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# Ameliorating effects of sunflower residues-derived biochar on mitigating the adverse effects of Pb and Cd on mung bean

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**ABSTRACT-** Lead (Pb) and cadmium (Cd) are trace elements known for potentially harming plants. Their toxicity can lead to increased oxidative harm, disturbance in plant metabolism, and deformation in plant structure. As a soil amendment, biochar (BC) effectively reduces heavy metal toxicity in polluted soils. To investigate the effects of BC derived from sunflower residues on mitigating the adverse impacts of Pb and Cd in mung bean (*Vigna radiata* Wilczek), a factorial experiment was conducted based on a completely randomized design with three replications in greenhouse conditions. The factors included Pb at three levels (0, 100, and 200 mg kg$^{-1}$ as Pb $(NO_3)_2$), Cd at three levels (0, 10, and 20 mg kg$^{-1}$ soil as Cd $(NO_3)_2$), and BC at three levels (0%, 1%, and 3% by weight). The results indicated that Pb- and Cd-induced stresses reduced all growth characteristics, such as shoot and root dry weights, and the number of active root nodules. The maximum soluble sugars (0.46 mg kg$^{-1}$) and proline content (44.7 µmol g$^{-1}$) were observed in the treatment with 200 mg kg$^{-1}$ of Pb and 20 mg kg$^{-1}$ of Cd without BC. Furthermore, applying BC decreased the concentration of Pb and Cd in the shoot by 19.1% and 13.8%, respectively, while enhancing all growth traits. Therefore, applying BC derived from sunflower residues is recommended as a promising and environmentally friendly approach to alleviating Pb- and Cd-induced stress in mung bean.

## INTRODUCTION

Heavy metals present significant environmental challenges due to their persistence and adverse impacts on organisms (Igiri et al., 2018). Lead (Pb), a common and highly toxic element in polluted soils, is readily absorbed by plant roots and is ten times more toxic than other heavy metals (Ferronato and Torretta, 2019). Elevated Pb levels in plants can impede growth by diminishing enzyme activities and photosynthesis and disrupting mineral nutrient balance (Baldi et al., 2021). Similarly, cadmium (Cd) is highly toxic to both organisms and humans, making it a matter of significant concern (Chellaiah, 2018). Undesirable effects of Cd on plants include root corking, reduced water and nutrient uptake, lowered chlorophyll levels, and enzyme activity disruption (Zulfiqar et al., 2021).

Because heavy metals are non-biodegradable, their removal from contaminated soils presents a significant challenge. Conventional physical and chemical methods for extracting these metals from the environment incur high costs and harm soil biological health (Raffa et al., 2021). As a cost-effective and eco-friendly alternative, the application of biochar (BC), derived from the pyrolysis of biomass and rich in carbon, offers a promising solution to alleviate heavy metal stress in plants (Liang et al., 2021).

BC, characterized by its stability as an organic compound and its affordability as an adsorbent, holds considerable promise for stabilizing Pb and Cd in soil by elevating soil pH (Guo et al., 2020). This pH elevation primarily stems from alkali and alkaline earth metals in BC ash. Moreover, the utilization of BC may impact crop growth and yield, likely attributed to its high cation exchange capacity (CEC), which enhances nutrient availability in the soil. Furthermore, integrating BC into the soil enhances soil organic carbon (SOC) levels, thereby enhancing soil physical properties (Hafeez et al., 2022).

Some studies underscore the positive effects of BC on plant growth and soil properties in calcareous soils of Iran, highlighting its potential as a sustainable agricultural practice in the region. In their study on the impact of BC on soil nutrient levels, microbial activity, and carbon storage potential in two calcareous soils, Khadem et al. (2021) found that BC, as an organic amendment, enhances soil properties, potentially leading to improved soil fertility and quality, ultimately resulting in increased plant yield. Osooli et al. (2022) investigated the BC effect on several physical properties of soil, the crop water stress index, and wheat yield in sandy loam soil. The findings indicated that BC enhanced soil air capacity, macro-pores, and plant available water promoting wheat growth by improving nutrient availability in the soil.

As per Mondal et al. (2015), studies have shown that heavy metals can negatively impact the growth,

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nodulation, and nitrogen fixation processes in *Vigna radiata*. Nodulation plays a vital role in leguminous plants, as it involves the formation of nodules on the roots by nitrogen-fixing bacteria to convert atmospheric nitrogen into a form that can be utilized by the plant. The occurrence of heavy metals interferes with this process, leading to decreased nodulation and compromised nitrogen fixation. In a distinct investigation by Jalal et al. (2024), it has been proposed that using BC can benefit the soil microorganism population, increasing nodule density. The research found the greatest nodule density in areas where BC was applied at a rate of 30 t $ha^{-1}$, while plots without BC had the lowest nodule density.

Numerous studies have demonstrated the ability of BC on reducing the bioavailability and toxicity of heavy metals in the soil, thereby alleviating the adverse effects on plant growth and development. Research conducted by Zhang et al. (2020) examined the impact of BC amendment on reducing cadmium and lead uptake in *Triticum aestivum*. The study found that the presence of BC in the soil significantly decreased the accumulation of cadmium and lead in the plant tissues, leading to improved growth and overall health of the plants. Similarly, a study by Nigam et al. (2019) investigated using BC to reduce lead stress in *Mentha arvensis*. The researchers observed a substantial reduction in Pb uptake by the plants when BC was added to the contaminated soil. This reduction enhanced photosynthetic activity, biomass production, and plant lead toxicity tolerance. Furthermore, research by Tang et al. (2022) focused on the role of BC in mitigating heavy metal toxicity in plants. The findings revealed that BC application effectively immobilized heavy metals in the soil, preventing plant uptake and reducing the negative impact on growth and yield.

The effectiveness of BC in mitigating contaminated soils hinges on factors such as its CEC, surface area, and the source materials utilized in its production (Liang et al., 2021). Research suggests that BC derived from dairy manure exhibits significant potential for extracting Cd and Pb from the soil solution (Chen et al., 2019). In contrast, BC sourced from pine cannot reduce heavy elements (Chemerys and Baltrėnaitė-Gedienė, 2017).

Every year, a large amount of heavy metals enters arable lands through agricultural practices, mainly due to chemical fertilizers, pesticides, livestock manures and compost, sewage-sludge-based bio-solids, and irrigation (Raffa et al., 2021). Given the increasing demand for food production and the consequent expansion of agricultural areas, the Pb and Cd accumulation risk in soil is a pressing concern. In Iran, where sunflowers are manually harvested, utilizing sunflower residues to produce BC via pyrolysis presents an economically viable and environmentally friendly approach to improve Pb- and Cd-induced stress in crop plants. Thus, this study aims to assess the efficacy of sunflower residues-derived BC on mitigating the adverse effects of Pb and Cd in mung beans (*Vigna radiata* Wilczek), offering a simple and cost-effective method for soil remediation.

## MATERIALS AND METHODS

A factorial experiment was conducted based on a completely randomized design with three replications in the greenhouse of Razi University, Kermanshah, Iran. The factors included BC at three levels of 0%, 1%, and 3% by weight, Pb at three levels of 0, 100, and 200 mg $kg^{-1}$ soil as Pb $(NO_3)_2$, and Cd at three levels of 0, 10, and 20 mg $kg^{-1}$ soil as Cd $(NO_3)_2$.

The BC application rates selected for this research study encompass the recommended intervals proposed by Sayyadian et al. (2018), allowing for an exploration of the impact of different BC application rates on soil characteristics and plant growth. The concentrations of Pb and Cd were determined based on prior research findings (Khdair et al., 2023), ensuring an adequate amount of plant material for laboratory analysis.

The solutions containing Cd-$(NO_3)_2$ and Pb-$(NO_3)_2$ were sprayed uniformly on the sub-samples separated from the original soil sample according to the desired concentrations. The samples were kept moist for 30 days at moisture conditions close to field capacity. Then, the amount of BC needed for each pot was calculated and thoroughly mixed with soil sub-samples. Finally, 7 kg of soil samples were transferred to pots and ten mung bean (*Vigna radiata* Wilczek) seeds were planted in September 2022. Irrigation was performed according to the plant water requirement. In this regard, the crops were irrigated based on about 75% field capacity moisture. Pots with 10 kg soil capacity, 20 cm height, 23 cm span diameter, and 18 cm floor diameter were used for the experiment.

A composite soil sample was taken from the surface layer (0-30 cm) of agricultural land with a latitude of 34º19′14″ N and a longitude of 47º6′14″ E. The percentage of sand, silt, and clay in the inorganic fraction of soil was measured using the hydrometer method. The soil pH was measured using a pH meter (Metrohm 691, Switzerland), and electrical conductivity (EC) was measured with an EC meter (Jenway, model 401, UK) in the paste saturation. Organic carbon (OC) through the wet ash method, calcium carbonate equivalent (CCE) by neutralizing with 1 N HCl, and CEC via the ammonium acetate method were determined. Phosphorous was measured using the Olsen method and a spectrophotometer (Apel, model PD 303S, Japan). Total nitrogen was analyzed using the Kjeldahl method. Potassium (in ammonium acetate extract) was determined using a flame photometer (Jenway, model PFP7, Japan) (Jones, 2001). Additionally, soil sample's total lead and cadmium content was determined by the DTPA method using an atomic absorption spectrophotometer (Varian SpectrAA 220FS). Table 1 indicates some physical and chemical properties of the soil sample. Based on the soil moisture characteristic curve (SMCC) of the study area, the values of permanent wilting point (PWP) and field capacity (FC) were obtained as 26% and 12%, respectively.

The BC used in this study was obtained from sunflower residues. It was prepared using a laboratory-scale furnace in a pyrolysis process at a temperature of 400 °C for 2 h. During the pyrolysis process, the feedstock material was subjected to controlled heating in an oxygen-limited environment to facilitate the conversion of the organic matter into BC. The heating rate, gas atmosphere, and cooling process were carefully monitored and controlled to ensure the successful production of high-quality BC with desired properties. After cooling, the BC was ground, sieved (mesh size: 0.5 mm), and stored in a plastic container for further use. The BC pH was 8.7, and the EC was 2.5 dS $m^{-1}$. The OC content of BC was 33.7 %, and the CEC was 79.3 $cmol_c$ $kg^{-1}$. The Mg, Ca, K, Na, and Cd were 42, 89, 98, 56, and 0.11 mg $kg^{-1}$, respectively. Table 2 displays some physical and chemical properties of the used BC.

In the three-leaf stage, five seedlings remained in each pot. Then, 1 g of urea (65.7 mg $kg^{-1}$ N) along with irrigation water and 1 g of triple superphosphate (68.5 mg $kg^{-1}$ $P_2O_5$) as powdered form were added separately to the soil in each pot (7 kg). The fertilizer treatment was determined according to the soil test results (Table 1). The greenhouse temperature was adjusted to 20-25 °C during the day and 14-18 °C at night. Plants were harvested at the beginning of the flowering stage (about 60 days after the planting date). Then, some characteristics were determined. Before harvesting the crops, plants from each plot were tagged, and their heights measured using a meter rule. The shoots and roots were oven-dried at 75 °C for 72 h to a constant weight. After cooling, the dry weight was measured using an analytical balance. Root length was measured using a meter rule. Root volume was determined using Archimedes' law. For this purpose, the root volume was measured by placing the roots in graduated cylinders and determining the amount of water level change. Total soluble carbohydrates were determined following the procedure outlined by Dubois et al. (1956). Proline contents were assessed following the method described by Bates et al. (1973). The detached nodules from the plants were retrieved from the soil, while those still attached to the roots of the plants were left undisturbed. Each nodule per mung bean plant was cut to determine its activity. Pinkish nodules indicated nitrogen-fixing activity and were considered active, while non-pinkish nodules were discarded as non-active. All plant parameters were then averaged for each pot.

Also, the concentration of Pb and Cd in extracts obtained from the digestion of leaf tissues were measured by a Varian AA220 atomic absorption spectrophotometer. Furthermore, pot soils were analyzed for SOC, EC, CEC, and pH. The analysis of variance (ANOVA) and comparison of means (Duncan's multiple range test) were performed using SPSS-16 software.

## RESULTS AND DISCUSSION

### Effects of Pb, Cd, and BC on the aerial parts of mung bean

The ANOVA revealed that the interaction of heavy metals (Pb and Cd) and BC significantly affected shoot dry weight, shoot Pb and Cd concentrations, proline content ($P < 0.01$), and soluble sugars ($P < 0.05$) (Table 3).

### Pb and Cd concentrations in leaf tissues

The highest concentrations of Pb (5.4 mg $kg^{-1}$) and Cd (4.2 mg $kg^{-1}$) in extracts obtained from the digestion of leaf tissues were obtained under severe stresses (200 mg $kg^{-1}$ of Pb, and 20 mg $kg^{-1}$ Cd, and BC control) (Table 4). The shoot Pb concentration increased by 17.4 and 31.8 times in the presence of 100 and 200 mg $kg^{-1}$ of Pb, respectively. Since Pb is the least active heavy element in the soil and accumulates in the surface layer of the soil, it is easily absorbed through the roots and enters the aerial parts of plants (Baldi et al., 2021). The shoot Cd concentration increased by 16.3 and 35 times at contamination levels of 10 and 20 mg $kg^{-1}$ of Cd, respectively.

However, BC application significantly reduced shoot Pb and Cd concentrations ($P < 0.01$). The Pb concentration decreased by 13.8% and 24.1% in the 1% and 3% (w/w) BC treatments, respectively. Similarly, the Cd concentration decreased by 10.2% and 21.4% in the 1% and 3% BC treatments, respectively. Consistent with these findings, Karami et al. (2011) also reported that BC significantly reduced Pb uptake in ryegrass by reducing Pb mobility in the soil. In other words, BC can decrease the availability of heavy metals by increasing soil pH. The results demonstrated that BC application significantly increased soil pH ($P < 0.01$). The highest pH value (8.17) was recorded in the 3% BC treatment. The presence of alkali and alkaline earth metals in the ash of BC has been identified as the main reason for the soil pH increase (Guo et al., 2020).

The ash of used BC contains high amounts of Ca, Mg, Na, and K (Table 2). Moreover, BC application significantly elevated the electrical conductivity (EC) in the soil solution under mung bean cultivation ($P < 0.01$). This increase was 14.1% and 22.6% in the 1% and 3% (w/w) BC applications, respectively.

Additionally, due to the high CEC and surface area, BC reduces the availability of heavy metals (Hayyat et al., 2016). The CEC and surface area of the BC used were high (79.3 $cmol_c$ $kg^{-1}$ and 59.3 m2 $g^{-1}$, respectively) (Table 2). Liang et al. (2021) noted that the presence of carboxyl, phenolic, hydroxyl, and other functional groups on the BC surfaces results in the formation of stable complexes with heavy metals over an extended period.

### Proline content

The highest proline content (44.7 µmol $g^{-1}$) was observed in 200 mg $kg^{-1}$ Pb and 20 mg $kg^{-1}$ Cd treatments, without BC (Table 4). In other words, Pb and Cd stress led to the production and accumulation of proline in the aerial parts of mung bean. The production of proline is a typical response in plants to heavy metals-induced stresses (Verbruggen and Hermans, 2008). Proline acts as a cell membrane protector, mitigating the adverse effects of abiotic stresses on plants. It enhances plant tolerance to stress by safeguarding enzymes from protein synthesis denaturation (Hosseinifard et al., 2022). Additionally, the primary deleterious impacts of heavy metals on plants is the induction of oxidative stress, generating reactive oxygen radicals. Plants utilize proline as a non-enzymatic antioxidant to combat these free radicals. Proline diminishes the toxicity of heavy metals in plants by forming a complex with heavy metals (Hayyat et al., 2016).

**Table 1.** Some characteristics of the soil used in this experiment

| Clay (%) | Silt (%) | Sand (%) | Soil texture | EC (dS m$^{-1}$) | pH | SOC (%) | $CaCO_3$ (%) | CEC (cmol$_c$ kg$^{-1}$) | $N_t$ (%) | $P_{avb}$ (mg Kg$^{-1}$) | $K_{avb}$ (mg Kg$^{-1}$) | Fe (mg Kg$^{-1}$) | Zn (mg Kg$^{-1}$) | Cu (mg Kg$^{-1}$) | Mn (mg Kg$^{-1}$) | Total Pb (mg kg$^{-1}$) | Total Cd (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 64.5 | 10.5 | Si L | 0.28 | 7.8 | 0.83 | 31.2 | 18.0 | 0.09 | 7.6 | 182 | 9.5 | 1.8 | 1.3 | 7.6 | 0.17 | 0.12 |

Si L, EC, SOC, and CEC represent silty loam, electrical conductivity, soil organic carbon, and cation exchange capacity, respectively.

**Table 2.** Physical and chemical properties of the biochar (BC) used in this experiment

| Cd (mg kg$^{-1}$) | Pb (mg kg$^{-1}$) | Density (g cm$^{-3}$) | Surface area (m$^2$ g$^{-1}$) | EC (dS m$^{-1}$) | pH | OC (%) | CEC (cmol$_c$ kg$^{-1}$) | Mg (mg kg$^{-1}$) | Ca (mg kg$^{-1}$) | K (mg kg$^{-1}$) | Na (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.11 | 0.14 | 0.29 | 59.3 | 2.5 | 8.7 | 33.7 | 79.3 | 42 | 89 | 98 | 56 |

EC, OC, and CEC represent electrical conductivity, organic carbon, and cation exchange capacity, respectively.

**Table 3.** Results of analysis of variance for the effects of Cd, Pb, and biochar (BC) on characteristics of aerial parts of mung bean

| Source of variations | Degree of Freedom | Pb concentration | Cd concentration | Proline content | Soluble sugars | Shoot dry weight | Plant height |
|---|---|---|---|---|---|---|---|
| BC | 2 | 5.86** | 1.62** | 321.5** | 0.039** | 4.242** | 191.04** |
| Cd | 2 | 0.17* | 84.64** | 908.5** | 0.165** | 4.933** | 512.3** |
| BC*Cd | 4 | 0.047ns | 0.52** | 21.37** | 0.003* | 0.112ns | 1.48ns |
| Pb | 2 | 131.25** | 0.06 ns | 2790** | 0.149** | 6.624** | 622.44** |
| BC*Pb | 4 | 1.937** | 0.05ns | 31.38** | 0.001ns | 0.147ns | 5.27ns |
| Cd*Pb | 4 | 0.127* | 0.02ns | 87.41** | 0.003* | 0.83** | 115.81** |
| BC*Cd*Pb | 8 | 0.11* | 0.06 ** | 10.67** | 0.002* | 0.377** | 6.58** |
| Error | 54 | 0.042 | 0.02 | 3.58 | 0.001 | 0.106 | 2.91 |
| CV (%) | | 9.20 | 9.50 | 10.05 | 10.10 | 9.41 | 8.54 |

ns: Represents non-significant; ** and *: Represent significant at the probability levels of 1% and 5%, respectively.
Note: Values given for parameters are mean of squares obtained from ANOVA.

**Table 4.** Mean comparisons of effects of biochar (BC), Cd, and Pb stresses on characteristics of mung bean

| BC Level (%) | Cd level (mg/kg) | Pb level (mg/kg) | Pb concentration (mg/kg) | Cd concentration (mg/kg) | Proline content (μmol/g) | Soluble sugars (mg/kg) | Shoot dry weight (g/pot) | Plant height (cm) | Root dry weight (g/pot) | Root volume ($cm^3$/pot) | Root length (cm/pot) | Total nodules (nodules/plant) | Active nodules (nodules/plant) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0.17$^{h}$ | 0.12$^{f}$ | 6.1$^{mn}$ | 0.15$^{kl}$ | 3.90$^{c}$ | 30.2$^{c}$ | 2.13$^{bc}$ | 27.7$^{cd}$ | 18.4$^{c}$ | 22.8$^{c}$ | 17.5$^{b}$ |
| | | 100 | 2.96$^{c}$ | 0.12$^{f}$ | 15.1$^{hij}$ | 0.23$^{ijk}$ | 3.51$^{c-g}$ | 21.5$^{fgh}$ | 1.49$^{klm}$ | 22.6$^{fgh}$ | 14.3$^{efg}$ | 15.6$^{def}$ | 12.0$^{ef}$ |
| | | 200 | 5.40$^{a}$ | 0.12$^{f}$ | 22.3$^{def}$ | 0.30$^{fg}$ | 2.72$^{hi}$ | 13.4$^{kl}$ | 1.31$^{m}$ | 15.9$^{klm}$ | 11.5hij | 12.5$^{g-k}$ | 9.7$^{ghi}$ |
| | 10 | 0 | 0.17$^{h}$ | 1.96$^{d}$ | 11.9$^{jk}$ | 0.20$^{ijk}$ | 3.65$^{c-f}$ | 19.6$^{ghi}$ | 1.73$^{e-k}$ | 25.2$^{def}$ | 15.6$^{de}$ | 16.3$^{de}$ | 13.5$^{cde}$ |
| | | 100 | 2.92$^{c}$ | 1.92$^{d}$ | 23.8$^{d}$ | 0.32$^{ef}$ | 3.21$^{e-h}$ | 16.4$^{ijk}$ | 1.68$^{g-l}$ | 20.3$^{hij}$ | 12.1$^{g-i}$ | 14.1$^{i-i}$ | 11.0$^{fg}$ |
| | | 200 | 5.31$^{a}$ | 1.89d | 35.6$^{b}$ | 0.40$^{bc}$ | 2.91$^{ghi}$ | 13.1$^{l}$ | 1.63$^{h-l}$ | 14.3$^{mn}$ | 10.3$^{j}$ | 10.6$^{klm}$ | 7.8$^{jkl}$ |
| | 20 | 0 | 0.17$^{h}$ | 4.17$^{a}$ | 12.5$^{ijk}$ | 0.33$^{def}$ | 3.11$^{fgh}$ | 14.5$^{kl}$ | 1.42$^{lm}$ | 21.6$^{ghi}$ | 12.7$^{f-i}$ | 13.6$^{f-j}$ | 10.6$^{fgh}$ |
| | | 100 | 2.31$^{de}$ | 4.11$^{a}$ | 29.8$^{c}$ | 0.39$^{bc}$ | 2.75$^{hi}$ | 16.4$^{ijk}$ | 1.62$^{i-l}$ | 18.1$^{jkl}$ | 11.3$^{hij}$ | 9.7$^{lm}$ | 6.4$^{lm}$ |
| | | 200 | 5.27$^{a}$ | 4.20$^{a}$ | 44.7$^{a}$ | 0.46$^{a}$ | 2.14$^{j}$ | 10.2$^{m}$ | 1.51$^{j-m}$ | 12.7$^{n}$ | 10.6$^{ij}$ | 8.3$^{m}$ | 5.5$^{m}$ |
| 1 | 0 | 0 | 0.15$^{h}$ | 0.11$^{f}$ | 5.8$^{mn}$ | 0.13$^{l}$ | 4.70$^{b}$ | 34.5$^{b}$ | 2.31$^{b}$ | 31.3$^{b}$ | 20.7$^{ab}$ | 25.5$^{b}$ | 18.3b |
| | | 100 | 2.52$^{d}$ | 0.10$^{f}$ | 11.5$^{k}$ | 0.21$^{ij}$ | 3.63$^{c-f}$ | 24.2$^{def}$ | 1.71$^{f-k}$ | 25.3$^{def}$ | 15.7$^{de}$ | 16.1$^{def}$ | 13.1de |
| | | 200 | 4.21$^{b}$ | 0.10$^{f}$ | 19.8$^{efg}$ | 0.27$^{fgh}$ | 3.24$^{d-h}$ | 16.1$^{jkl}$ | 1.50$^{j-m}$ | 17.8$^{jkl}$ | 12.7$^{f-i}$ | 13.7$^{f-i}$ | 11.0$^{fg}$ |
| | 10 | 0 | 0.16$^{h}$ | 1.52$^{e}$ | 9.8$^{kl}$ | 0.19$^{jk}$ | 3.80$^{cde}$ | 22.0$^{efg}$ | 1.98$^{c-f}$ | 28.3$^{cd}$ | 18.0$^{c}$ | 18.0$^{d}$ | 14.1$^{cd}$ |
| | | 100 | 2.11$^{ef}$ | 1.61$^{e}$ | 19.2$^{fg}$ | 0.29$^{fg}$ | 3.42$^{c-g}$ | 18.5$^{hij}$ | 1.91$^{c-h}$ | 22.7$^{fgh}$ | 13.2$^{fgh}$ | 16.1$^{def}$ | 12.0$^{ef}$ |
| | | 200 | 4.22$^{b}$ | 1.42$^{e}$ | 31.2$^{c}$ | 0.38$^{bcd}$ | 3.22$^{e-h}$ | 16.0$^{jkl}$ | 1.86$^{c-i}$ | 16.0$^{klm}$ | 11.4$^{hij}$ | 12.5$^{g-k}$ | 8.6$^{ijk}$ |
| | 20 | 0 | 0.15$^{h}$ | 3.76$^{b}$ | 11.0$^{kl}$ | 0.30$^{fg}$ | 3.25$^{d-h}$ | 19.6$^{ghi}$ | 1.62$^{i-l}$ | 24.4$^{efg}$ | 13.8$^{efg}$ | 14.7$^{efg}$ | 11.1$^{fg}$ |
| | | 100 | 1.94$^{f}$ | 3.71$^{b}$ | 22.8$^{de}$ | 0.37$^{cde}$ | 3.10$^{fgh}$ | 16.4$^{ijk}$ | 1.84$^{d-i}$ | 20.2$^{hij}$ | 12.5$^{f-j}$ | 11.2$^{jkl}$ | 7.2$^{klm}$ |
| | | 200 | 4.12$^{b}$ | 3.21$^{c}$ | 35.6$^{b}$ | 0.43$^{ab}$ | 2.36$^{ij}$ | 14.3$^{kl}$ | 1.72$^{f-k}$ | 14.3$^{mn}$ | 11.5$^{hij}$ | 9.8$^{lm}$ | 6.3$^{lm}$ |
| 3 | 0 | 0 | 0.13$^{h}$ | 0.10$^{f}$ | 5.4$^{n}$ | 0.07$^{m}$ | 5.61$^{a}$ | 37.5$^{a}$ | 2.65$^{a}$ | 34.6$^{a}$ | 22.5$^{a}$ | 29.6$^{a}$ | 23.0$^{a}$ |
| | | 100 | 1.16$^{g}$ | 0.09$^{f}$ | 9.9$^{kl}$ | 0.17$^{jkl}$ | 3.77$^{cde}$ | 26.5$^{d}$ | 1.78$^{e-j}$ | 27.3$^{cde}$ | 17.5$^{cd}$ | 17.6$^{d}$ | 13.5$^{cde}$ |
| | | 200 | 4.17$^{b}$ | 0.10$^{f}$ | 15.6$^{hi}$ | 0.22$^{hij}$ | 3.69$^{c-f}$ | 19.6$^{ghi}$ | 1.58$^{i-l}$ | 19.0$^{ijk}$ | 14.2$^{efg}$ | 14.2$^{e-h}$ | 11.0$^{fg}$ |
| | 10 | 0 | 0.12$^{h}$ | 1.53$^{e}$ | 8.0$^{lmn}$ | 0.17$^{jkl}$ | 3.99$^{c}$ | 24.6$^{de}$ | 2.10$^{bcd}$ | 30.2$^{bc}$ | 18.8$^{bc}$ | 18.0$^{d}$ | 15.0$^{c}$ |
| | | 100 | 1.14$^{g}$ | 1.49$^{e}$ | 16.5$^{gh}$ | 0.27$^{fgh}$ | 3.64$^{c-f}$ | 20.3$^{gh}$ | 2.01$^{cde}$ | 24.5$^{efg}$ | 14.7$^{ef}$ | 16.1$^{def}$ | 12.3$^{ef}$ |
| | | 200 | 4.13$^{b}$ | 1.43$^{e}$ | 28.4$^{c}$ | 0.33$^{def}$ | 3.87$^{cd}$ | 18.6$^{hij}$ | 1.96$^{c-g}$ | 17.3$^{j-m}$ | 12.6$^{f-i}$ | 11.7$^{i-l}$ | 9.2$^{hij}$ |
| | 20 | 0 | 0.14$^{h}$ | 3.25$^{c}$ | 9.1$^{klm}$ | 0.25$^{ghi}$ | 3.93$^{c}$ | 21.3$^{fgh}$ | 1.73$^{e-k}$ | 25.9$^{de}$ | 14.3$^{efg}$ | 15.0$^{ef}$ | 11.8$^{ef}$ |
| | | 100 | 1.11$^{g}$ | 3.14$^{c}$ | 18.3$^{gh}$ | 0.28$^{fg}$ | 3.57$^{c-f}$ | 18.6$^{hij}$ | 1.95$^{c-g}$ | 21.7$^{ghi}$ | 13.8$^{efg}$ | 11.8$^{h-l}$ | 7.5$^{kl}$ |
| | | 200 | 4.16$^{b}$ | 3.19$^{c}$ | 29.0$^{c}$ | 0.32$^{ef}$ | 2.92$^{ghi}$ | 16.1$^{jkl}$ | 1.86$^{c-i}$ | 15.2$^{lmn}$ | 12.8$^{f-i}$ | 10.5$^{klm}$ | 6.7$^{lm}$ |

In each column, means with similar letters are not significantly different (Duncan's test, $P < 0.05$)

The lowest amount of proline (5.4 µmol $g^{-1}$) was recorded under 3% (w/w) BC treatment, without Pb and Cd. Younis et al. (2015) reported that the application of BC can reduce proline production by enhancing nutrient uptake and improving plant growth parameters under heavy metal stress. Additionally, BC can enhance plant growth by decreasing the availability of heavy metal in soil solutions. BC mitigates the adverse effects of heavy metals on plants by forming complexes between carbonate, sulfate, and phosphate in BC and heavy metals in the soil (Park et al., 2013).

### Soluble sugars

The highest soluble sugar content (0.46 mg $kg^{-1}$) was detected in the 200 mg $kg^{-1}$ of Pb and 20 mg $kg^{-1}$ of Cd treatments without BC (Table 4). This suggests that Pb and Cd in the root zone increased soluble sugars in mung bean plants. Therefore, the elevation of soluble sugars under heavy metal stress represents a plant adaptation mechanism to regulate the cell water potential in the cytosolic fraction (Aldoobie and Beltagi, 2013).

Conversely, the lowest amount of soluble sugars (0.07 mg $g^{-1}$) was observed in 3% (w/w) BC treatment without Pb and Cd. This indicates that soluble sugars in mung bean decreased in the presence of BC. Soluble sugars typically increase under water deficit conditions. However, since BC can enhance relative water content in leaves by improving soil moisture conditions and increasing available water content (%) (Hafeez et al., 2017), a lower amount of soluble sugars was found with BC application.

### Shoots dry weight

The interaction effect of BC, Pb, and Cd on shoot dry weight indicated that this parameter decreased by increasing Pb- and Cd-induced stresses and increased with an appropriate amount of BC (3%, w/w). The lowest dry weight of shoots (2.14 g per pot) was observed in the treatment with 200 mg $kg^{-1}$ Pb and 20 mg $kg^{-1}$ Cd, without BC (Table 4).

A decline in plant growth parameters due to the heavy metals-induced stresses may be attributed to the reduced metabolic processes, inhibition of cell division and elongation (Benavides et al., 2005), primarily caused by the irreversible disability of the proton pump responsible for the cell growth (Liu et. al. 2010), and/or the sensitivity of the carbon sequestration cycle enzymes involved in the photosynthesis process (Sheoran et al., 1990). For example, the presence of Pb in plants increases chlorophyllase activity, leading to the decomposition of chlorophyll or a reduction in its production, disrupting the photosynthesis process, and reducing the biomass of the aerial parts and roots (Baldi et al., 2021).

Another reason for shoots' dry weight decreases in the presence of heavy metals is likely the excessive production of reactive oxygen species, such as superoxide and hydrogen peroxide radicals. While plants naturally produce these reactive oxygen species as part of their metabolic activities, their production increases in the presence of heavy metals in the soil (Demiral and Turkan, 2005).

The highest dry weight of shoots (5.43 g $pot^{-1}$) was achieved with 3% (w/w) BC and in the controls for Pb and Cd. The impact of BC on the shoot dry weight could be linked to an increase in SOC and CEC. BC's enhancement in soil nutritional conditions improves the plant roots' availability and nutrient uptake (Biederman and Harpole, 2013). The results demonstrated that BC at 1% and 3% (w/w) significantly increased SOC ($P < 0.01$) by 85% and 140.1%, respectively (Table 4). Similar results, on the effect of BC on increasing SOC and CEC have been reported by Herath et al. (2013) and Hafeez et al. (2022). The porous structure, large surface area, and surface charge of BC enhance the soil CEC and retention of nutrients (Biederman and Harpole, 2013).

Furthermore, the effect of BC application on shoot dry weight can be attributed to an increase in soil pH, which reduces the availability and uptake of Cd and Pb by plant roots, leading to a decrease in Cd and Pb mobility in the soil solution (Biria et al., 2017). The positive effect of BC in alleviating the adverse effects of Cd (Beesley et al., 2011) and Pb (Karami et al., 2011) on plant growth has been documented.

### Plant height

Plant height was decreased by 28.9% and 56.4% at contamination levels of 100 and 200 mg $kg^{-1}$ of Pb and by 35.2% and 52.1% at levels of 10 and 20 mg $kg^{-1}$ of Cd, respectively (Table 4). The presence of Pb in the root environment reduces photosynthesis in plants. Therefore, the growth parameters decrease due to the reduced water uptake by the roots (Amin et al., 2018). Reducing water in the tissues can result in decreased cell division and elongation. It has also been reported that Cd's reduction of plant growth parameters may be due to the inhibiting some metabolic activities, such as oxidative stress, photosynthesis, and respiration (Benavides et al., 2005). Additionally, plant height increased significantly ($P < 0.01$) by 14.3% and 23.9% due to the BC application at levels of 1% and 3% (w/w), respectively.

### Leaf area and number of leaves

The results displayed that contamination levels of 200 mg $kg^{-1}$ of Pb and 20 mg $kg^{-1}$ of Cd decreased leaf area by 32.5% and 49.5% and the number of leaves by 12.4% and 17.4%, respectively. The effect of heavy metals on reducing the leaf area of spinach (*Spinacia oleracea*) has been reported by Alia et al. (2015). They found that the accumulation of heavy metals in cellular cytoplasm disrupted metabolic activities like cell division, leading to a decrease in the plant growth parameters.

Using 3% (w/w) BC increased leaf area and the number of leaves in mung beans by 21.5% and 17.4%, respectively. Due to the porous structure, BC can increase soil water-holding capacity (Guo et al., 2020). Additionally, it contains nutrients that can enrich the soil and improve the nutritional status of the plant growth (Liu et al., 2013; Kim et al., 2016).

### Effect of Pb, Cd, and BC on mung bean root characteristics

The ANOVA showed that Pb, Cd, and BC interaction significantly affected root volume, dry weight, and number of total and active nodules ($P < 0.01$) (Table 5).

**Table 5.** Results of analysis of variance for the effects of Cd, Pb, and biochar (BC) on root characteristics of mung bean

| Source of variations | Degree of Freedom | Root dry weight | Root volume | Root length | No. of total nodules | No. of active nodules |
|---|---|---|---|---|---|---|
| BC | 2 | $0.817^{**}$ | 117.1** | $409.6^{**}$ | 38.19** | $21.34^{**}$ |
| Cd | 2 | $0.228^{**}$ | $187.3^{**}$ | $98.99^{**}$ | 331.5** | $261.9^{**}$ |
| BC*Cd | 4 | $0.002^{ns}$ | $1.18^{ns}$ | $0.89^{ns}$ | 4.715* | $2.853^{*}$ |
| Pb | 2 | $0.637^{**}$ | $953.9^{**}$ | $189.7^{**}$ | 416.7** | $303.5^{**}$ |
| BC*Pb | 4 | $0.008^{ns}$ | $3.63^{ns}$ | $0.536^{ns}$ | $4.868^{*}$ | $2.62^{*}$ |
| Cd*Pb | 4 | $0.757^{**}$ | $8.008^{*}$ | $20.61^{**}$ | 51.89** | $16.18^{**}$ |
| BC*Cd*Pb | 8 | $0.007^{ns}$ | $7.282^{*}$ | $4.287^{**}$ | 4.476* | $2.861^{**}$ |
| Error | 54 | 0.021 | 2.96 | 1.411 | 1.703 | 0.901 |
| CV (%) | | 8.02 | 7.82 | 8.28 | 8.69 | 8.38 |

### Nodulation

The results indicated that adding Cd and Pb greatly inhibited the nodulation of mung bean roots. The lowest nodulation (8.3 nodules plant$^{-1}$) was observed under contamination levels of 200 mg kg$^{-1}$ of Pb and 20 mg kg$^{-1}$ of Cd treatments, without BC (Table 4). Additionally, Pb at 100 and 200 mg kg$^{-1}$ levels reduced active nodules by 31.6% and 45.3%. Furthermore, Cd at 10 and 20 mg kg$^{-1}$ levels decreased this parameter by 28.3% and 39.3%, respectively. In the early stages of nodulation, the cell wall composition of rhizobium plays a vital role in connecting bacteria to the root hairs. Heavy metals may bind to the cell wall of rhizobium and reduce their connection to the roots (Chen et al., 2003). Moreover, the weight ratio of root to leaf in mung bean decreased as the Cd or Pb concentrations increased, which may explain the reduction of nodulation. However, the uptake and accumulation of Cd and Pb in roots may have deleterious effects on root systems (Cheng and Huang, 2006).

The number of total and active nodules of roots was significantly improved in BC treatment. The highest total and active nodules (29.6 and 23 nodules per plant, respectively) were obtained with 3% (w/w) BC, without Pb and Cd. The number of nodules reflects the symbiosis of the plant with rhizobium. Due to the positive effects of BC on soil moisture and SOM (Guo et al., 2020; Kim et al., 2016), and the subsequent increase in the soil microbial population, the nodulation of mung bean roots was significantly induced by addition of BC. However, the effect of high levels of BC on improving the number of nodules in mung bean was higher at high concentrations of Cd than Pb.

### Root dry weight

The lowest root dry weight in mung bean (1.51 g per pot) was observed under contamination levels of 200 mg kg$^{-1}$ of Pb and 20 mg kg$^{-1}$ of Cd (Table 4). Some studies reported that the reduced dry weight of plant roots exposed to Cd might be due to the root necrosis (Cheng and Huang, 2006). However, Alia et al. (2015) found that the adverse effects of heavy metals such as Cd on root dry weight are mainly due to a decrease in the synthesis of plant hormones, especially cytokinins, which play a significant role in cell division and plant growth. Moreover, contact of plant roots with high concentrations of heavy metals reduces photosynthesis, thereby reducing the overall growth of the plant.

The highest root dry weight (2.65 g per pot) was obtained with a 3% (w/w) BC treatment. Adding BC to the soil also mitigated the adverse effects of Pb and Cd on the root dry weight in mung beans. Biria et al. (2017) reported that BC increased the growth and development of roots by improving soil physical conditions, resulting in an increased root dry weight. As mentioned earlier, BC can increase SOC and CEC, ultimately leading to the enhanced root development in the soil.

### Root volume and length

The root volume and length decreased 38.7% and 37.6% at 200 mg kg$^{-1}$ of Pb and by 30.8% and 30.6% at 20 mg kg$^{-1}$ of Cd, respectively (Table 4). A decrease in the plant growth and root parameters due to the Pb- and Cd-induced stresses may be related to the reduced cell division and elongation, mainly caused by the irreversible disability of the proton pump responsible for cell growth (Liu et al., 2010). Additionally, amending soil with BC increased root volume and length by 23.5% and 22.6%, at a 3% (w/w), respectively. Similarly, the effect of BC on increasing root parameters in corn has been reported by Biria et al. (2017). Using BC effectively increases plant biomass, photosynthetic efficiency, root length, surface area, and total volume by improving the soil's physical structure and nutrient uptake (Hafeez et al., 2022).

## CONCLUSION

It was found that applying BC as a soil amendment could substantially mitigate the adverse effects of Pb- and Cd-induced stress in *Vigna radiata* Wilczek by reducing heavy metals uptake, enhancing soil conditions, and promoting the plant growth. Furthermore, the results indicated that BC can be considered a promising and environmentally friendly approach for improving plant growth under heavy metals stress.

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## CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization, Ali Ashraf Amirinejad and Zahra Timori; methodology, Zahra Timori; software, Mokhtar Ghobadi; investigation, Zahra Timori; data curation, Ali Ashraf Amirinejad; writing—original draft preparation, Ali

Ashraf Amirinejad; writing—review and editing, Mokhtar Ghobadi; supervision, Ali Ashraf Amirinejad.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interest.

## ETHICAL STATEMENT

This work is not related to experimental animals or specific human diseases that requires publication and approval of publication ethics.

## DATA AVAILABILITY

All data analyzed and generated during this study are included in this published article.

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