



Unveiling Role of Poultry Litter-Derived Biochar and Boron on Chickpea Growth, Soil Chemistry, Nutrient Availability and Heavy Metal Accumulation

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Abstract

This study explores the combined effects of biochar and boron on chickpea growth, soil chemistry and nutrient composition offering a novel approach to sustainable agriculture. A controlled experiment was conducted using biochar derived from poultry litter at four doses (0%, 2%, 4%, and 8%) and boron supplementation at three levels (0, 1 and 2 mg kg⁻¹ boron). Growth attributes, soil chemistry, macro and micro nutrient status and heavy metals accumulation were determined in chickpea (*Cicer arietinum*). Phosphorus, potassium, magnesium and calcium uptake were promoted with higher biochar doses by 20.2%, 16.6%, 20.2% and 25% over control, respectively. Similarly, boron and copper picked with 8% of biochar, while nickel had the highest value with 2% of it. Boron treatments increased magnesium and manganese uptake, whereas they reduced calcium and copper accumulations in plant tissues. biochar inhibited lead, chromium and aluminum absorption at low doses (2%), but increased at higher doses. The highest phosphorus concentration (2458 mg kg⁻¹) was observed with 8% biochar, while magnesium and potassium contents increased by 28.9% and 15.6%, respectively. Principal component analysis showed strong positive correlations between heavy metal uptake and biochar application, highlighting potential shifts in bioavailability. Plant growth parameters responded positively to biochar but negatively to excessive boron. Soil EC increased by 47% under 8% biochar, underscoring the need for careful application to avoid salinity issues. Biochar and boron application rates can promote soil nutrient bioavailability and chickpea growth, and restrict heavy metal accumulation in plants. However, high applications limit plant growth due to intense heavy metal accumulation and ion imbalances in the plant. The application of 2% biochar and 1 mg kg⁻¹ boron are considered optimal because it enhances nutrient uptake, minimizes heavy metal accumulation, and maintains low salinity risk.

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Graphical Abstract



Keywords Biofortification · Grain legume · Nutrient uptake · Nutritional composition · Sustainable agriculture

1 Introduction

Chickpea (*Cicer arietinum* L.), a member of *Fabaceae*, has a critical position in human nutrition and plays a unique role in agroecosystems. Chickpea, which has a high protein content (13–27%), is consumed as a basic food especially in developing countries where protein deficit is high (Jadhav et al., 2015). In addition, chickpea increases soil fertility by fixing atmospheric nitrogen (N_2) through symbiotic *Rhizobium* bacterias on roots and provides a natural nitrogen source for next crops (Zhang et al. 2024). However, chickpea growth and development is largely directly related to soil fertility, irrigation regimes and agricultural inputs. In particular, deficiency of micro- and macro-nutrients negatively affects growth process. Studies show that lack of appropriate nutrients in the soil significantly reduces chickpea biomass production and grain yield (Abebe And Beyene 2021).

The use of micronutrients such as boron (B), which has a significant effect on the physiological characteristics, is gaining more attention among yield-enhancing practices in chickpea cultivation (Pereira et al. 2023). It is essential for processes such as nucleic acid and protein synthesis, chlorophyll production, carbohydrate transport, pollen tube

growth, and root development (Ozturk et al. 2010). Boron reduces heavy metal toxicity in plants by inhibiting toxic accumulation and decreasing oxidative stress (Gheshlaghpour et al. 2021). Boron significantly increases nutrient uptake and translocation (Vishekaii et al. 2019), while B deficiency is well-documented to cause issues such as abnormal seedling growth (Day And Aasim 2020), reduced photosynthesis and CO_2 assimilation (Cong et al. 2015). Moreover, B treatments lead to reduce plant stress under saline conditions (Açıkbaş et al. 2024). Boron deficiency impacts various structural and functional activities of a plant's vascular system, including root initiation, starch metabolism, sugar transport, and nitrogen digestion (Li et al. 2023). B availability in soil is influenced by factors such as pH, the solubility of B sources, adsorption, complexation, and transport processes involving organic matter, metal hydroxides, and clay minerals (Das And Purkait 2020). Therefore, variable availability of B in soil underscores the need for amendments that improve nutrient retention, such as biochar.

Biochar is recognized as a product revealed with pyrolysis of carbon-rich solid materials. Biomasses are thermally degraded under oxygen-limited conditions during pyrolysis (Haider et al. 2022). Biochar enhances plant growth and yield, supports carbon sequestration, regulates greenhouse

gas emissions (GHS), and mitigates environmental contamination (Nguyen et al. 2023; Ji et al. 2025). Its highly aromatic carbon structure and high surface area and porosity, improves soil properties by increasing nutrient availability, cation exchange capacity, and water-holding capacity (Adhikari et al. 2022). Biochar also improves the nitrification process in less acidic soils, largely due to its influence on autotrophic nitrifying bacteria (Wang et al. 2015). Additionally, BCH stimulates nitrogen fixation and nitrogen mineralization, as its high hydrogen-to-carbon ratio facilitates the decomposition of soil minerals, releasing nitrogen for plant uptake while also providing a carbon source for soil microbiota (Dai et al. 2016). Furthermore, BCH plays a significant role in promoting precipitation reactions in soil, facilitating the conversion of minerals into insoluble forms. Given these multifaceted benefits, biochar derived from nutrient-rich poultry litter may interact with B to further influence plant nutrition and soil chemical Dynamics.

This study is based on the hypothesis that poultry litter-derived BCH and B treatments can alter nutrient uptake, soil chemistry and heavy metal accumulation in chickpea plants. It is postulated that suitable doses of BCH and B can enhance the availability and uptake of macro- and micro-nutrients while concurrently mitigating the translocation of toxic heavy metals into plant tissues. In addition, BCH can be produced from each organic material and generally used for soil designer. However, poultry waste is also a rich material in terms of nutrients. Therefore, it was estimated that poultry litter-derived BCH may lead to enhance plant growth and nutrient uptake via rich substrate composition as well as boosting soil characterization. Accordingly, the primary aim of this research is to comprehensively evaluate the individual and interactive effects of varying BCH and B doses on chickpea growth performance, soil chemical properties, nutrient accumulation, and heavy metal dynamics. Moreover, the study seeks to provide practical insights into the use of organic waste-derived amendments and nutrient strategies for improving soil health and crop nutrition within the scope of sustainable agriculture.

2 Materials and Methods

2.1 Experimental Area

The study was conducted in the climate chamber at Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Seed Science and Technology, Bolu, Türkiye. Light was provided using artificial illumination with 16 h of light and 8 h of darkness in the climate chamber. The intensity of the light sources was adjusted to 400–450 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The ambient temperature and humidity were

maintained at 24 ± 2 °C and 70% humidity throughout the experiment, respectively.

2.2 Experimental Materials

The Azkan chickpea (*Cicer arietinum*) variety of the Desi type was used in the experiment. Boric acid (H_3BO_3) was used for B treatments. BCH material was produced from chicken litter waste obtained from an organic poultry farm.

2.3 Characterization of Experimental Soil and Biochar

The chicken litter waste was pyrolyzed at 800 °C for 30 min in order to produce electrical energy, the biochar obtained was ground and passed through a <0.1 mm sieve (Çiğ et al. 2021). Experimental soil and BCH were analyzed for pH, EC, lime, organic matter, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), boron (B), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu). Texture was also determined in soil by Bouyoucos hydrometer method (Bouyoucos 1962). The soil reaction (pH) and salinity (EC) were determined as outlined in “Soil Survey Laboratory Staff” using the 1:2.5 soil-water mixture method (Burt 1992). Lime was detected using the calcimeter method (Howard 1917). Soil organic matter was determined using the Walkley Black wet combustion method (De Vos et al. 2007). Macro and micronutrient analyses in soil and biochar samples were determined by the ICP-OES spectrophotometric method according to Hansen et al. (2012).

Classification of experimental soil was calcisol according to Food and Agriculture Organization of the United Nations (FAO) classification criterias (FAO 2022). The pH of the experimental soil is neutral, textured loamy, salt-free, lime content is high, organic matter and N are very low, P is medium, K and Ca are very high, Fe, Mn and Cu is sufficient, and Zn and B contents are low (Tüzüner, 1990). The pH of BCH was alkaline, salty, organic matter content was high and total macro and micro element contents were very high. Physicochemical properties of experimental soil and biochar were determined in the laboratory and presented in supplementary file (Table S1).

2.4 Experimental Design

Eight seeds were planted in each pot, and thinning was performed to leave four plants after emergence. Different doses of BCH and boric acid were used in the experiment. Four doses of BCH material (BCH0: control, BCH1: 2%, BCH2: 4%, and BCH3: 8%) produced from poultry waste and three doses of boric acid (B0: control, B1: 1 mg kg^{-1} B, and B2: 2 mg kg^{-1} B) were applied to the pots. Thus, the experiment

was conducted according to completely randomized factorial design with four replications. Since the applied BCH doses were planned as a pot experiment, they were given in percentages. Considering field conditions, they approximately correspond to 50 tons ha⁻¹ for BCH1, 100 tons ha⁻¹ for BCH2, and 150 tons ha⁻¹ for BCH3. The doses were determined based on previous research on BCH application in field crops (Jalal et al. 2020; Bangash et al. 2023). Boron doses were dissolved in pure water and added to the pots. Pots with a capacity of 2 kg of soil were used in the study. The soil was obtained from a field in the region, air-dried, sieved through a 2 mm mesh, weighed at calculated amounts, and mixed with BCH according to volumetric ratios. Boron doses were given under seed bed before sowing. Boron granules were covered with soil to avoid contact with seeds. Homogenous ten seeds were sown to each pot and they were thinned to 5 homogenous seedlings after emergence. Before sowing, soil samples were taken and analyzed under laboratory conditions, with the physicochemical properties presented in supplementary file (Table S1). All pots were supplied with 200 mg N kg⁻¹ as ammonium sulfate due to very low N content of experimental soil. Irrigation was performed according to 80% field capacity. The method used by Çiğ et al. (2022) was utilized to calculate the field capacity. During the experiment, no symptoms indicating disease or pest effects were observed. Harvesting was done at the end of 45 days from the starting of experiment.

2.5 Experimental Observations and Analyses

At the end of the experiment, the plants removed from the pots were gently shaken to remove coarse soil. Additionally, sufficient soil samples were taken from each pot, and changes in soil reaction (pH) and electrical conductivity (EC) were observed depending on the treatments. Soil pH and EC were determined before sowing and after harvest using the standard 1:2.5 soil-to-water suspension method. Subsequently, the plants were cut at the root collar using scissors. Root length (RL), plant height (PLH), seedling fresh weights (SFW) and root fresh weight (RFW) were measured. The plants were then rinsed with pure water, placed in paper bags, and dried in an oven set to 70 °C until they reached a constant weight. The dried samples were weighed to determine seedling dry weight (SDW) and root dry weight (RDW). Finally, the samples were ground using a plant grinding mill to prepare them for analysis. Changes in macro and micro nutrients, and heavy metal contents in the above ground plant samples (leaf+stem) were determined. For nutrient element analysis, extracts were prepared using the dry ash method according to Hansen et al. (2012) and element readings were performed using an ICP-OES spectrophotometer instrument.

The quality of all chemical analyses was ensured by following standardized laboratory protocols. Calibration curves were generated prior to each analytical run using multi-element standard solutions. Quality control procedures included the measurement of reagent blanks, the use of certified reference materials (CRM) for accuracy verification, triplicate readings of each sample, and instrument drift correction after every ten samples.

2.6 Specific Analyses

Pearson correlation analysis was used to assess dual relationships of experimental observations using “*PerformanceAnalytics*” package (Peterson et al. 2014). Hierarchical clustering heatmap analysis indicated representations of experimental characteristics by treatments and was drawn using “*lattice*” package. Principle component analysis (PCA) was subjected to investigate multi-relationships of experimental treatments and characteristics (Çiğ et al. 2024). Visualization of statistical results was carried out using R (V 4.3.2).

2.7 Statistical Analysis

The data were subjected to the Shapiro-Wilk normality test. Normal distributing data were analyzed using two-way analysis of variance (ANOVA) according to completely randomized factorial design to assess statistical significance ($p < 0.05$ or 0.01). The analyses were conducted to evaluate the main effects of BCH and B (3×4) factors, as well as their BCH $\times$ B interaction effects. The means of the dependent variables were grouped using Tukey’s Honestly Significant Difference (HSD) test, implemented with the *agricolae* package in R software. (Mendiburu 2010).

3 Results

Four doses of BCH, three doses of B and interactive impacts of them were subjected to soils growing chickpea seedlings were investigated in the experiment. According to ANOVA results, any doses of BCH and B caused a significant differences in Fe, PLH, SFW and SDW. While Mn significantly ($p < 0.05$) change by only B treatment, P and EC were significantly ($p < 0.01$) affected by only BCH. Zinc content and pH were not significantly affected by BCH or B, however, BCH $\times$ B interaction caused statistically significant differences on them at 1% and 5%, respectively. Boron and BCH $\times$ B treatments led to statistically significant differences on RL at 5% and 1%, respectively, but no BCH. On the other hand, K and nickel (Ni) concentrations were just significantly affected ($p < 0.05$) by BCH treatment. Biochar

and BCH×B interaction caused statistically significant differences at 1% on Cu, however, B at 5%. Biochar and B treatment were significant at 1% for RFW, but BCH×B interaction had no impact on them. Biochar treatment significantly ($p < 0.01$) affected RDW, whereas B had no significant effect. Finally, Mg, Ca, Al, As, Cr and Pb were significantly affected ($p < 0.01$) by BCH, B and BCH×B treatment (Supplementary file, Table S2).

3.1 Macro Nutrients

Phosphorus concentration changed between 2045 and 2458 mg kg⁻¹ and 2163–2336 mg kg⁻¹ according to BCH and B treatments, respectively. The lowest P was determined in control plants while the highest one was observed in 8% BCH treated plants. The lowest K content (1.528%) was determined in no BCH treatment while the highest one (1.766%) was observed in 8% of BCH treated plants, therefore, it increases K content by 15.6%. The lowest Mg content (5733 mg kg⁻¹) was determined in 2% BCH treated plants whereas the highest one (7393 mg kg⁻¹) was observed with 8% of BCH under different BCH concentrations. 1 mg kg⁻¹ B treatment reduced Mg content while the 2 mg kg⁻¹ B increased by 8.5% over control. The lowest Ca content (16.2 mg kg⁻¹) was determined in 2% of BCH

treated plants while the highest one (24.76 mg kg⁻¹) was observed with 8% of BCH. However, B treatment reduced Ca concentration in plants, i.e., 1 and 2 mg kg⁻¹ B were decreased it by 26.7% and 15.1%, respectively (Fig. 1).

3.2 Micro Nutrients

Iron and B concentrations increased with rising BCH doses, therefore, 8% of BCH caused the highest Fe (350 mg kg⁻¹) and B (86.3 mg kg⁻¹) content. The lowest Fe (195.5 mg kg⁻¹) was determined in 2% of BCH, but it was in the same statistical group with control plants. The 8% of BCH treatment increased Fe and B concentrations in plants at 77.8% and 15.4% compared with non-BCH treatment plants, respectively. Manganese and Zn concentrations were changed between 66.58 and 74.11 mg kg⁻¹ and 95.1–98.8 mg kg⁻¹ by BCH treatments, respectively, i.e., alterations were not significant. The lowest Cu (6.66 mg kg⁻¹) was determined in control plants whereas the highest one (12.94 mg kg⁻¹) was observed in 6% of BCH treated plants. Copper content in plant tissues increased with 6% of BCH by 94.3% over control. Nickel content in plant tissues significantly increased with 2% of BCH and achieved at the highest value (36.78 mg kg⁻¹), however it decreased under higher doses (Fig. 2).

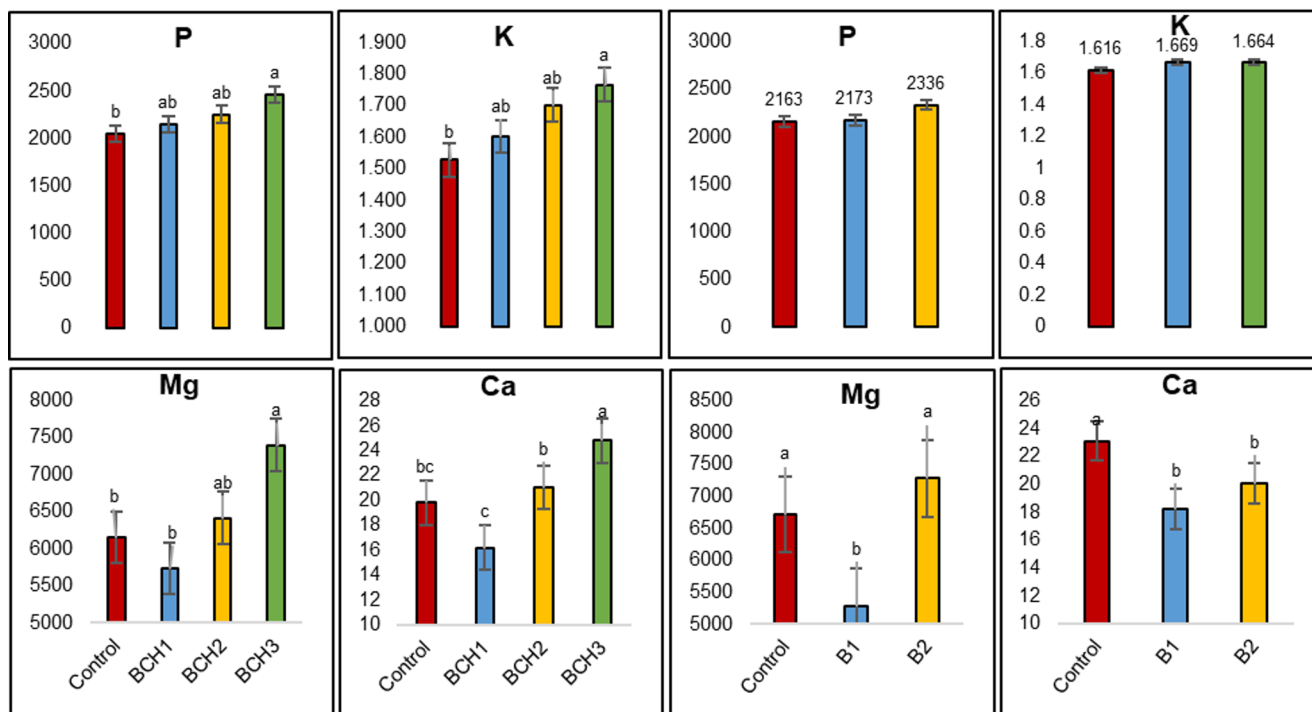


Fig. 1 Macronutrient status in chickpea under biochar and boron treatments (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between groups are

indicated by different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No biochar treatment, BCH1: 2%, BCH2: 4%, BCH3: 8%, Control: No boron treatment, B1: mg kg⁻¹ B, B2: 2 mg kg⁻¹ B, P: Phosphorus (mg kg⁻¹), K: Potassium (%), Mg: Magnesium (mg kg⁻¹), Ca: Calcium (mg kg⁻¹))

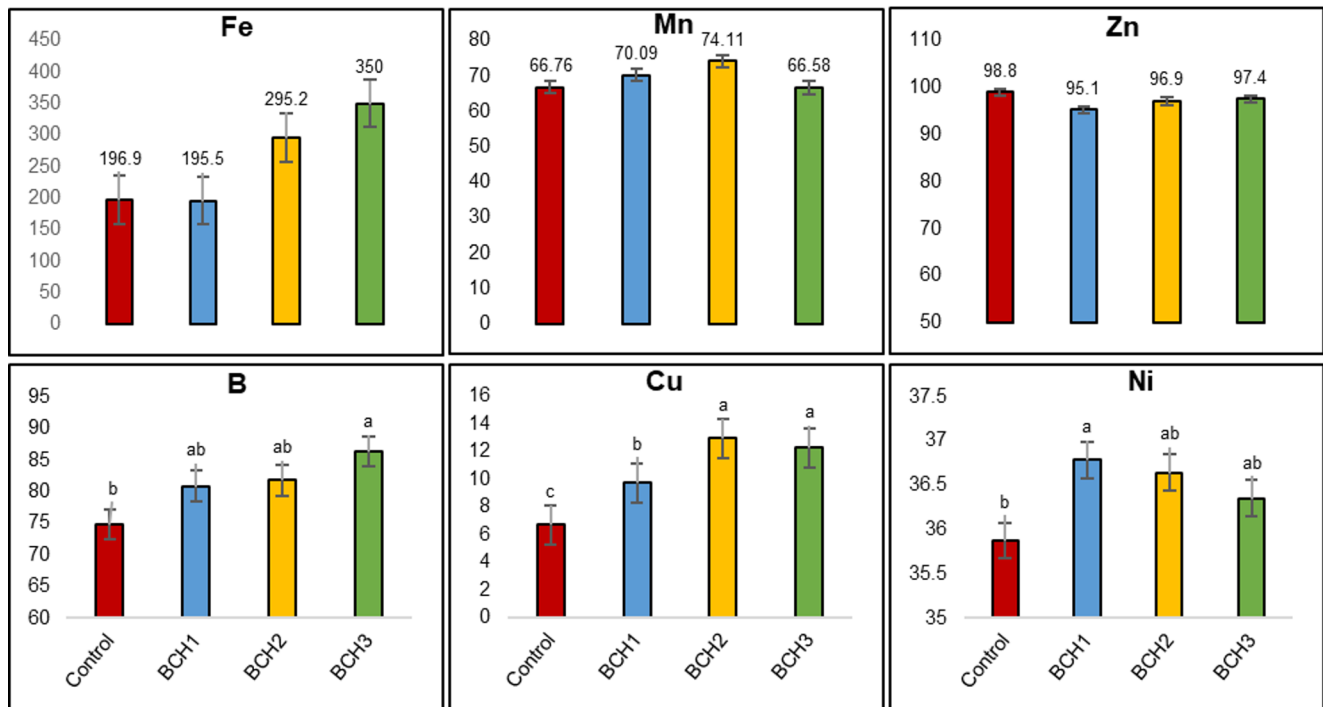


Fig. 2 Micronutrient status in chickpea under biochar treatments (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between groups are indicated by

different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No biochar treatment, BCH1: 2%, BCH2: 4%, BCH3: 8%, Fe: Iron (mg kg⁻¹), Mn: Manganese (mg kg⁻¹), Zn: Zinc (mg kg⁻¹), B: Boron (mg kg⁻¹), Cu: Copper (mg kg⁻¹), Ni: Nickel (mg kg⁻¹)

According to B treatments, Fe, Zn and Ni concentrations did not significantly changed and varied between 203 and 317.1 mg kg⁻¹, 93.6–102.3.6.3 mg kg⁻¹ and 36.25–36.64 (mg kg⁻¹), respectively. Manganese content increased with rising B treatment. 2 mg kg⁻¹ B increased Mn content by 21.4% compared with control plants. Similarly, B treatment boosted B uptake and translocation to plant tissues compared with control in which the highest B content (81.32 mg kg⁻¹) was obtained from 2 mg kg⁻¹ B plants while the lowest B (70.57 mg kg⁻¹) was determined in control plants. In contrast, B treatment reduced Cu concentration in plant tissues. Copper content was determined as 11.19 mg kg⁻¹ in control plants, however, it decreased by 8.6% and 4% with 1 and 2 mg kg⁻¹ B treatments, respectively (Fig. 3).

3.3 Toxic Heavy Metals

Biochar treatment significantly affected all toxic heavy metals. The lowest aluminum (Al) value (275 mg kg⁻¹) was determined in 2% of BCH whereas the highest one (2640 mg kg⁻¹) was observed in 8% of BCH, i.e., increased among 6-fold over control plants. Similarly, arsenic (As) concentration in plant tissues increased with rising BCH doses. Thus the lowest (0.104 mg kg⁻¹) and highest (0.365 mg kg⁻¹) As concentration were determined in control and 8%

of BCH treated plants, respectively. The 2% of BCH treatment reduced chromium (Cr) concentration (0.345 mg kg⁻¹) by 44% over control, however, higher doses increased it up to nearly starting level (0.652 mg kg⁻¹) again. Although low dose of BCH treatment (2%) restricted lead (Pb) concentration (1.238 mg kg⁻¹) in plant tissues, higher doses re-increased up to control plant's level. Boron treatment also significantly affected toxic heavy metal uptake in chickpea plants. According to results, 2 mg kg⁻¹ B promoted Al, As, Cr and Pb concentrations in plant tissues compared with control and 1 mg kg⁻¹ B treated plants. On the other hand, 1 mg kg⁻¹ B restricted toxic heavy metal contents over control except for As (Fig. 4).

3.4 Soil Reaction and Electrical Conductivity

Soil pH varied between 7.32 and 7.39 depending on BCH and B treatments, however, difference was not statistically significant. On the other hand, EC exhibited a significant alteration according to different BCH, but B treatments. Soil EC was 0.44 dS m⁻¹ at the beginning of the experiment. Rising BCH doses increased soil EC. The lowest EC (1.096 dS m⁻¹) was seen in control plots while the highest one (1.612 dS m⁻¹) was determined in 8% of BCH treated ones at the end of the experiment (Fig. 5).

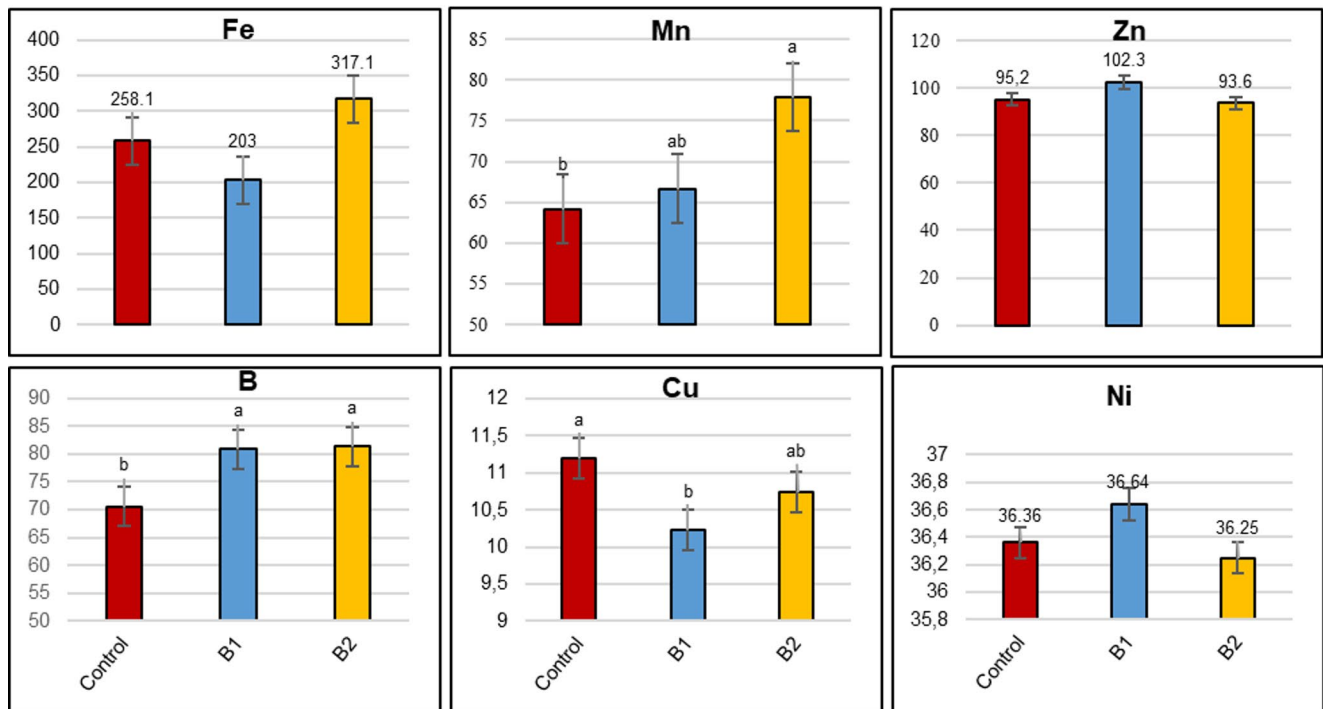


Fig. 3 Micronutrient status in chickpea under boron treatments (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between groups are indicated by

different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No boron treatment B1: mg kg^{-1} B, B2: 2 mg kg^{-1} B, Fe: Phosphorus (mg kg^{-1}), Mn: Potassium (mg kg^{-1}), Zn: Magnesium (mg kg^{-1}), B: Calcium (mg kg^{-1}), Cu (mg kg^{-1}), Ni (mg kg^{-1}))

3.5 Plant Growth, Biomass and Dry Matter Accumulation

According to BCH treatments, PLH ranged from 23.4 to 25.4 cm, RL from 18.1 to 19.8 cm, SFW from 2.612 to 3.000 g and SDW from 0.505 to 0.531 g. In terms of root biomass, 2% and 4% of BCH reduced RFW, however, 8% of BCH increased over control. The lowest RFW (2.781 g) was seen in 4% of BCH treated plans whereas the highest one (4.100 g) was determined in 8% of BHC treated ones. Therefore, 8% of BCH increased root biomass up to 26.7% over control. Similarly, BCH treatment boosted dry matter accumulation in roots. The lowest RDW (0.319 g) was determined in control plants while the highest one (0.472 g) was observed in 2% of BCH treated plants, however, it was in the same statistical group with 8% of BCH treated ones (Fig. 6).

In terms of B treatment, PLH ranged from 23.7 to 24.7 cm, SFW from 2.450 to 2.902 g, SDW from 0.513 to 0.531 g and RDW from 0.377 to 0.556 g. On the other hand, B treatment reduced root growth at a ratio of 14.4% and 13.7% with 1 and 2 mg kg^{-1} B treatments, respectively. Similarly, rising B doses decreased RFW in which the lowest value (2.799 g) was determined in 2 mg kg^{-1} B treated

plants while the highest one (3.898 g) was observed in control plants (Fig. 7).

3.6 Effect of BCH×B Interaction on Investigated Characteristics

BCH×B interaction caused statistically significant differences ($p < 0.05$ or 0.01) on Mg, Ca, Zn, Cu, Al, As, Cr, Pb, pH and RL. The lowest Mg content (4363 mg kg^{-1}) was observed in 1 mg kg^{-1} B and 2% of BCH treated plants whereas the highest Mg concentration (10081 mg kg^{-1}) was determined in 2 mg kg^{-1} B and 4% of BCH treated ones. Co-application of BCH and B reduced Ca concentration in plants, i.e., the highest Ca (27 mg kg^{-1}) was seen in control plants while the lowest one (15.4 mg kg^{-1}) was determined in 1 mg kg^{-1} B and 2% of BCH treated plants. According to BCH×B interaction, the lowest Zn (74.9 mg kg^{-1}) was determined in 2% of BCH treated plants without B application while the highest Zn (119.4 mg kg^{-1}) was observed in 4% of BCH treated plants without B application. Co-application of BCH and B reduced Cu concentration in plants. The lowest Cu content (14.85 mg kg^{-1}) was seen in 1 mg kg^{-1} B treatment without BCH addition whereas the lowest Cu (5.73 mg kg^{-1}) was determined in 8% of BCH and 1 mg kg^{-1} B treated plants. Co-application of 8% BCH and 2 mg kg^{-1} B

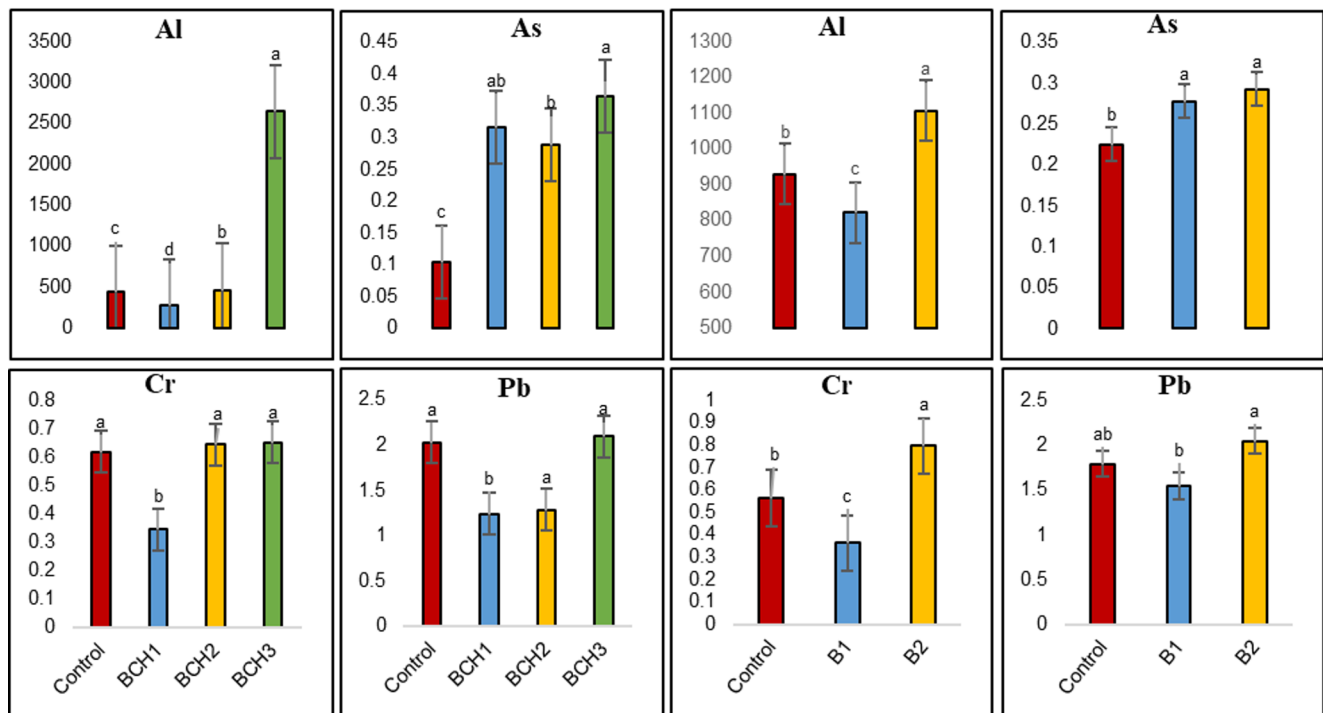


Fig. 4 Toxic heavy metal status in chickpea under biochar and boron treatments (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between groups are indicated by different letters. Groups without letter anno-

tations do not show significant differences based on the treatments. Control: No biochar treatment, BCH1: 2%, BCH2: 4%, BCH3: 8%, Control: No boron treatment, B1: mg kg⁻¹ B, B2: 2 mg kg⁻¹ B, Al: (Aluminum (mg kg⁻¹), As: Arsenic (mg kg⁻¹), Cr: Chromium (mg kg⁻¹), Pb: Lead (mg kg⁻¹))

kg⁻¹ B treatment caused the highest Al content (3628 mg kg⁻¹) whereas 2% of BCH and 2 mg kg⁻¹ B decreased Al content at the lowest value (191 mg kg⁻¹). In terms of another toxic heavy metal (As), rising application of BCH and B increased As concentration in plants (0.469 mg kg⁻¹) while the lowest As (0.061 mg kg⁻¹) was determined in control (No BCH or B) plants. Cr concentration in plant tissues exhibited an abnormal distribution depending on Co-application of BCH and B where 4% of BCH with 1 mg kg⁻¹ B caused the lowest Cr (0.079 mg kg⁻¹) whereas 4% of BCH with 2 mg kg⁻¹ B led to the highest Cr (1.276 mg kg⁻¹). The scenario was similar for Pb concentration. The 2% of BCH without B treatment caused the lowest (0.926 mg kg⁻¹) while 4% of BCH with 2 mg kg⁻¹ B caused the highest Pb (2.768 mg kg⁻¹) concentration. In general, co-application of treatments caused to increase in soil pH. The lowest (7.29) and highest (7.51) pH was determined in control plants (No BCH or B) and 8% of BCH without B treatment, respectively. Finally, the lowest RL (15.2 cm) was observed in 1 mg kg⁻¹ B without BCH treated plants whereas the highest RL (23.3 cm) was determined with 4% of BCH without B treatment (Supplementary file, Table S3). Characteristics that were statistically significant depending on BCH×B interaction were visualized by column charts

facilitate understanding relationships between experimental treatments (Fig. 8).

3.7 Correlation Matrix of Experimental Observations and Treatments

Heatmap indicated that treatments were divided into two main clusters. The first main cluster (lower part of heatmap) contained high BCH with or without B treatments exhibiting high levels of Mn, P, Al, Fe, Pb, Cr, Mg, RFW, EC, K, As and SFW. The first main cluster bifurcated into two sub-clusters. Lower one of them exhibited high positive correlation with Mn, P, Al, Fe, Pb, Cr and Mg while the upper one (divided into two sub-groups) indicated high level with RFW, EC, K, As and SFW. The second main cluster (upper part of heatmap) was divided into two sub-clusters exhibiting high levels of shoot and root growth characteristics as well as micronutrients and As. Control and B1 treatments exhibited high similarities and were involved in the same sub-cluster, therefore, sole B1 treatment has no significant difference with control except for PLH and Cu concentration. Treatments located in the second sub-cluster (B2, Bio2-B2, Bio2-B1, Bio2, Bio4, Bio8-B1) had how mean values with macro nutrients (P, K, Mg and Ca), toxix heavy

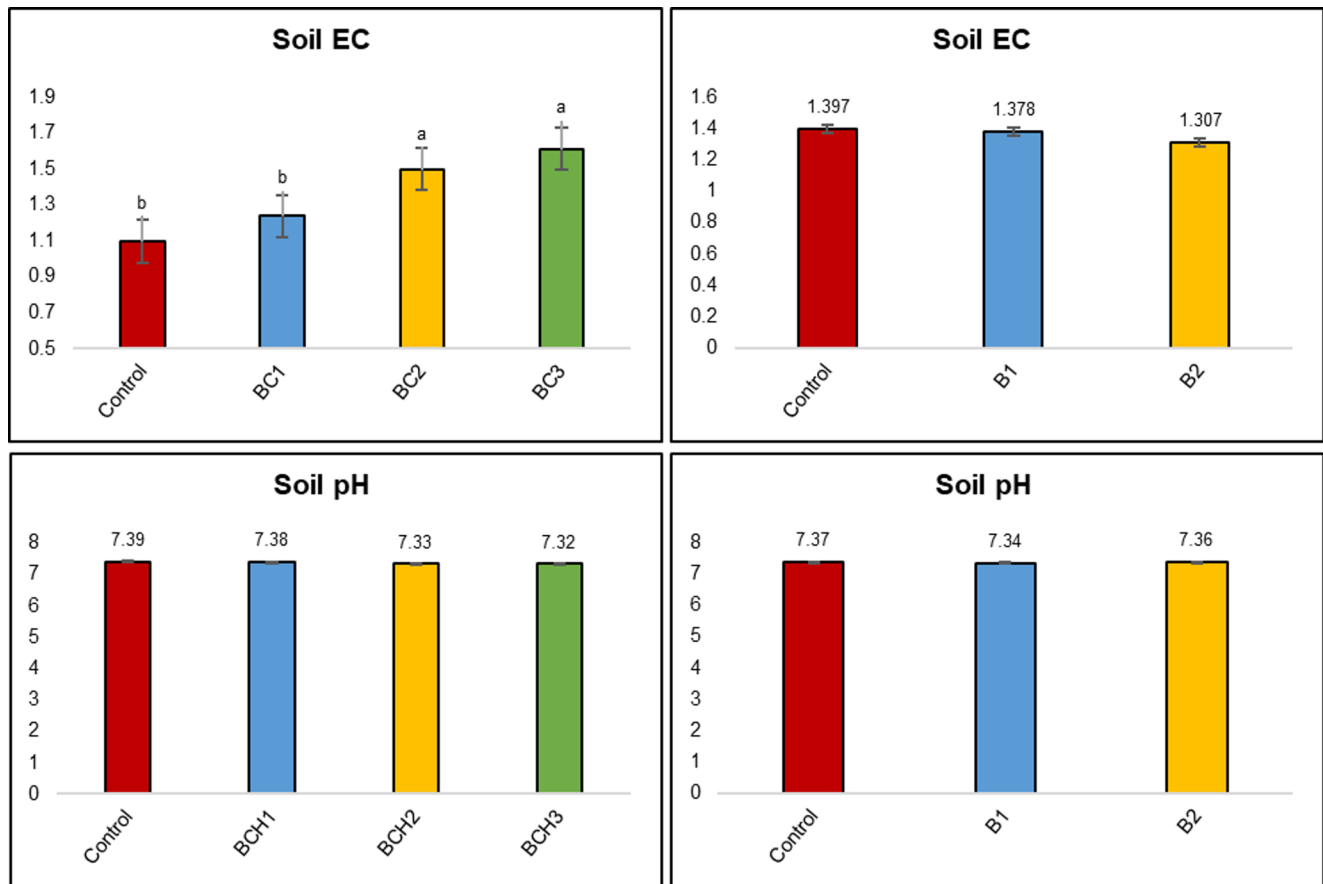


Fig. 5 Soil reaction and electrical conductivity under biochar and boron treatments (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between

groups are indicated by different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No biochar treatment, BCH1: 2%, BCH2: 4%, BCH3: 8%, Control: No boron treatment, B1: mg kg⁻¹ B, B2: 2 mg kg⁻¹ B)

metals (Al, Pb, Cr and As), soil pH and EC. According to heatmap, the highest mean values were obtained by combined application of high BCH and B treatments (Fig. 9).

RDW exhibited negative strong correlation with PLH ($p < 0.01$) and SFW ($p < 0.05$). PLH showed significant negative correlation with B ($p < 0.05$) while it had positive significant correlation with SFW ($p < 0.05$). Fe had strong positive correlations with Pb ($p < 0.01$), P ($p < 0.05$) and Al ($p < 0.05$). Cr concentration in plant tissues had strong negative correlation with Ni ($p < 0.05$), whereas it had strong positive correlation with Fe ($p < 0.05$), Pb ($p < 0.001$) and Mg ($p < 0.01$). Cu status was noteworthy interactive with other elements. Cu concentration has negative correlations with Al ($p < 0.01$), P ($p < 0.001$), B ($p < 0.01$), K ($p < 0.05$), As ($p < 0.05$), Ca ($p > 0.05$), RL ($p < 0.05$) and soil EC ($p < 0.01$). K status played a pivotal role on soil pH so that there was a strong positive correlation ($p < 0.05$) among them. Al had strong positive correlations with P ($p < 0.001$), EC ($p < 0.05$), Ca ($p < 0.05$) and Fe ($p < 0.05$). Soil EC was a variable characteristics since it had a strong negative correlation with

Cu ($p < 0.01$), whereas it had a strong positive correlations with K ($p < 0.05$), As ($p < 0.05$), B ($p < 0.05$), P ($p < 0.05$) and Al ($p < 0.05$). Zn exhibited significant negative correlation ($p < 0.01$) just with Mg (Fig. 9).

According to PCA, the first two PC accounted for 51.4% of total variance. The first PC that accounted for 30.2% of total variance exhibited high positive correlation among P and Al with the highest values for these traits being represented by Bio8 (8% BCH) and Bio8B2 (8% BCH+B2). A high positive correlation was found among EC, K, B and As in the positive load values of the first PC and these characteristics were represented by Bio8B1 (8% BCH+B1). Cu had the highest mean value in the negative load of the first PC. It was represented by B1 and had strong negative correlation with P and Al. The second PC accounted for 21.1% of total variation, which exhibited high positive correlation with Pb and Cr followed by PLH and pH. These characteristics were represented by Bio4B2 (4% BCH+B2) and control treatments, respectively. Ni had the highest value in the negative load of PC2 followed by RDW, RFW, As and B.

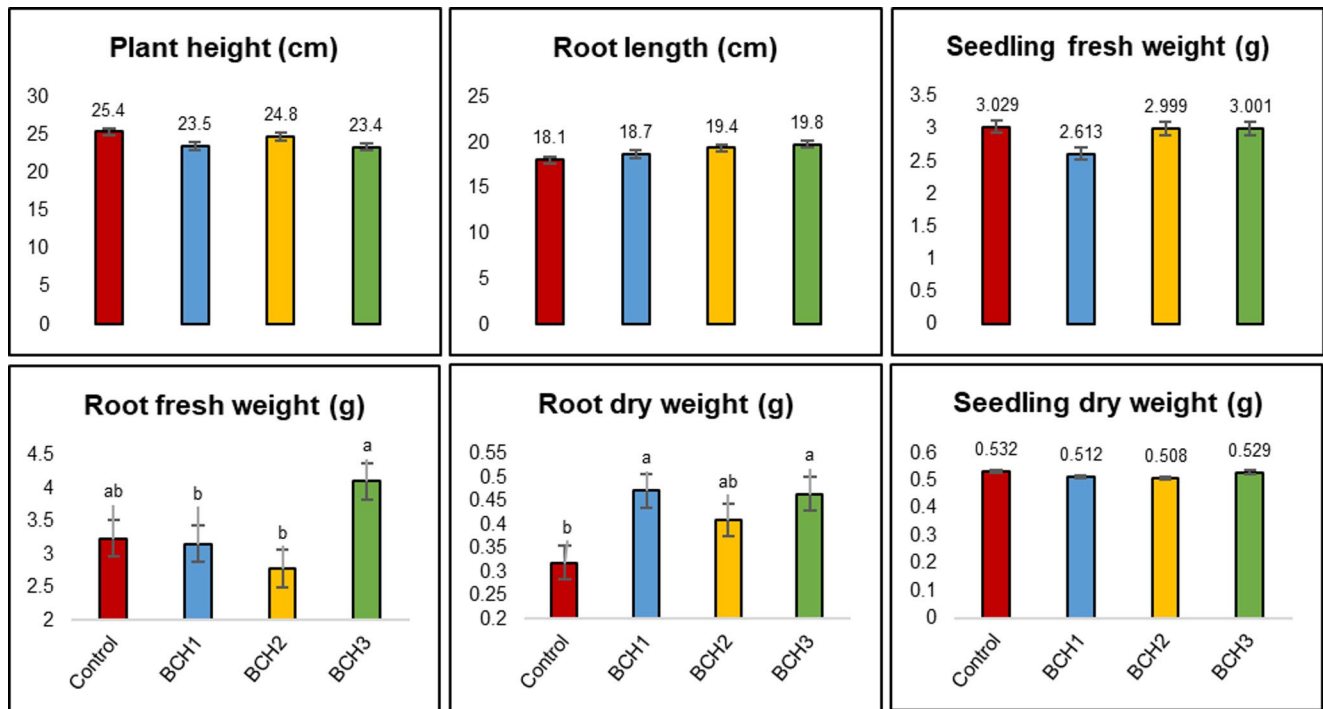


Fig. 6 Effect of biochar doses on chickpea growth attributes (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases.

Statistically significant differences between groups are indicated by different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No biochar treatment, BCH1: 2%, BCH2: 4%, BCH3: 8%)

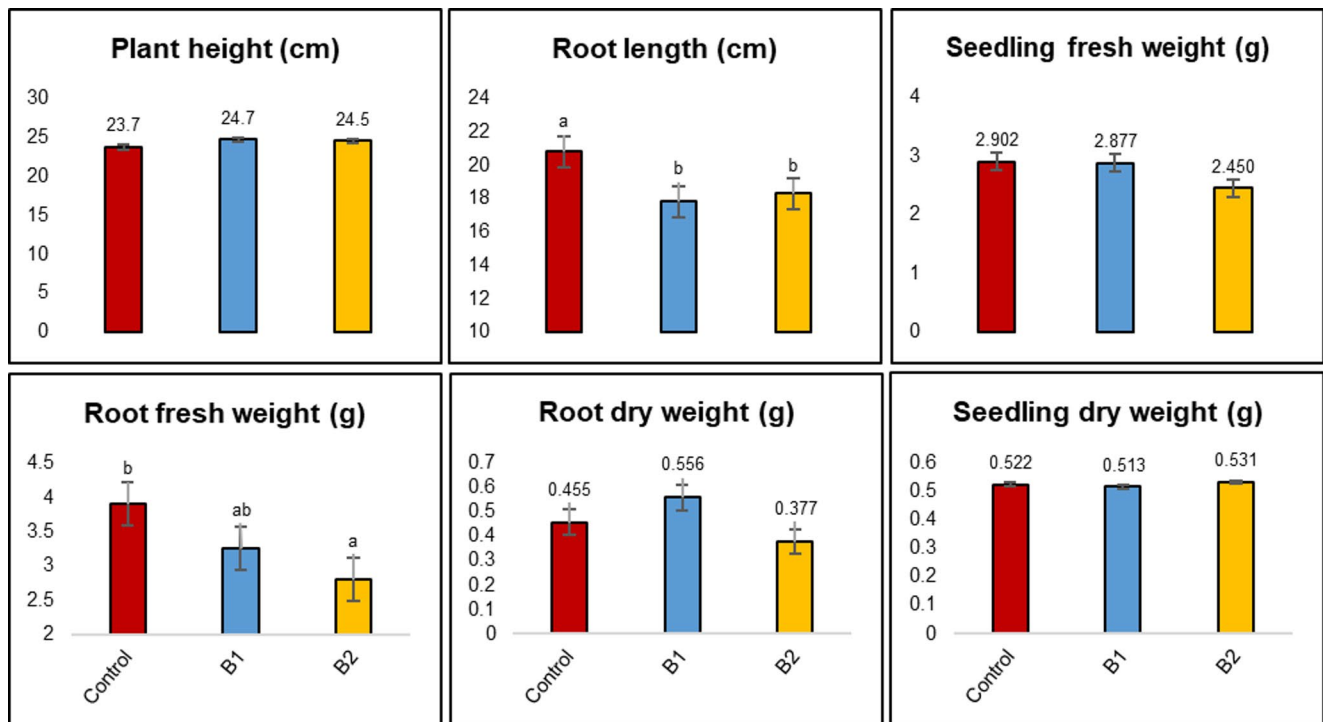


Fig. 7 Effect of boron treatments on alteration of seedling growth (The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases.

Statistically significant differences between groups are indicated by different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No boron treatment, B1: mg kg⁻¹ B, B2: 2 mg kg⁻¹ B)

These traits were represented by Bio2 (2% BCH), Bio4 (4% BCH) and Bio4B1 (4% BCH+B1) treatments in descending order (Fig. 10).

As and B are located in the lower right region of the graph and are positively correlated with soil EC. It shows that the mobility of B and As increases with some biochar doses. Ni and RDW are located in the lower left region and are particularly associated with treatments with high biochar doses, such as Bio8B1. This suggests that in some cases biochar treatments may increase Ni deposition. Pb and Cr are located in the upper right region and show a strong relationship with the Bio4B2 treatment. This suggests that the combination of high BCH (Bio4) and B (B2) increases Pb and Cr concentrations. Mn and Fe show a similar trend to Pb and Cr and are correlated with treatments with high BCH content. This represents that BCH may increase the mobility of these metals in soil. Zn and Cu were more abundant in treatments with low BCH doses such as Bio2B1 and Bio2B2. It can be assumed that Zn and Cu bind with BCH and their mobility decreases. High B content (B2 treatments) was positively correlated with Pb, Cr and Mn uptake. This suggests that B enhance the transport of some heavy metals. The correlation between B and EC suggests that As and K transport change with increasing salinity. It means that high B applications can mobilize toxic elements (Fig. 11).

4 Discussion

Plant growth responses to biochar and boron application reflected contrasting above- and below-ground allocation patterns. Above-ground parts of plants were not affected by BCH and B treatments. The lack of effect of BCH application on PLH, total biomass and dry matter accumulation is due to the slow-release organic structure of BCH material. During the experiment (45 days), it was concluded that although the EC value increased in the soil and showed significant effects on the accumulation of most of the elements in plant tissues, it did not have a positive effect on plant development morphologically due to the fact that the nutrients taken have not yet participated in metabolic activities. Another argument supporting this situation is the significant increase in RFW and RDW characters with increasing BCH doses. Xiang et al. (2017) reported in the meta analysis on the effect of BCH on root growth that BCH promoted root volume (29%), root biomass (32%), root length (52%) and root surface area (39%). However, the effects of BCH on root characteristics is depend on BCH production process, i.e., pyrolysis time and temperature. The increase of 26.7% and 45.8% in RDW and RFW was seen suitable according to previous experiment. Wan et al. (2023) reported that biochar-amended soils produced plants exhibiting a greater

total root length, area, diameter, and volume in maize. Joseph et al. (2021) indicated in the systematic review that biochar application can create beneficial conditions for root development by increasing pH, porosity, and water availability. On the other hand, Xiao et al. (2016) pointed out the critical process on effect of BCH on root and shoot growth. Biochar treatment had no significant increase on the accumulation of shoot dry matter during the first three sampling periods, however, it boosted root and seedling growth later stages. However, This root-oriented response contrasts with the effects observed under boron application.

In contrast to BCH, B treatment exhibited a negative impact on root growth in the experiment. Previous studies reported that high B concentrations reduce root growth, but the most common effect of excess B is the accumulation of B in the shoot since it has a low immobility in plants (Dinh et al. 2022). Thus, increasing B doses inhibited root growth and reduced RL and RFW in plants. On the other hand, although B application had a negative effect on root growth, it did not have a significant effect on B accumulation in leaves, which can be explained by its low mobility and the duration of the experiment did not allow sufficient transport via xyleme. Silva et al. (2015) reported that suitable B doses increased root dry weight and number of nodules in addition to herbage production up to 2.8 kg ha⁻¹, however, higher B amendments had negative effects on white clover production due to toxicity issues. Taken together, these findings indicate that BCH promotes early-stage root development without immediate above-ground biomass gains, whereas High B supply shifts biomass allocation unfavorably by suppressing root growth.

Soil chemical responses, particularly EC and pH, play a central role in mediating the effects of BCH on nutrient availability and uptake. Although small variations in soil pH can influence nutrient uptake and microbial activity (Kennedy 2022; Acikbas 2024), soil pH was not significantly affected by either BCH or B treatments in the experiment. In contrast, EC strongly responded to increasing BCH doses in which 8% BCH increased EC from 1.096 to 1.612 dS m⁻¹ (47%). Pre-experiment analyses indicated EC values of 0.44 dS m⁻¹ for soil and 10.82 dS m⁻¹ for BCH, making this increase a foreseeable outcome. Therefore, a rapid increase of soil EC is a foreseeable scenario. Fernandes et al. (2019) attracted attention that BCH production from some materials such as poultry litter has high salt concentration, therefore, one has to be careful when using BCH due to rapid increasing of EC. Zolfi Bavariani et al. (2019) determined that biochars produced at higher temperatures (400–500 °C) exhibited high electrical conductivity, likely due to dissolved salts. Similarly, Rodriguez et al. (2020) reported that high pyrolysis temperature generally increases the electrical conductivity, ash contents, and soluble cations in BCH.

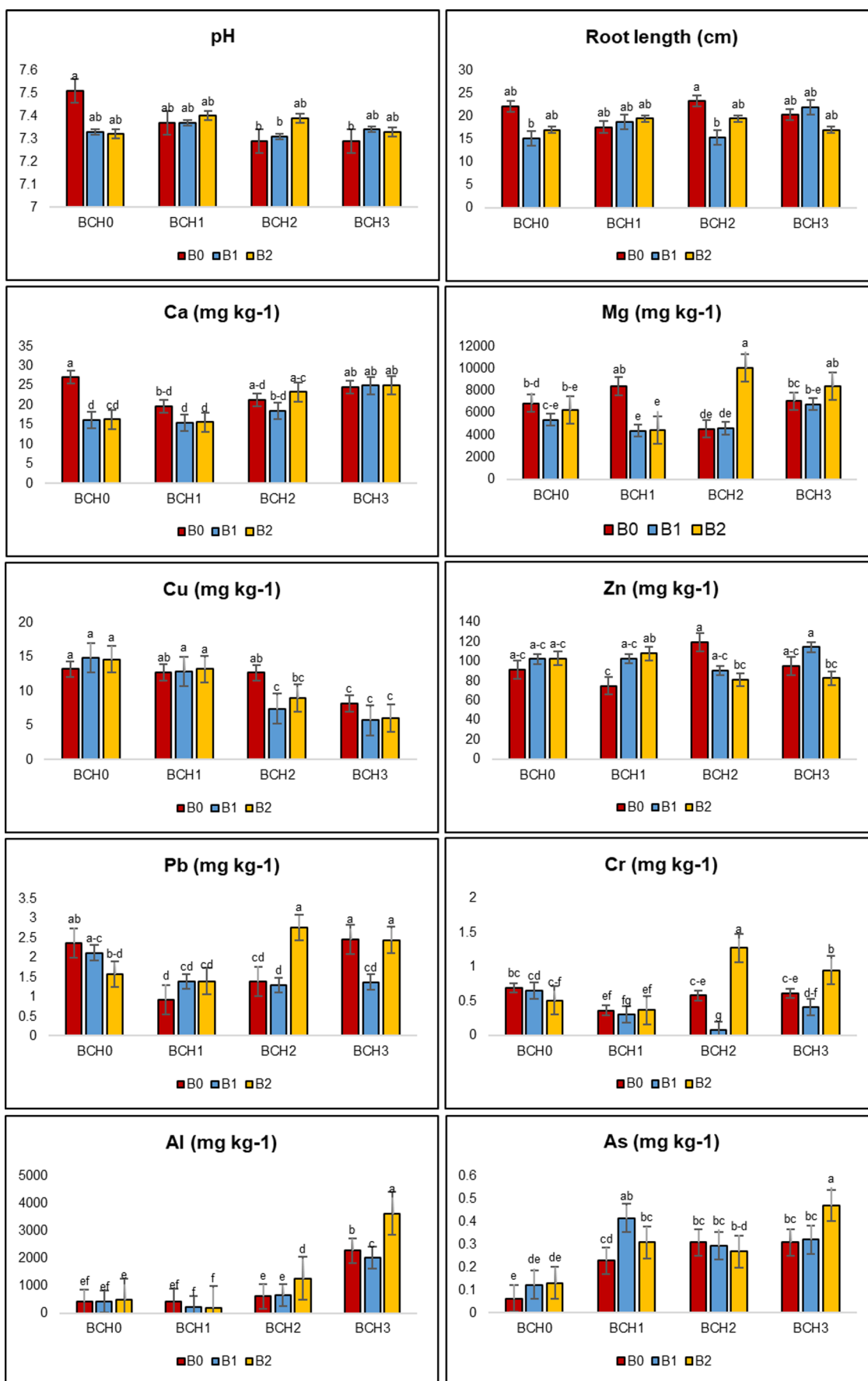


Fig. 8 Effect of Biochar×Boron interaction on some experimental characteristics (Statistically significant characteristics were given in the figure. The bars in the graphs represent the standard error (SE) of the mean. SE indicates the reliability of each mean value; as SE increases, variability among measurements is higher and confidence in the mean decreases. Statistically significant differences between groups are indicated by different letters. Groups without letter annotations do not show significant differences based on the treatments. Control: No boron/biochar treatment, B1: mg kg^{-1} B, B2: 2 mg kg^{-1} B, BCH1: 2%, BCH2: 4%, BCH3: 8%)

Accordingly, the high pyrolysis temperature (800°C for 30 minutes) likely contributed to the elevated EC. Taken together, the significant effect of BCH on soil EC, rather than on soil pH, suggests that changes in ionic strength and soluble nutrient pools may underlie the pronounced BCH-driven responses observed in plant nutrient uptake.

Biochar application markedly altered macronutrient acquisition in chickpea and major cations showed a clear dose-dependent response. The significant increase in P concentration in plant tissues can be directly linked to the high P content ($13,450 \text{ mg kg}^{-1}$) of BCH (Supplementary file, Table S1). Previous studies highlighted the pivotal role of BCH on improving P uptake via its high surface functional groups, large specific surface area, porous structure, and slow-release behavior (An et al. 2020; Ahmed et al. 2024). Beyond phosphorus dynamics, BCH treatment promoted the accumulation of major cations including K, Mg, and Ca in plants. Despite classical expectations of antagonistic interactions among these elements (Gransee and Führs 2013), higher accumulation of Ca in plant tissues with increasing BCH concentration can be explained by its high Ca content. However, K, Ca and Mg concentrations in plants increased with 8% BCH in the experiment. This deviation can be attributed to the dissolution of nutrient-rich mineral fractions from BCH and their efficient transport into the rhizosphere, where they become readily available for root uptake (El-Naggar et al. 2019). Similarly, previous studies indicated that BCH from poultry litter is particularly rich in K due to mineral composition (Hossain et al. 2020). Another effective point on K biofortification via poultry litter derived BCH in plants is pyrolysis temperature. Many researchers reported that pyrolysis of poultry litter-derived BCH at 250°C and 550°C increased K content by approximately 4.16–5.93%, while poultry litter-derived biochar produced at 400°C and 600°C similarly yielded K contents ranging from 3.88% to 5.88% (Subedi et al. 2016; Xiao et al. 2018). Although BCH addition led to a non-significant reduction in soil pH, this shift (likely driven by enhanced microbial activity) may have further facilitated cation uptake by improving nutrient solubility in the rhizosphere (Alam et al. 1999). This interpretation is further supported by multivariate analyses, where PCA and Pearson correlation revealed strong positive associations between Ca and

Mg uptake, predominantly driven by higher BCH doses (Figs. 2 and 3). Overall, these results demonstrate that poultry litter-derived BCH functions not only as a direct nutrient source but also as a rhizosphere modifier that partially overrides classical cation antagonisms, thereby enhancing macronutrient uptake under the calcareous soil conditions of the present study.

Boron application influenced macronutrient uptake patterns primarily by modifying cation balance rather than uniformly enhancing nutrient acquisition. This conclusion is based on the selective response of cations (Mg increased and Ca decreased) under B application, in contrast to the unchanged P and K uptake and stable soil pH. The highest Mg was observed in 2 mg kg^{-1} B treated plants, while the highest Ca was found in control plants and it decreased with B doses. Because soil pH remained largely unchanged under B treatments, the observed Mg–Ca antagonism is more likely attributable to ionic competition than to pH-mediated solubility effects. Given the low initial boron content of the experimental soil (1.01 mg kg^{-1}), B fertilization likely alleviated a latent micronutrient deficiency, thereby contributing to a re-equilibration of nutrient dynamics in the soil-plant system. This interpretation is further supported by heatmap analysis, which showed that maximum Mg accumulation coincided with BCH-containing treatments, whereas Ca uptake remained highest in control plants, consistent with antagonistic behavior (Fig. 1). Similar responses reported that boron supplementation enhanced the uptake of multiple macro- and micronutrients without additional N–P–K inputs (Lou et al. 2003; Lai et al. 2023). Many researchers demonstrated that B supplementation has synergistic effects with other nutrients, therefore, co-application of B with other nutrient sources (BCH in this experiment) caused positive interactions on other element uptake mechanisms (Zhao et al. 2020; Long And Peng 2023; Ghimire et al. 2025). The selective macronutrient responses to boron application highlight the need to evaluate its effects on micronutrient dynamics.

Micronutrient responses to BCH and boron application showed a more heterogeneous pattern compared with macronutrients, with manganese exhibiting a distinct sensitivity to boron supply. Aref (2010) reported that B fertilization increased Mn concentration in corn grain. Aref (2012) reported that there is an antagonistic relationship between B and Mn. The contrasting reports on B–Mn interactions suggest that Mn uptake may be highly context-dependent, varying with species, B dose, and soil chemical conditions. In parallel, BCH can alter the bioavailability of micronutrients due to its high surface area, porosity and cation exchange capacity. In particular, BCH supports plant nutrition by increasing the mobility of micronutrients such as Fe, Mn, Zn and Cu in soil (Akanji et al. 2021). According to

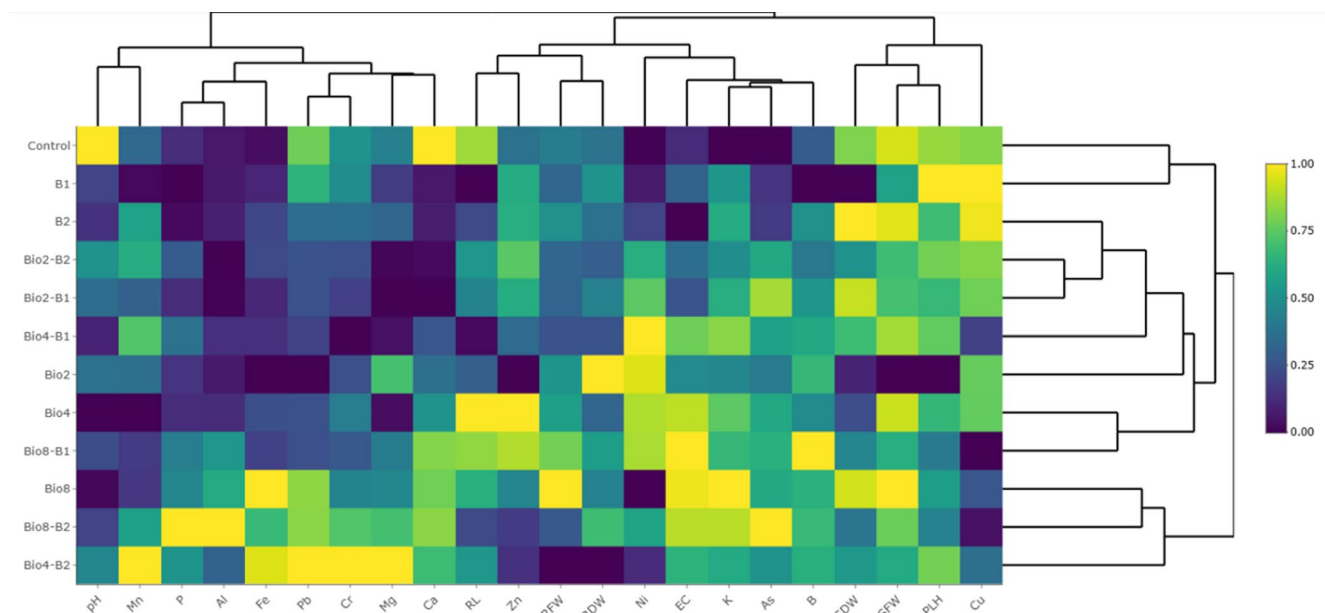


Fig. 9 Hierarchical clustering heatmap analysis showing relative concentrations of nutrients, growth attributes and soil traits depending on different biochar and boron treatments (RDW: Root dry weight, RFW: Root fresh weight, SDW: Shoot dry weight, SFW: Shoot fresh weight, PLH: Plant height, RL: Root length, P: Phosphorus, K: Potassium,

Ca: Calcium, Mg: Magnesium, Al: Aluminum, Fe: Iron, Pb: Lead, Cr: Chromium, Zn: Zinc, Ni: Nickel, EC: Electrical conductivity, As: Arsenic, B: Boron, Cu: Copper, Bio2: 2% of biochar, Bio4: 4% of biochar, Bio8: 8% of biochar, 8%, B1: mg kg^{-1} B, B2: 2 mg kg^{-1} B)

the results, BCH applications increased the concentrations of B, Cu and Ni, but made no statistically significant difference for Fe, Zn and Mn. The absence of significant changes in Fe and Zn concentrations (despite increasing of Cu and Ni) likely reflects element-specific adsorption and binding mechanisms on biochar surfaces. It is noteworthy that micronutrients such as Zn and Cu show a certain affinity with heavy metals in PCA (Fig. 3). According to Marschner (2012), Zn and Cu may show antagonistic interactions with toxic metals such as Pb and Cr. Here Zn and Cu are positioned in different directions and not in the opposite direction to Pb and Cr, so the antagonistic interaction is not clearly visible here. It is estimated that micronutrient–heavy metal interactions in this system are governed by complex and non-linear processes.

Boron plays a critical role in many physiological processes such as cell wall stability and sugar transport. However, high doses can cause toxicity (Kohli et al. 2022). As indicated by the study findings, B treatment did not significantly change Fe, Zn and Ni concentrations, but increased Mn and B concentrations. The increased Mn accumulation in the presence of B is thought to result from Mn's role as a cofactor in cellular enzymes and its synergistic interaction with B (Vera-Maldonado et al. 2024). Long and Peng (2023) published a review on the interaction of B with other elements in which they indicated that B promotes Mn accumulation in plants. On the other hand, both of B doses (1 and 2 mg kg^{-1}) significantly increased B status in plants.

Starter B level was low in the soil, therefore, increasing of B uptake by plants is a predicted scenario. Songsriin et al. (2023) investigated the effect of B fertilization in rice via soil amendment and foliar spraying under controlled and field conditions. Across both field and pot experiments, soil application of B resulted in the highest total B uptake, increasing uptake by 42.4% in the field and by 66.7% in the pot experiment compared with the control and foliar B treatments. Similarly, Javed et al. (2021) reported that B fertilization increased B level in soil as well as B concentration of shoots in sorghum and cowpea plants. Findings indicated that B availability plays a key role in regulating Mn and B accumulation without broadly altering other micronutrient concentrations under low initial B availability and calcareous soil conditions.

Biochar exerted contrasting effects on the uptake of toxic heavy metals (Pb, Cr, As, and Al) depending on application doses. Biochar can reduce the solubility of heavy metals by increasing soil pH (Meng et al. 2023). Therefore, high doses of BCH may have increased toxic heavy metal uptake. However, it has been reported that in some cases high BCH doses may increase heavy metal mobility. This is due to the fact that heavy metals adsorbed on the surface of BCH dissolve over time and are taken up more by plants (Liu et al. 2025). Recent studies show that BCH types produced at different temperatures can significantly affect the heavy metal adsorption capacity (Gotore et al. 2024). The vector aspects of heavy metals such as Pb and Cr reveal that these elements

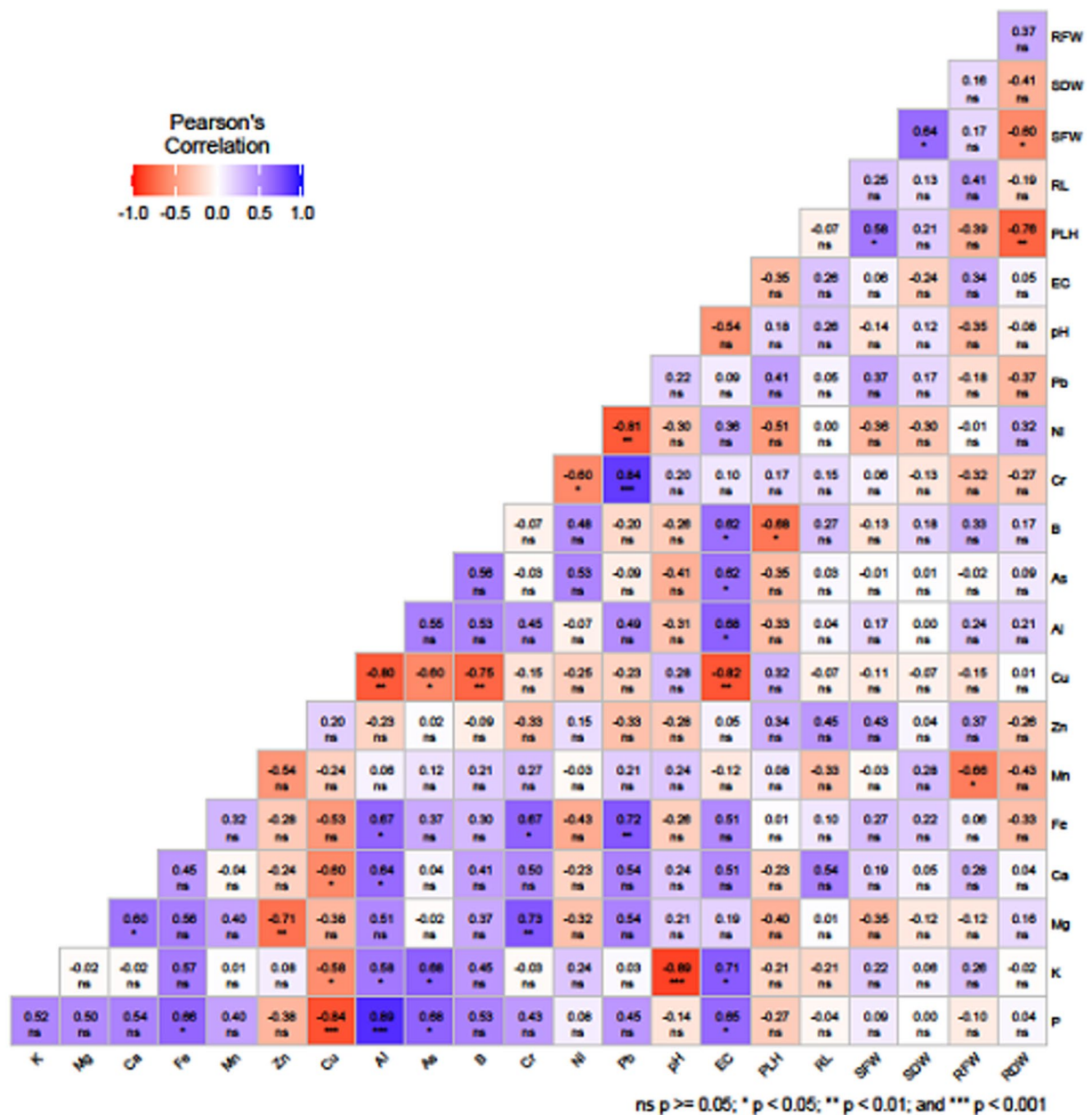


Fig. 10 Pearson correlation analysis showing dual relationships of experimental characteristics (RDW: Root dry weight, RFW: Root fresh weight, SDW: Shoot dry weight, SFW: Shoot fresh weight, PLH: Plant height, RL: Root length, P: Phosphorus, K: Potassium, Ca: Cal-

cium, Mg: Magnesium, Al: Aluminum, Fe: Iron, Pb: Lead, Cr: Chromium, Zn: Zinc, Ni: Nickel, EC: Electrical conductivity, As: Arsenic, B: Boron, Cu: Copper)

often show an inverse relationship with plant growth due to oxidative stress (Mansoor et al. 2023). Likewise, Pb and Cr are located in opposite directions to RDW and RFW in this PCA analysis (Fig. 3). These findings indicate that heavy metal responses to BCH application are dose- and context-dependent, with potential trade-offs between metal immobilization and increased mobility under certain conditions. Moreover, PCA patterns confirm that increased Pb and Cr accumulation is resulted with reduced root growth,

highlighting the potential phytotoxic risks under specific BCH conditions.

Effects of B application on heavy metal uptake are more complex. It has been reported that high doses of B increase accumulation of metals such as Al, As, Cr and Pb, while low doses may inhibit toxic metal uptake (Chernobrovkina et al. 2012). It has been suggested that B can form complex compounds with toxic heavy metals, thus limiting plant uptake. Previous publications have supported the complex action

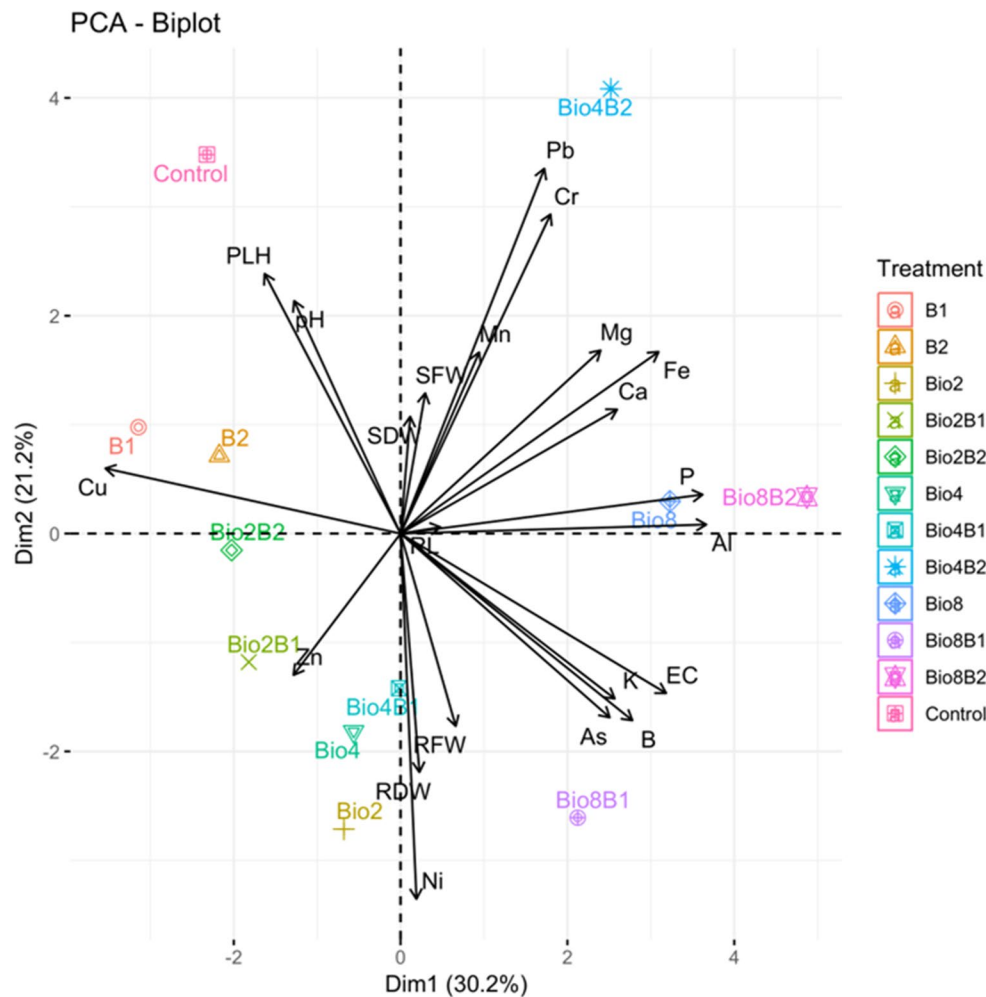


Fig. 11 Principal component analysis showing relative concentrations of nutrients, growth attributes and soil traits depending on different biochar and boron treatments (Black arrows (vectors) represent the loadings of the measured variables on the first two principal components (PC1 and PC2). The direction of each arrow indicates the relationship between the variable and the principal components, while the length reflects the magnitude of its contribution to the explained variance. Variables with arrows pointing in similar directions are positively correlated, whereas arrows pointing in opposite directions indicate negative correlations; approximately perpendicular arrows

suggest weak or no correlation. The proximity of treatment points to vector directions indicates stronger associations between those treatments and the corresponding variables. RDW: Root dry weight, RFW: Root fresh weight, SDW: Shoot dry weight, SFW: Shoot fresh weight, PLH: Plant height, RL: Root length, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Al: Aluminum, Fe: Iron, Pb: Lead, Cr: Chromium, Zn: Zinc, Ni: Nickel, EC: Electrical conductivity, As: Arsenic, B: Boron, Cu: Copper, Bio2: 2% of biochar, Bio4: 4% of biochar, Bio8: 8% of biochar, B1: mg kg⁻¹ B, B2: 2 mg kg⁻¹ B)

mechanism between B and heavy metal uptakes. Boron treatment restricted cadmium accumulation by suppressing Cd uptake and transport in rice (Chen et al. 2019), pepper (Xin et al. 2023), spinach (Huang et al. 2023), *Brassica napus* (Wu et al. 2020) and wheat (Qin et al. 2020). Yu et al. (2009) reported that B supplementation alleviated Al toxicity in pea roots and shoots by decreasing Al content in the root tips and cell walls. In another experiment, co-application of B and selenium (Se) in a hydroponic system significantly reduced the accumulation of Pb in the shoots of tobacco plants (Jin et al. 2025). On the other hand, some researchers highlight that B stress, whether due to deficiency or toxicity, becomes more severe when combined with other stresses

such as heavy metal contamination, which increases plant sensitivity to boron imbalance (García-Sánchez et al. 2020). Bariya et al. (2014) indicated that the interaction of B with other elements is complex and may exert antagonistic/synergistic effects, which may be specific for species, growth medium, and different environments. Overall, boron–heavy metal interactions display complex, dose-dependent antagonistic and synergistic behavior.

The divergent responses observed under individual BCH and B applications suggest that their co-application may trigger non-linear and dose-dependent interactions, warranting a detailed assessment of combined BCH–B effects on nutrient dynamics and heavy metal mobility. Rather than acting

additively, BCH×B interaction demonstrated both synergistic and antagonistic outcomes across various parameters of chickpea physiology and soil chemistry. A clear synergistic interaction was observed for Mg uptake. The highest Mg concentration (10081 mg kg⁻¹) was detected under the 4% BCH and 2 mg kg⁻¹ B treatment (BCH2 × B2), suggesting a coordinated mechanism where biochar enhances Mg availability via increased cation exchange capacity and surface sorption (Adhikari et al. 2022), while B plays a regulatory role in membrane stability and ion transport (Li et al. 2023). This combined effect is in line with studies reporting that biochar can significantly improve the availability of secondary macronutrients like Mg under calcareous or nutrient-poor soils (El-Naggar et al. 2019). Co-application of BCH and B resulted in non-additive and dose-dependent interactions, with clear synergistic effects on Mg uptake.

Conversely, antagonistic responses were observed for Ca and Cu. The lowest Ca (15.6 mg kg⁻¹) and Cu (5.73 mg kg⁻¹) levels were found in BCH1 × B1 and BCH3 × B1 treatments, respectively. This is likely due to competitive interactions among divalent cations (Ca²⁺, Mg²⁺, Cu²⁺) at the root uptake sites, as also reported by Gransee and Fühns (2013). In particular, high doses of BCH may immobilize Cu due to surface adsorption or promote Cu precipitation at elevated pH, thereby limiting its bioavailability (Laird et al. 2010). Notably, these antagonistic nutrient responses coincided with pronounced changes in heavy metal behavior under the same BCH×B combinations. Alongside these antagonistic nutrient responses, a particularly concerning outcome was the substantial increase in toxic heavy metals under specific BCH×B combinations. The BCH3 × B2 treatment resulted in the highest accumulation of Al (3628 mg kg⁻¹), As (0.469 mg kg⁻¹), and Pb (2.447 mg kg⁻¹), while Cr peaked under BCH2 × B2 (1.276 mg kg⁻¹). These results suggest that at high doses, biochar may desorb previously adsorbed metals, especially under conditions of elevated EC or through interactions with mobile boron species. Similar findings were reported by Liu et al. (2025), who demonstrated that biochar produced under high pyrolysis temperatures can release adsorbed heavy metals when exposed to complexing agents or pH shifts. Additionally, Chernobrovkina et al. (2012) demonstrated that excessive B can enhance the translocation of heavy metals into plants by altering membrane permeability and forming boron-metal complexes. Co-application of BCH and B revealed a trade-off between antagonistic nutrient interactions and increased heavy metal accumulation at higher doses.

Soil chemical changes induced by BCH × B interaction offer important insights into the coupled responses of metal mobility and root growth. The pH was highest under the BCH3 × B0 treatment (7.51), consistent with the alkaline nature of poultry litter-derived biochar (Haider et al.

2022). Elevated pH can increase the solubility of certain heavy metals like Cr and As, especially in soils where redox changes are minimal (Meng et al. 2023). In terms of root morphology, root length (RL) was maximized under moderate biochar (BCH2 × B0) without B application, reaching 23.33 cm. This aligns with previous meta-analyses indicating that biochar enhances root volume, length, and surface area through improved soil aeration and nutrient retention (Xiang et al. 2017). However, the addition of B under the same BCH dose significantly reduced RL, confirming findings by Dinh et al. (2022), who noted that excess B can suppress root elongation by generating oxidative stress and disrupting mitotic activity. Moreover, the interaction between B and heavy metals is supported by the PCA in this study, where increased B levels coincided with higher Cr, Pb, and Al uptake. This was also observed by Mansoor et al. (2023), who reported that stress induced by heavy metal accumulation under micronutrient interactions can impair root biomass and disturb overall plant metabolic functioning. Finally, these findings demonstrate that BCH × B interaction simultaneously modifies soil chemical conditions and metal mobility, translating into contrasting effects on root development under specific dose combinations.

5 Conclusion

This experiment clearly achieved that poultry litter-derived biochar and soil-treated boron regulate plant growth, nutrient uptake and metal behavior in chickpea through dose-dependent and interacting mechanisms. Biochar improved nutrient availability at low application rates by modifying rhizosphere chemical conditions, whereas higher doses increased toxic heavy metal accumulation. Boron exerted a selective regulatory role by enhancing manganese and boron uptake under low initial boron availability, but, higher doses promoted heavy metal accumulation and root growth inhibition. These findings confirm that the beneficial effects of biochar and boron are restricted to well-defined application ranges and highlight the non-linear nature of their combined effects. In conclusion, moderate poultry litter-derived biochar application rates (approximately 2%) combined with low boron supply (around 1 mg kg⁻¹) emerged as the most suitable strategy to enhance nutrient uptake while limiting toxic heavy metal accumulation in particular under calcareous soil conditions.

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Statistical analysis were subjected by [Ferit Sönmez] and [Figen Ceritoglu]. Visualization of manuscript was realized by [Figen Ceritoglu] and [Mustafa Ceritoglu]. The first draft of the manuscript was written by [Mustafa Ceritoglu] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability All data was presented in this paper.

Declarations

Financial interests All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Conflict of interest The authors declare that there are no conflicts of interest.

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6. References

- Abebe TN, Beyene S (2021) Growth limiting nutrient(s) and their effects on the yield and nutrient uptake of chickpea (*Cicer arietinum* L.) in Nitisols, Southern Ethiopia. *Environ Earth Sci* 11:7–20
- Acikbas S (2024) Evaluation of nutrient content of different harvest stages in switchgrass (*Panicum virgatum* L.) cultivars. *PeerJ* 12:e18570. <https://doi.org/10.7717/peerj.18570>
- Adhikari S, Timms W, Mahmud MP (2022) Optimising water holding capacity and hydrophobicity of biochar for soil amendment—a review. *Sci Total Environ* 851:158043. <https://doi.org/10.1016/j.scitotenv.2022.158043>
- Ahmed N, Deng L, Wang C et al (2024) Advancements in biochar modification for enhanced phosphorus utilization in agriculture. *Land* 13:644. <https://doi.org/10.3390/land13050644>
- Akanji MA, Usman ARA, Al-Wabel MI (2021) Influence of acidified biochar on CO₂-C efflux and micronutrient availability in an alkaline sandy soil. *Sustainability* 13:5196. <https://doi.org/10.3390/su13095196>
- Alam SM, Naqvi SSM, Ansari R (1999) Impact of soil pH on nutrient uptake by crop plants. In: Pessarakli M (ed) *Handbook of plant and crop stress*, 2nd edn. CRC Press, Boca Raton, pp 51–60
- An X, Wu Z, Yu J et al (2020) High-efficiency reclaiming phosphate from an aqueous solution by bentonite modified biochars: a slow release fertilizer with a precise rate regulation. *ACS Sustainable Chemistry & Engineering* 8:6090–6099. <https://doi.org/10.1021/acssuschemeng.0c01112>
- Aref F (2010) Zinc and boron fertilization on concentration and uptake of iron and manganese in the corn grain. *J Am Sci* 6:236–242
- Aref F (2012) Manganese, iron and copper contents in leaves of maize plants (*Zea Mays* L.) grown with different Boron and zinc micro-nutrients. *Afr J Biotechnol* 11:896–890. <https://doi.org/10.5897/AJB11.165>
- Bangash SH, Ullah F, Azam S et al (2023) Residual effect of biochar and legumes on soil fertility, yield and yield components of wheat. *J Soil Plant Environ* 2:47–62. <https://doi.org/10.56946/jspae.v2i2.275>
- Bariya H, Bagtharia S, Patel A (2014) Boron: A promising nutrient for increasing growth and yield of plants. *Nutrient use efficiency in plants*. Plant ecophysiology, vol 10. Springer, Cham, pp 153–170
- Bouyoucos GJ (1962) Hydrometer method improved for making particle size analyses of soils. *Agron J* 54(5):464–465. <https://doi.org/10.2134/agronj1962.00021962005400050028x>
- Burt R (1992) *Soil survey laboratory methods manual*. USDA
- Chen D, Chen D, Xue R et al (2019) Effects of boron, silicon and their interactions on cadmium accumulation and toxicity in rice plants. *J Hazard Mater* 367:447–455. <https://doi.org/10.1016/j.jhazmat.2018.12.111>
- Chernobrovkina NP, Titov AF, Robonen EV, Morozov AK (2012) Effect of boric acid on the ability of plants to accumulate heavy metals. *Russ J Ecol* 43:42–46. <https://doi.org/10.1134/S1067413612010031>
- Çığ F, Erman M, El Sabagh A et al (2024) Screening of lentil genotypes during germination and early growth stages under PEG-induced drought stress. *Chil J Agr Res* 84:326–337. <https://doi.org/10.4067/S0718-58392024000300326>
- Çığ F, Erman M, İnal B et al (2022) Mitigation of drought stress in wheat by bio-priming by PGPB containing ACC deaminase activity. *Atatürk Univ J Agric Fac* 53:51–57. <https://doi.org/10.54614/AUAF.2022.972753>
- Çığ F, Sönmez F, Nadeem MA, Sabagh AE (2021) Effect of biochar and PGPR on the growth and nutrients content of einkorn wheat (*Triticum monococcum* L.) and post-harvest soil properties. *Agronomy* 11:2418. <https://doi.org/10.3390/agronomy11122418>
- Cong XL, Jing HY, Lin N et al (2015) Boron deficiency affects cell morphology and structure of young leaves of radish. *Acta Physiol Plant* 37:247–257. <https://doi.org/10.1007/s11738-015-2004-7>
- Dai L, Li H, Tan F et al (2016) Biochar: a potential route for recycling of phosphorus in agricultural residues. *GCB Bioenergy* 8:852–858. <https://doi.org/10.1111/gcbb.12365>
- Das AK, Purkait A (2020) Boron dynamics in soil: classification, sources, factors, fractions, and kinetics. *Commun Soil Sci Plant Anal* 51:2778–2790. <https://doi.org/10.1080/00103624.2020.1849261>
- Day S, Aasim M (2020) Role of boron in growth and development of plant: deficiency and toxicity perspective. *Plant Micronutrients: Deficiency and Toxicity Management*. Springer, Cham, pp 435–453. https://doi.org/10.1007/978-3-030-49856-6_19
- De Vos B, Lettens S, Muys B, Deckers JA (2007) Walkley-black analysis of forest soil organic carbon: recovery, limitations and uncertainty. *Soil Use Manage* 23:221–229. <https://doi.org/10.1111/j.1475-2743.2007.00084.x>
- Dinh AQ, Naeem A, Mühling KH (2022) Growth and distribution of boron in oilseed rape (*Brassica napus* L.) as affected by boron supply. *Plants* 11:2746. <https://doi.org/10.3390/plants11202746>
- El-Naggar A, El-Naggar AH, Shaheen SM et al (2019) Biochar composition-dependent impacts on soil nutrient release, carbon mineralization, and potential environmental risk: a review. *J Environ Manage* 241:458–467. <https://doi.org/10.1016/j.jenvman.2019.02.044>

- FAO, World Reference Base for Soil Resources (2022) 2022, update 2022. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 202. FAO, Rome. Available at: <https://www.fao.org/soils-portal/oil-survey/soil-classification/world-reference-base/en/>
- Fernandes JD, Chaves LHG, Mendes J et al (2019) Alterations in soil salinity with the use of different Biochar doses. *Rev Ciênc Agrár* 42:89–98. <https://doi.org/10.19084/RCA18248>
- García-Sánchez F, Simon-Grao S, Martínez-Nicolas JJ et al (2020) Multiple stresses occurring with boron toxicity and deficiency in plants. *J Hazard Mater* 397:122713. <https://doi.org/10.1016/j.jhazmat.2020.122713>
- Gheshlaghpour J, Asghari B, Khademian R, Sedaghati B (2021) Silicon alleviates cadmium stress in basil (*Ocimum basilicum* L.) through alteration of phytochemical and physiological characteristics. *Ind Crops Prod* 163:113338. <https://doi.org/10.1016/j.indcrop.2021.113338>
- Ghimire P, Gairhe JJ, Kafle K, Khanal S (2025) Nutrient synergy in wheat: impacts of nitrogen and boron on productivity, accumulation, and soil nutrient retention. *PLoS One* 20:e0334042. <https://doi.org/10.1371/journal.pone.0334042>
- Gotore O, Masere TP, Muronda MT (2024) The immobilization and adsorption mechanisms of agro-waste based biochar: a review on the effectiveness of pyrolytic temperatures on heavy metal removal. *Environ Chem Ecotoxicol* 6:92–103. <https://doi.org/10.1016/j.enceco.2024.04.002>
- Gransee A, Führs H (2013) Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant Soil* 368:5–21. <https://doi.org/10.1007/s11104-012-1567-y>
- Haider FU, Coulter JA, Cai L et al (2022) An overview on biochar production, its implications, and mechanisms of biochar-induced amelioration of soil and plant characteristics. *Pedosphere* 32:107–130. [https://doi.org/10.1016/S1002-0160\(20\)60094-7](https://doi.org/10.1016/S1002-0160(20)60094-7)
- Hansen TH, de Bang TC, Laursen KH et al (2012) Multielement plant tissue analysis using ICP spectrometry. *Plant mineral nutrients methods and protocols*. Springer, New Jersey, pp 121–141
- Hossain MZ, Bahar MM, Sarkar B et al (2020) Biochar and its importance on nutrient dynamics in soil and plant. *Biochar* 2:379–420. <https://doi.org/10.1007/s42773-020-00065-z>
- Howard LP (1917) Determination of the lime requirements of soils by the use of calcium bicarbonate. *J AOAC Int* 3:141–144. <https://doi.org/10.1093/jaoac/3.1.141>
- Huang Y, Shen C, Wang X et al (2023) Boron decreases cadmium accumulation in water spinach by enhancing cadmium retention in the root cell walls. *Environ Sci Pollut Res* 30:101168–101177. <https://doi.org/10.1007/s11356-023-29447-z>
- Jadhav AA, Rayate SJ, Mhase LB et al (2015) Marker-trait association study for protein content in chickpea (*Cicer arietinum* L.). *J Genet* 94:279–286. <https://doi.org/10.1007/s12041-015-0529-6>
- Jalal F, Arif M, Akhtar K et al (2020) Biochar integration with legume crops in summer gape synergizes nitrogen use efficiency and enhance maize yield. *Agronomy* 10:58. <https://doi.org/10.3390/agronomy10010058>
- Javed MB, Malik Z, Kamran M et al (2021) Assessing yield response and relationship of soil boron fractions with its accumulation in sorghum and cowpea under boron fertilization in different soil series. *Sustainability* 13:4192. <https://doi.org/10.3390/su13084192>
- Ji H, Zhou C, Li P (2025) Applications of biochar in the remediation of soil microplastic pollution: a review. *Soil Sci Soc Am J* 89:e70078. <https://doi.org/10.1002/saj2.70078>
- Jin L, Qiao Q, Yang L, Gao Y (2025) Integrated physiological, transcriptomic, and metabolomic analyses reveal the response mechanisms of selenium (Se) and Boron (B) under lead (Pb) stress in tobacco. *Plant Cell Environ* 48:8878–8897. <https://doi.org/10.1111/pce.70172>
- Joseph S, Cowie AL, Van Zwieten L et al (2021) How biochar works, and when it doesn't: a review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy* 13:1731–1764. <https://doi.org/10.1111/gcbb.12885>
- Kennedy SO (2022) Soil pH and its impact on nutrient availability and crop growth. *Int J Geogr Geol Environ* 4:236–238
- Kohli SK, Kaur H, Khanna K et al (2022) Boron in plants: uptake, deficiency and biological potential. *Plant Growth Regul* 100:267–282. <https://doi.org/10.1007/s10725-022-00844-7>
- Lai CM, Huang YM, Liu CP, Su T-H (2023) Optimum boron supplementation enhances growth and nutrient uptake of broad-leaved tree seedlings. *J Soil Sci Plant Nutr* 23:3761–3774. <https://doi.org/10.1007/s42729-023-01296-2>
- Laird D, Fleming P, Wang B, Horton R, Karlen D (2010) Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma* 158:436–442. <https://doi.org/10.1016/j.geoderma.2010.05.012>
- Li S, Yan L, Venuste M et al (2023) A critical review of plant adaptation to environmental boron stress: uptake, utilization, and interplay with other abiotic and biotic factors. *Chemosphere* 338:139474. <https://doi.org/10.1016/j.chemosphere.2023.139474>
- Liu X, Jiang Y, Yang W et al (2025) Effects of low molecular weight organic acids on adsorption of Cd (II) by *Auricularia auricula* spent substrate-derived biochar: types and reaction sequence. *Environ Chem* 7:154–163. <https://doi.org/10.1016/j.enceco.2024.09.001>
- Long Y, Peng J (2023) Interaction between boron and other elements in plants. *Genes* 14:130. <https://doi.org/10.3390/genes14010130>
- Lou Y, Liang Y, Yang Y, Bell RW (2003) Effect of fertilization on plant growth and nutrient uptake in oilseed rape under varying Boron supply. *Commun Soil Sci Plant Anal* 34:1059–1075. <https://doi.org/10.1081/CSS-120019109>
- Mansoor S, Ali A, Kour N et al (2023) Heavy metal induced oxidative stress mitigation and ROS scavenging in plants. *Plants* 12:3003. <https://doi.org/10.3390/plants12163003>
- Marschner H (2012) Marschner's mineral nutrition of higher plants. Academic Press, London
- Mendiburu F (2010) *Agricolae*: Statistical procedures for agricultural research. R package version 1.1-8. <https://cran.r-project.org/web/packages/agricolae/agricolae.pdf> (Available at: 12.07.2024)
- Meng F, Huang Q, Cai Y et al (2023) A comparative assessment of humic acid and biochar altering cadmium and arsenic fractions in a paddy soil. *J Soils Sediments* 23:845–855. <https://doi.org/10.1007/s11368-022-03385-8>
- Nguyen T-B, Sherpa K, Bui X-T et al (2023) Biochar for soil remediation: a comprehensive review of current research on pollutant removal. *Environ Pollut* 337:122571. <https://doi.org/10.1016/j.envpol.2023.122571>
- Ozturk M, Sakcali S, Gucel S et al (2010) Boron and plants. *Plant adaptation and phytoremediation*. Springer Netherlands, Dordrecht, pp 275–311
- Pereira GL, Nascimento VL, Omena-Garcia RP et al (2023) Physiological and metabolic changes in response to Boron levels are mediated by ethylene affecting tomato fruit yield. *Plant Physiol Biochem* 202:107994. <https://doi.org/10.1016/j.plaphy.2023.107994>
- Peterson BG, Carl P, Boudt K et al (2014) PerformanceAnalytics: Econometric tools for performance and risk analysis, R package version 2.0.4. <https://cran.r-project.org/web/packages/PerformanceAnalytics/PerformanceAnalytics.pdf> (Available at: 12.07.2024)
- Qin S, Liu H, Rengel Z et al (2020) Boron inhibits cadmium uptake in wheat (*Triticum aestivum*) by regulating gene expression. *Plant Sci* 297:110522. <https://doi.org/10.1016/j.plantsci.2020.110522>

- Rodriguez JA, Lustosa Filho JF, Melo L et al (2020) Influence of pyrolysis temperature and feedstock on the properties of biochars produced from agricultural and industrial wastes. *J Anal Appl Pyrolysis* 149:104839. <https://doi.org/10.1016/j.jaap.2020.104839>
- Silva MD, Assmann TS, Martin TN, Caldas TDS (2015) White clover roots production and nodulation submitted to boron levels. *Agric Sci* 31:65–72. <https://doi.org/10.14393/BJ-v31n1a2015-18142>
- Songsriin J, Yamuangmorn S, Lordkaew S et al (2023) Efficacy of soil and foliar boron fertilizer on boron uptake and productivity in rice. *Agronomy* 13:692. <https://doi.org/10.3390/agronomy13030692>
- Subedi R, Taupe N, Pelissetti S et al (2016) Greenhouse gas emissions and soil properties following amendment with manure-derived biochars: influence of pyrolysis temperature and feedstock type. *J Environ Manag* 166:73–78. <https://doi.org/10.1016/j.jenvman.2015.10.007>
- Tüzüner A (1990) Soil and water analysis laboratories handbook. General Directorate of Rural Services, Ankara, pp 148–165. (In Turkish)
- Vera-Maldonado P, Aquea F, Reyes-Díaz M et al (2024) Role of boron and its interaction with other elements in plants. *Front Plant Sci* 15:1332459. <https://doi.org/10.3389/fpls.2024.1332459>
- Vishekaii ZR, Soleimani A, Fallahi E, Ghasemnezhad M, Hasani A (2019) The impact of foliar application of boron nano-chelated fertilizer and boric acid on fruit yield, oil content, and quality attributes in olive (*Olea europaea* L.). *Sci Hort* 257:108689. <https://doi.org/10.1016/j.scienta.2019.108689>
- Wan H, Liu X, Shi Q et al (2023) Biochar amendment alters root morphology of maize plant: its implications in enhancing nutrient uptake and shoot growth under reduced irrigation regimes. *Front Plant Sci* 14:1122742. <https://doi.org/10.3389/fpls.2023.1122742>
- Wang Z, Zong H, Zheng H et al (2015) Reduced nitrification and abundance of ammonia-oxidizing bacteria in acidic soil amended with Biochar. *Chemosphere* 138:576–593. <https://doi.org/10.1016/j.chemosphere.2015.06.084>
- Wu X, Song H, Guan C, Zhang Z (2020) Boron alleviates cadmium toxicity in *Brassica napus* by promoting the chelation of cadmium onto the root cell wall components. *Sci Total Environ* 728:138833. <https://doi.org/10.1016/j.scitotenv.2020.138833>
- Xiang Y, Deng Q, Duan H, Guo Y (2017) Effects of biochar application on root traits: a meta-analysis. *GCB Bioenergy* 9:1563–1572. <https://doi.org/10.1111/gcbb.12449>
- Xiao R, Wang JJ, Gaston LA et al (2018) Biochar produced from mineral salt-impregnated chicken manure: fertility properties and potential for carbon sequestration. *Waste Manag* 78:802–810. <https://doi.org/10.1016/j.wasman.2018.06.047>
- Xin J, Yuan H, Yang L et al (2023) Effect of boron supply on the uptake and translocation of cadmium in *Capsicum annuum*. *Ecotoxicol Environ Saf* 257:114925. <https://doi.org/10.1016/j.ecoenv.2023.114925>
- Yu M, Shen R, Xiao H et al (2009) Boron alleviates aluminum toxicity in pea (*Pisum sativum*). *Plant Soil* 314:87–98. <https://doi.org/10.1007/s11104-008-9708-z>
- Zhang J, Wang J, Zhu C, Singh RP, Chen W (2024) Chickpea: its origin, distribution, nutrition, benefits, breeding, and symbiotic relationship with mesorhizobium species. *Plants* 13:429. <https://doi.org/10.3390/plants13030429>
- Zhao Z, Wang S, White PJ et al (2020) Boron and phosphorus act synergistically to modulate absorption and distribution of phosphorus and growth of *Brassica napus*. *J Agric Food Chem* 68:7830–7838. <https://doi.org/10.1021/acs.jafc.0c02522>
- Zolfi Bavariani M, Ronaghi A, Ghasemi R (2019) Influence of pyrolysis temperatures on FTIR analysis, nutrient bioavailability, and agricultural use of poultry manure biochars. *Commun Soil Sci Plant Anal* 50:402–411. <https://doi.org/10.1080/00103624.2018.1563101>

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