higher TF values were determined in control treatments. TF of Cd were decreased by Cd treatment (Figure 6). Biochar applications decreased the TF value of the Cd metal, especially in root tissue of plant. This results also show the remarkable effects of biochar applications on metal stability and metal transfer to plant. MU value was higher in shoot tissue of plant and as expected, metal uptake of plants was increased by Cd application. Biochar application decreased MU of tomato plant (Figure 7). Metal uptake rate of Cd was increased about more than 50 fold by the treatments compared to control. The decrease in TF values due to Cd applications is considered to be due to the relative decrease in the uptake of Cd metal applied to the soil at a high level.

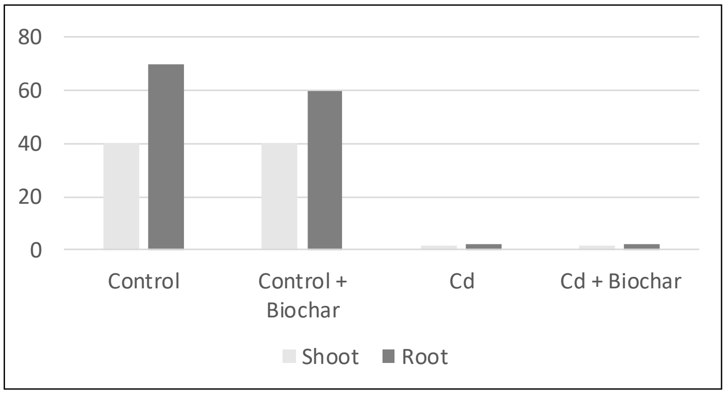

Figure 6: Metal transfer factor of tomato plant in treatments.

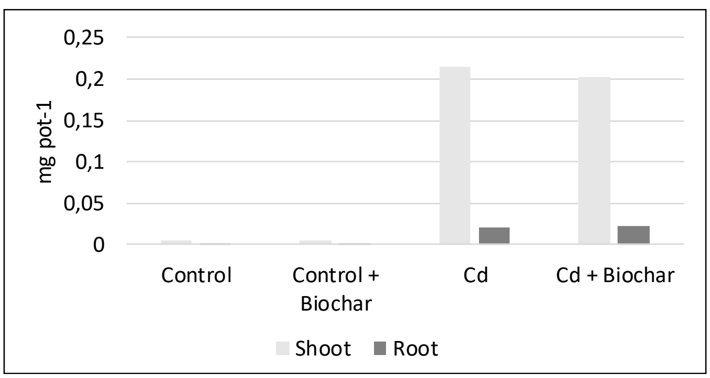

Figure 7: Metal uptake of tomato plant in treatments.

3. 5. Variation in plant stress parameters in treatments

Total polyphenol, flavonoid and proline contents of tomato plant increased with Cd applications to soil. The concentration of these three compounds was determined at higher levels in the shoot tissues of the plant. Biochar applied to the soil significantly reduced these stress indicators in the tomato plant (Figure 8, 9 and 10). The increase in total polyphenol, flavonoid and proline contents of tomato plant due to Cd metal applications shows that the plant is exposed to significant stress, which also caused a decrease in the dry matter value of the plant. The accumulation of phenolic compounds in tomato plant under heavy metal stress conditions has been reported by many studies (Khanna et al., 2019; Ullah et al., 2019).

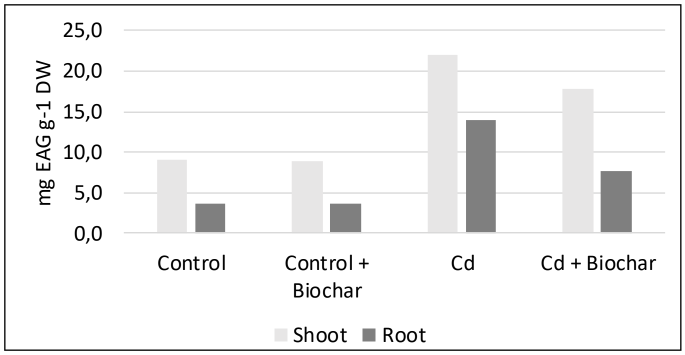

Figure 8: Total polyphenol contents of tomato plant in treatments.

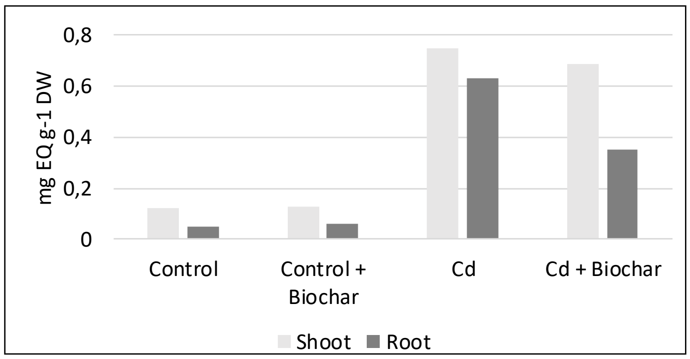

Figure 9: Total flavanoid contents of tomato plant in treatments.

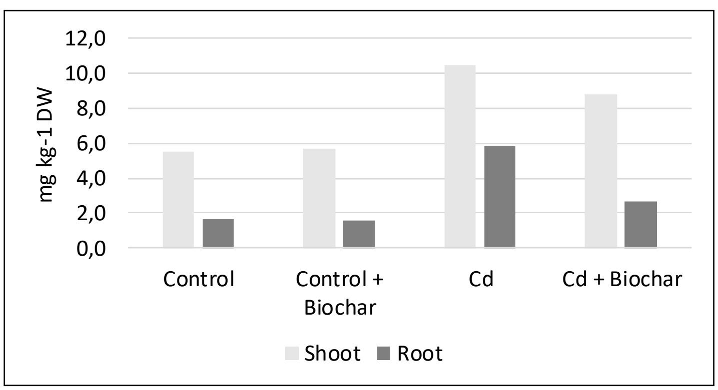

Figure 10: Total proline contents of tomato plant in treatments.

4 Conclusion

Cd metal applied to the soil increased the stress indicators in tomato plant and decreased the plant biomass values significantly. The bioavailable Cd content in the soil and the soluble and exchangeable Cd amounts in the soil fractions, the Cd content and the Cd uptake in tomato plant significantly increased depending on the Cd applications to the soil. Biochar applications significantly reduced the soluble and exchangeable forms of Cd fractions and Cd mobility factor in Cd applied soil. Depending on the biochar applications, the biomass values of the plant was increased, Cd concentration and Cd uptake were decreased. The results show that Biochar applications are very promising in providing metal stabilization in soil, reducing metal availability, reducing plant stress and metal uptake. Considering the availability of a wide range of natural resources that can be used in the production of biochar and its economic and environmentally friendly properties, it can be considered as a remarkable alternative soil conditioner for the reclamation of polluted soils. Testing the findings obtained from experimental studies in different climatic and soil conditions, determining the agronomic and economic application doses and determining the unpredictable environmental effects are among the complementary research topics.

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Conflict of interests

The author declares that there are no competing interests.

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