Synergistic Effects of Biochar and *Arabidopsis helleri* on Soil Cd and Pb Bioavailability and Uptake and Disposition

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*Corresponding author: yjlyt@ycit.cn

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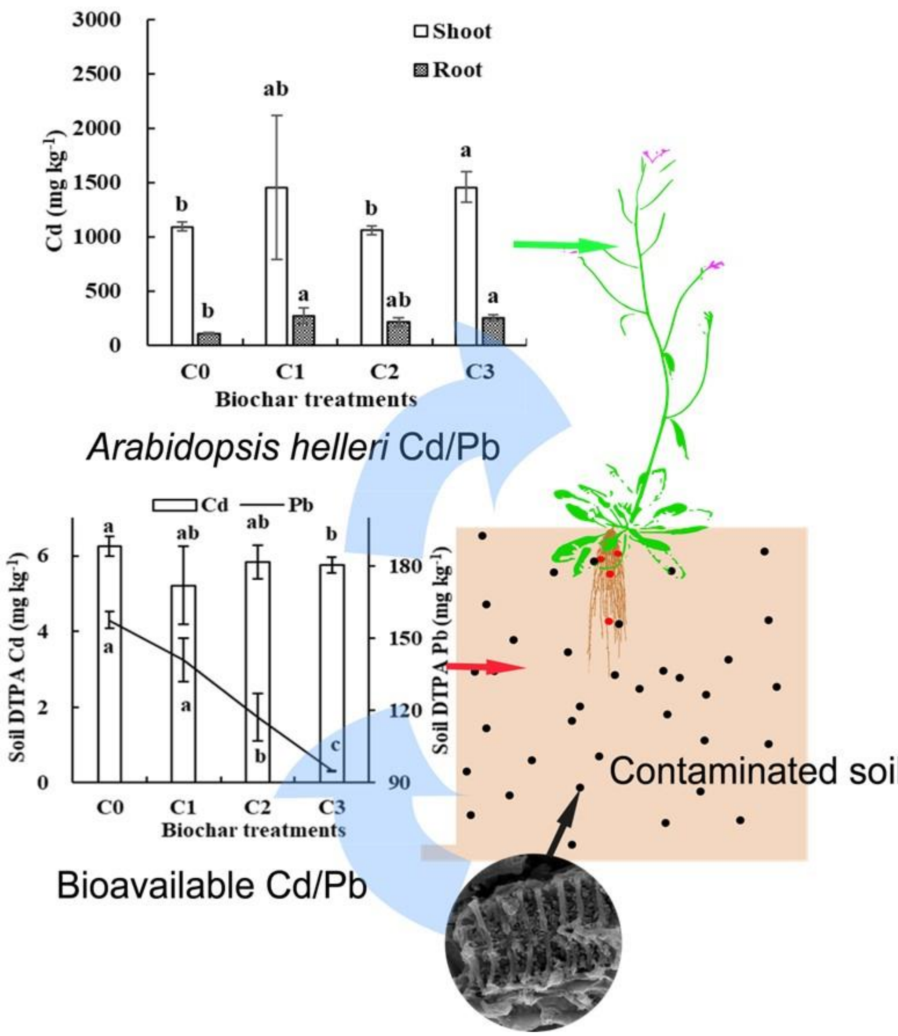
GRAPHICAL ABSTRACT



Synergistic Effects of Biochar and *Arabidopsis helleri* on Soil Cd and Pb Bioavailability and Uptake and Disposition

Liqiang Cui,[a,b] Guixiang Quan,[a,b] Jinlong Yan,[a,b,]* Fengfeng Sui,[a,b] Hui Wang,[a,b] Kiran Hina,[c] Shaukat Ali Abro,[d] Nobuyuki Kitajima,[e,f] Hiroshi Kubota,[e,f] Ama Tong,[e,f] Moeko Shinoda,[e,f] Syota Fukuro,[e,f] and Wenyu Zhang [e,f]

The potential effects of combining biochar with hyperaccumulator plants on heavy metal stabilization and uptake in Cd and Pb-contaminated soils have received limited attention. This study used biochar (BC) at levels ranging from 0 to 40 t ha^{-1} as soil amendments in Cd and Pb contaminated rice paddy soil. Biochar significantly decreased soil bioavailable Cd and Pb concentrations 6.7 to 16.6% and 10.6 to 39.7%, respectively, compared to the control. The shoot and root Cd concentrations in *Arabidopsis helleri* increased significantly by 33.5% and 133.1%, respectively, in the C3 treatment compared to the control. Similarly, shoot Pb concentrations showed a 57.5% increase compared to the control, but no significant changes were observed in root Pb concentrations. The *Arabidopsis helleri* bioconcentration factor (BCF) saw an increase of up to 40.5% for Cd and 57.8% for Pb with the C3 treatment. Conversely, the Cd translocation factor (TF) decreased 42.8 to 49.1%, while the Pb TF increased 32.8 to 96.6% with biochar application. The majority of *Arabidopsis helleri*-biochar Cd and Pb was found in the B3 fraction (organic fraction), constituting over 50%, and even over 80% for Cd. The *Arabidopsis helleri*-biochar primarily contained organic char binding Cd and exhibited slow-release characteristics.

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Keywords: Biochar; Cadmium; Lead; Arabidopsis helleri; Waste treatment

*Contact information: a: School of Environmental Science and Engineering, Yancheng Institute of Technology, Yancheng, 224051, China; b: Jiangsu Engineering Research Center of Biomass Waste Pyrolytic Carbonization & Application, Yancheng, 224051, P. R. China; c: Department of Environmental Sciences, Hafiz Hayat Campus, University of Gujrat, Gujrat, 54000, Pakistan; d: Department of Soil Science, Sindh Agriculture University, Tandojam, Pakistan; e: Technology Development Division, Fujita Corporation, Atsugi, 243-0125, Japan; f: Fujita (China) Construction Engineering Co., Ltd, Shanghai, 200030, China; *Corresponding author: yjlyt@ycit.cn*

INTRODUCTION

Cadmium (Cd) and lead (Pb) are heavy metals responsible for severe soil contamination worldwide. They are among the most hazardous elements found in soil, even though neither people nor plants require them for survival. Human activities, the use of phosphate fertilizers containing cadmium and lead impurities, and sewage sludge are key sources of contamination (Kudo *et al.* 2023). In China, agricultural soils were found to have an average Cd content of 0.35 mg/kg, higher than the background level of 0.097 mg/kg (Lin *et al.* 2022). Global Cd emissions have reached 2.2×10^5 tons, and Cd pollution is becoming increasingly problematic, especially in plant systems (Zveushe *et al.* 2023)

Phytoremediation is a cost-effective and environmentally friendly method for remediating Cd-contaminated soils, offering solution for the critical global problem of heavy metal pollution. It irreversibly eliminates toxins from the soil, making it an attractive technology. For widespread application to remediate contaminated Chinese agricultural soils, these technologies must be highly efficient and cost-effective (Zhao *et al.* 2015). Phytoextraction, using hyperaccumulators such as *Sedum alfredii*, *Sedum plumbizincicola*, or high Cd-accumulating rice cultivars, has been evaluated in limited-scale studies (Li *et al.* 2014). Economic viability will determine their potential for widespread application. *Arabidopsis halleri*, a model Cd-tolerant hyperaccumulator species, shows promise for remediating heavy metal-polluted soils (Baliardini *et al.* 2015). Chelator complexes play a key role regulating heavy metal absorption and tolerance in hyperaccumulators. Soil amendments such as biochar can enhance their efficacy (Shanmugam *et al.* 2013). Biochar achieves a mass balance by reducing soil Cd levels during plant growth while changing Cd's chemical form (Grignet *et al.* 2022).

Biochar can dramatically reduce the bioavailability and leachability of cationic metals and metalloids in soils. It also has been shown to improve soil structure, physicochemical properties, fertility, and revegetation, affecting elemental mobility (Narayanan and Ma 2022). Biochar’s efficiency in stabilizing heavy metals through chemisorption mechanisms has been demonstrated (Ippolito *et al.* 2012). For example, Zhao *et al.* (2019) found that barley grass biochar sorbed up to 95% of soil-borne lead, reducing lead levels in the soil. Qin *et al.* (2018) showed that biochar made from pig manure reduced leaching losses of bioavailable forms of lead and cadmium 38% and 71%, respectively, compared to a control. Other studies (Cui *et al.* 2011, 2012, 2016; Bian *et al.* 2014; Ippolito *et al.* 2017) demonstrated biochar’s ability to enhance soil pH and soil organic matter (SOM), leading to significant reductions in metal bioavailability and concentrations in rice and wheat grains over both short and long terms.

Several types of biochar, including phytoremediation plant (*Symphytum officinale* L)-biochar, show potential as additives for remediating strongly metal-affected soils and purifying water with low extractable heavy metal (Du *et al.* 2019; Gascó *et al.* 2019). Safe disposal of hyperaccumulator biomass presents challenges when harmful components are present after the process (Chai *et al.* 2022). Pyrolysis offers a risk-free way to dispose of waste from hyperaccumulators, immobilizing heavy metals in the resulting biochar (Huang *et al.* 2023). To ensure its safety, hyperaccumulator-derived biochar should undergo environmental impact testing before use (Cui *et al.* 2021b). Pyrolysis significantly reduces heavy metal leaching, making it a secure and valuable option for hyperaccumulator disposal (Wang *et al.* 2017).

Further research is needed to understand how biochar affects soil properties, heavy metal bioavailability, hyperaccumulator Cd and Pb uptake, and waste disposal. This investigation aimed to determine the effects of biochar on the uptake and disposal of lead and cadmium, using *Arabidopsis helleri* as a model heavy metals accumulator plant. The authors hypothesized that biochar would bind Cd and Pb, reducing the soil's bioavailable fraction, assisting *Arabidopsis helleri* in amending Cd and Pb polluted soil, and that pyrolysis would be the preferred waste disposal method to stabilize Cd and Pb.

EXPERIMENTAL

Experimental Design

The soil used in the experiment was collected from a paddy soil that has been contaminated by air fallout and effluent discharges from a nearby smelter since at least the 1970s in Jiangsu Province. The most common types of metal contaminants found in this area are cadmium and lead. The paddy soil (Ferric-accumulic Stagnic Anthrosol) was determined to be the sort of soil that was present in the paddy. A sieve with a 5-mm opening was used to prepare the soil for use after it had been air-dried and stones and other debris had been removed. At the Sanli New Energy Company in Henan province, China, the wheat straw used in the production of the biochar was pyrolyzed at temperatures of roughly 450 °C. In this study, a variety of treatments were used, each with a different application level of biochar (0, 10, 20, or 40 tons per hectare), and these treatments were designated by the following abbreviations: C0, C1, C2, and C3. In polyethylene plastic barrels with a diameter of 40 cm, a height of 40 cm, and a depth of 35 cm, the soil and biochar were mixed together. The seeds of *Arabidopsis helleri* were put in each container on November 12, 2021, with a total of 50 seeds. The biochar samples and the background soil samples were both collected and brought to the laboratory, where they were both ground to the point where they could pass through a sieve with a 1.5-mm opening. In accordance with the standard protocols outlined by Lu (2000), an investigation into the fundamental properties of both the biochar and the soil was done, and the results of this investigation are shown in Table S1.

Sample Collection and Analysis

Arabidopsis helleri whole plants were harvested on May 11, 2022 by carefully removing them from their pots. After removing the soil, the plant samples were cleaned in tap and deionized water, and their roots and shoots were separated. After that, the plant samples were dried at 105 °C for 30 min, then at 60 °C until totally dry. The *Arabidopsis helleri* biomass of the root and shoot were weighed before and after dried. The dried materials were ground into powder and placed in polyethylene bags.

A 0.50-g sample was placed in a 100-mL beaker. It was predigested in a solution of concentrated HNO_3 and $HClO_4$ (4:1, v:v) at room temperature overnight. The next day, the beakers were heated from 100 to 200 °C for 30 min, then to 250 °C until the solution became colorless, leaving around 2 mL. The solutions were filtered using a membrane filter with a 0.45-micron pore size after being cooled and brought to a final volume of 25 mL. The Cd and Pb concentrations in the solutions were then measured utilizing atomic absorption spectrometry (AAS; TAS-986, Persee, China).

Three soil cores (0 to 15 cm) were taken from each container after harvesting *Arabidopsis helleri*. After removing plant detritus, the soil was air-dried and processed to pass through a 2-mm screen. Analytical procedures described by the Lu (2000) were used to analyze soil pH, EC, SOM, and total N. A glass electrode and 1:2.5 soil-to-water ratio measured soil pH and EC. To measure total nitrogen and organic carbon, the semimicro-Kjeldahl and dichromate oxidation methods were used, respectively. Total soil phosphorus (total P) was determined using the NaOH fusion–Mo–Sb anti-spectrophotometric method. To measure total/DTPA Cd, Pb, and progressively extractable heavy metal fractions, an additional subsample of air-dried soil was powdered until it passed a 0.15-mm sieve. After digesting soil with aqua regia and hydrofluoric acid, atomic absorption spectrometry was used to measure the lead-to-cadmium ratio.

The *Arabidopsis helleri* biomass collected from C0 treatment was first air-dried, then crushed, and then dried in an oven at 105 °C. The pyrolysis process for converting the dried *Arabidopsis helleri* biomass into biochar was carried out in a vacuum tube furnace (model number: NBD-O1200, Henan, China) under a continuous flow of nitrogen gas (500 mL min^{-1}) to maintain an inert atmosphere. Two different pyrolysis temperatures, 400 and 500 °C, were investigated. For each temperature, the dried biomass was loaded into a ceramic crucible and placed inside the furnace. The furnace was heated from room temperature to the target pyrolysis temperature at a ramping rate of 10 °C min^{-1}. Once the desired temperature was reached, it was maintained for 4 hours to ensure complete carbonization of the biomass. After the 4-hour holding period, the furnace was allowed to cool down to room temperature under the continuous nitrogen flow. The resulting biochar was carefully collected from the crucible, weighed, and stored in airtight containers for further analysis.

According to the findings presented by Cui *et al.* (2020), the extraction methodology for the *Arabidopsis helleri*-biochar samples consisted of four stages and was based on the method developed by the European Community Bureau of Reference (BCR). Each stage of the process utilized a distinct reagent, beginning with 0.11 M acetic acid for the exchangeable fraction (B1), moving on to hydroxyl ammonium chloride for the iron and manganese oxyhydroxides portion (B2), hydrogen peroxide for the organic fraction (B3), and aqua regia for the residual fraction (B4). Before analyzing them on atomic absorption spectrometry (TAS-986, Persee, China), the final liquids from all four processes were filtered through a membrane with a pore size of 0.45 microns. This was done so that the levels of cadmium and lead could be accurately measured.

Statistical Analysis

The data were presented with the means as well as the standard deviation. A two-way analysis of variance (ANOVA) was carried out to examine the differences between the treatments, and P-values of less than 0.05 were taken as indicative of statistical significance. SPSS, version 20.0 (SPSS Institute, Chicago, IL, USA) was utilized for statistical analysis. Principal component analysis (PCA) is a statistical technique used for dimensionality reduction and exploratory data analysis. It is an orthogonal transformation that converts a set of potentially correlated variables into a new set of uncorrelated variables called principal components. These principal components are linear combinations of the original variables, arranged in descending order of variance explained. The first principal component (PC1) accounts for the maximum possible variance in the dataset, capturing the most significant pattern or trend. The second principal component (PC2) is orthogonal to the first and accounts for the second-largest portion of the remaining variance, representing the next most significant pattern. Subsequent principal components (PC3, PC4, and so on) are computed in a similar manner, each capturing progressively smaller portions of the remaining variance. In the present study, the original data was first normalized as a pre-treatment step to ensure that all variables were on a comparable scale. Subsequently, a correlation matrix (Pearson's correlation) was constructed using the normalized data. This correlation matrix served as the input for the PCA, which generated a new set of orthogonal principal components or coordinates. The PCA computation was performed using the statistical software SPSS (version 20.0), as described by Batista and Gomes (2021). The bioconcentration factor (BCF) was calculated by dividing the concentration of cadmium or lead found in the sections of the plant that grow above ground by the corresponding concentration of cadmium or lead found in the soil. In a similar manner, the translocation factor (TF) was determined by dividing the concentration of cadmium or lead in the above-ground portion of the plant by the concentration of cadmium or lead in the roots of the plant.

RESULTS AND DISCUSSION

Biochar Effects on Total and Bioavailable Cd and Pb in the Soil

Biochar reduced bioavailable Cd and Pb relative to the C0 treatment, but soil total Cd and Pb did not decrease (Fig. 1). Lead reductions were 10.6 to 39.7% and cadmium 6.7 to 16.6%. DTPA-extractable Cd and Pb were reduced as biochar was added to the soil. It was expected from other studies that the biochar would reduce the bioavailable heavy metals. For instance, Wang *et al.* (2019) found that rice straw biochar (5%, w:w) significantly reduced soil heavy metal solubility. Compared to other biochars, a maximum reduction of 58.9 mg kg^{-1} was found for Cu and 10.6 mg kg^{-1} was found for Pb. The 2% (w:w) rice straw biochar lowered Cd bioavailability due to soil improvements such as pH (Chen *et al.* 2019), which showed a similar trend. The 5% biochar significantly reduced DTPA-extractable Cd in soils with pot experiment (Li *et al.* 2019). Therefore, the findings in this study were well aligned with previous literature.

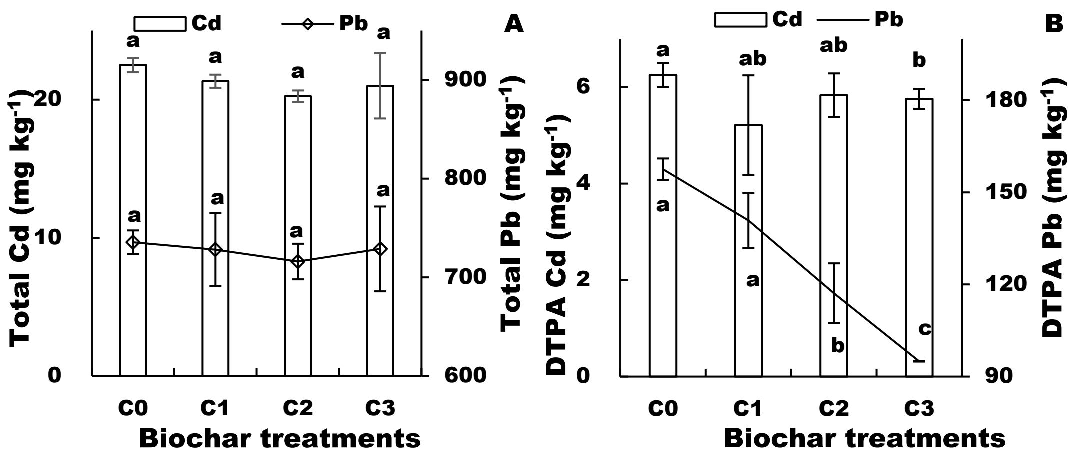

Fig. 1. Changes in soil total Cd/Pb(A) and EDTA Cd/Pb(B) with biochar application. Different small letters above error bars show significant treatment differences. The error bars indicate the mean standard deviation from three replicates (n = 3). A Tukey *post-hoc* test showed significant treatment differences at $p < 0.05$.

Biochar Effects on pH, EC, SOM, and Total N

The application rates of biochar were associated with changes in soil characteristics (Fig. 2A). Increasing biochar application rates resulted in a pH increase of 0.03 to 0.19 units for the soil, which had a direct impact on the heavy metal fractions during the study (Cui *et al.* 2019). So, the biochar had the tendency to increase soil pH with increasing biochar application ratio. For the C3 treatment, the soil's electrical conductivity (EC) decreased 27.5% (Fig. 2B), indicating that biochar can also adsorb water-soluble salts or promoted the plant uptake.

Biochar had a significant impact on the soil organic matter (SOM) content. The SOM was significantly increased by 15.8% to 46.6% with biochar application compared with control (Fig. 2C). This increase can be attributed to the fact that biochar is resistant to degradation and can help maintain stable SOM levels over the long term (Cui *et al.* 2019, 2020).

A similar trend was observed with the total nitrogen (N) content of the soil. The soil total nitrogen was significantly increased 1.4% to 19.3% with increasing biochar application ratio (Fig. 2D). The increasing total nitrogen content suggested that biochar also contributed to the nitrogen supply (Cui *et al.* 2021a). The higher biochar application ratios had higher N contribution in the soil. It was also found that biochar application can help reduced nitric nitrogen leaching with raising of the soil pH (Rees *et al.* 2020).

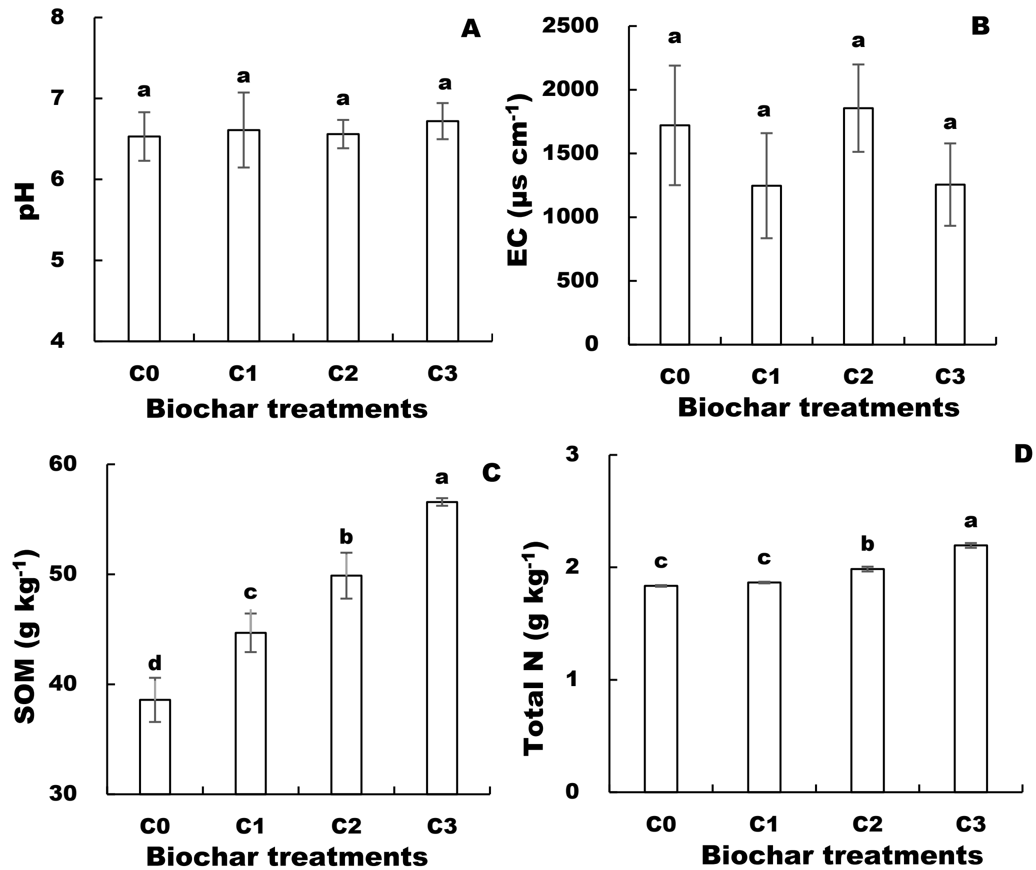

Fig. 2. Biochar alters soil pH (A), EC (B), SOM (C), and total N (D). Different small letters above error bars show significant treatment differences. The error bars indicate the mean standard deviation from three replicates (n = 3). A Tukey *post-hoc* test showed significant treatment differences at $p < 0.05$.

Biochar Effects on Cd and Pb transfer and growth in *Arabidopsis helleri*

The Cd and Pb concentrations and transfer ratio in *Arabidopsis helleri*, as a function of biochar application rate, are shown in Fig. 3. Following biochar application at 40 t ha^{-1}, the shoot and root Cd concentration significantly increased 33.5% and 133.1% compared with control, and shoot Pb concentrations increased 57.5% as compared to the control. Meanwhile, the root Pb concentrations exhibited no significant changes, and even tended to decrease. Lesser increases were observed with lower biochar application rates. Biochar significantly decreased Cd transfer from roots to shoot, but it increased the root Pb uptake. Similar findings were reported by Cui *et al*. (2011), where biochar amendments significantly affected the uptake, transfer, and growth of hyperaccumulators. Kudo *et al*.

(2021) found that the maximum Cd concentrations in shoots were 1890 mg kg^{-1} and in roots were 300 mg kg^{-1} on the 103rd day, respectively. Other studies also showed that 5% biochar amendment significantly enhanced the accumulation capacity of stem and leaf Cd, reaching a maximum of 355 mg kg^{-1} (Fu *et al.* 2021). It was also found that the corn straw biochar amendment with growing *Sedum plumbizincicola* was higher in decreases in extractable Cd 60.0% compared to that without biochar (Li *et al.* 2018). The biochar also increased of shoot production and promoted growth and enhancing extraction of Cd by hyperaccumulators of *Noccea caerulescens* on soil (Ree *et al.* 2015). The biochar addition promoted plant growth and increased shoot trace metal concentrations, consequently increasing the removal efficiency; soil trace metal removal by the hyperaccumulator further reduced the extractable trace metals in addition to immobilization by biochar (Su *et al.* 2021). It was found that the Cd uptake by *Sedum alfredii* roots substantially increased 118 to 187% with biochar addition (Chen *et al.* 2023). In addition to immobilizing metals, biochar may decrease competition with cations for metal uptake, thus enhancing extraction of metals by hyperaccumulators (Rees *et al.* 2015).

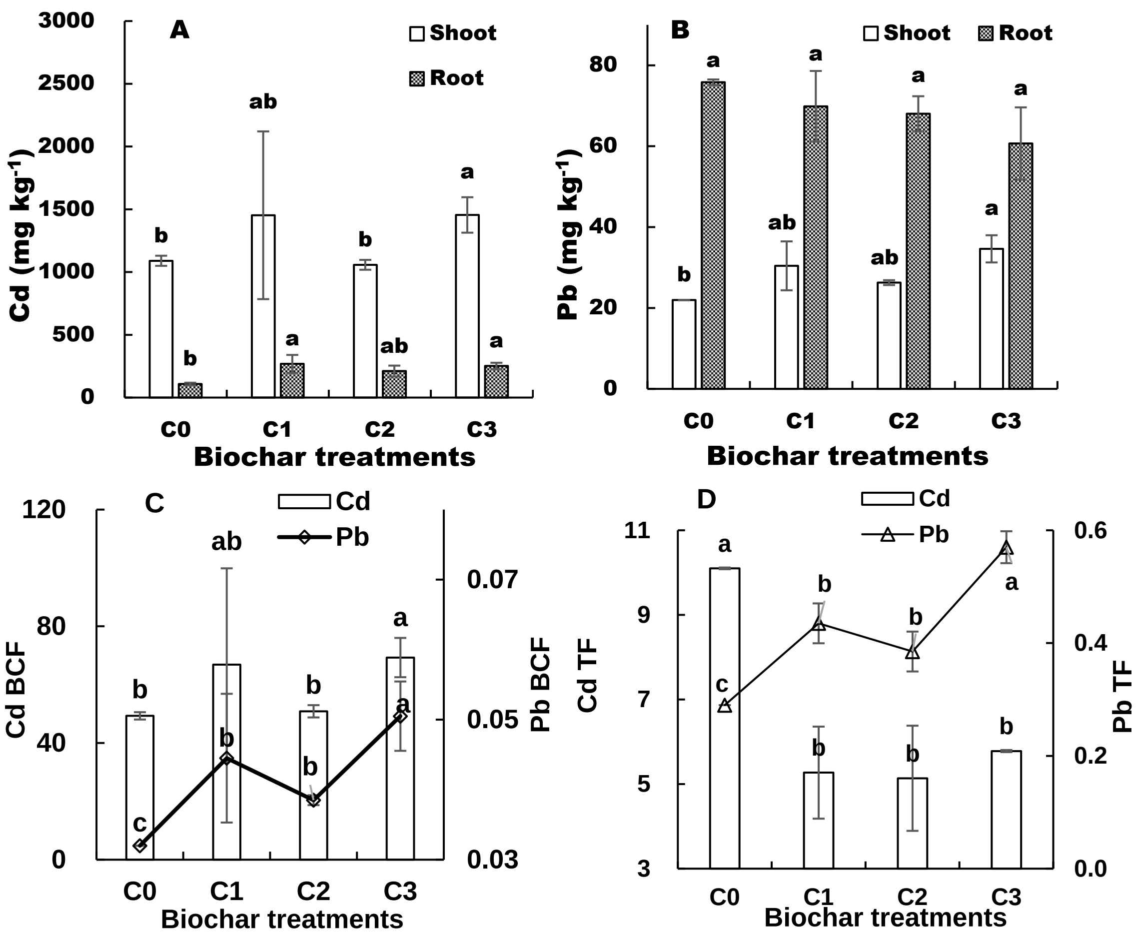

Fig. 3. Plant tissue Cd (A) and Pb(B) concentration and BCF (C) and TF (D) transfer ratio. Different small letters above error bars show significant treatment differences. The error bars indicate the mean standard deviation from three replicates (n = 3). A Tukey *post-hoc* test showed significant treatment differences at $p < 0.05$.

The BCF (Bioconcentration Factor, the ratio of shoot concentration to soil concentration) and TF (Translocation Factor, the ratio of shoot concentration to root concentration) were used to assess Cd and Pb transfer from the soil to the above-ground plant, and from plant roots to above-ground tissues, respectively (Wei *et al.* 2012). The BCF increased up to 40.5% (Cd) and 57.8% (Pb) with C3 treatment. The Cd TF decreased 42.8 to 49.1%, but the Pb TF increased 32.8 to 96.6% with biochar application. The changes of *Arabidopsis helleri* BCF and TF with increasing biochar application level indicated that biochar can improve the Cd and Pb transfer and uptake in the hyperaccumulator. In the presence of biochar, the plant BCF was decreased under the Cd and Pb contaminated soil, and the TF also was decreased, ranging from 49.6 to 61.0% (Cd) and 61.0 to 70.7% (Pb), respectively, as compared to a control (Mujtaba Munir *et al*. 2020). These findings suggest that biochar effectively improved heavy metal phases, leading to reduced metal translocation within plants. The biochar improved the polluted soil environment and probably saved the energy for hyperaccumulator, because Cd transfer from the hyperaccumulator root medium to the xylem is an energy-dependent process and translocation from roots to shoots in inorganic form. The combination of biochar with *hyperaccumulator* growth decreased the available amount of Cd and Pb in the soil, and the bioaccumulation factor (BAF) of Cd were particularly elevated (> 10) in the unamended soil and reached values higher than 1 for other elements (Gascó *et al.* 2019). The TF was reduced for Cd and Pb after biochar addition, indicating root accumulation of these metals. *Arabidopsis halleri* is a Cd hyperaccumulator, which accumulated 1500 mg kg^{-1} Cd in the shoot, and the Cd transfer from the root medium to the xylem, which was an energy-dependent process that is partly shared with Zn and/or Fe transport (Ueno *et al.* 2008).

Biochar Effects on *Arabidopsis helleri* Biomass and Cd/Pb Fractions in Biochar

Biochar improved soil characteristics and affected plant biomass (Fig. 4A). Similar conclusions were reached by Kudo *et al.* (2023) regarding metal accumulation in *Arabidopsis halleri* biomass. However, contrasting results were observed with the application of straw biochar at 5% concentration, which led to substantial increases in moso bamboo biomass (157% in leaves, 113% in roots, and 111% in stems) (Wang *et al.* 2019). Figure 4B presents the results of sequential BCR extractions on Cd and Pb fractions in *Arabidopsis helleri* biochar at different pyrolysis temperatures. The exchangeable Cd and Pb fractions (B1) accounted for approximately 10% of the total extracted Cd and Pb. As the pyrolysis temperature increased, the B1 fraction increased for Cd but decreased for Pb. The B2 fraction ratio of *Arabidopsis helleri* biochar Cd increased with higher pyrolysis temperatures, as did the B4 Cd fraction. However, the B2 and B3 Pb fraction ratios decreased with higher pyrolysis temperatures, while the B4 Pb fraction ratio increased, suggesting greater stability of Pb at higher temperatures. The majority of Cd and Pb in *Arabidopsis helleri* biochar were found in the B3 fraction (organic fraction), representing over 50% and even over 80% for Cd, indicating strong organic binding of Cd in the biochar. Pb was also mainly found in the B3 fraction (organic fraction). This suggests that pyrolyzing *Arabidopsis helleri* may be a promising strategy for heavy metal preservation or recycling. Wu *et al.* (2022) presented a new method for producing MnOx-loaded biochars from the biomass of a Mn-hyperaccumulator species (*Phytolacca americana*) that effectively remediated Cd-contaminated waterways in an environmentally friendly manner. Du *et al.* (2019) found that heavy metal extraction proportions decreased as pyrolysis temperature increased, and biochars produced at higher temperatures (over 550 °C) were deemed environmentally safe for surrounding ecosystems.

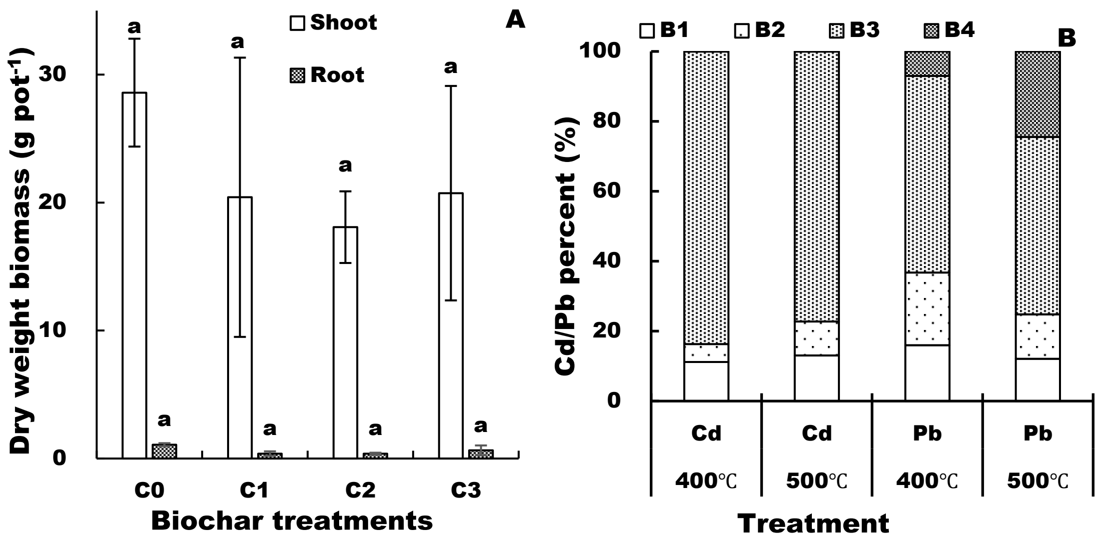

Fig. 4. The *Arabidopsis helleri* biomass (A) and Cd/Pb fractions in *Arabidopsis helleri* biochar (B) based on a BCR sequential extraction. B1 (exchangeable fraction); B2 (iron and manganese oxyhydroxides fraction); B3 (organic fraction) and B4 (residual fraction)

The Influence of Biochar on Soil and Plant Characteristics in Relation to Cd and Pb Contamination and Fraction

Principal component analysis and correlation analysis (Fig. 5) revealed that the application of biochar improved soil characteristics and facilitated metals transport and uptake in *Arabidopsis helleri*. The PCA indicated positive associations between biochar application levels and soil properties (*e.g.*, soil organic matter, soil pH, and soil nitrogen concentrations), as well as *Arabidopsis helleri* Cd/Pb concentrations in PC1 (63.8%). Conversely, PC1 showed negative associations between biochar application and DTPA, Cd, and Pb concentrations. PC2 primarily represented total Cd and Pb as well as EC, all linked to the amount of biochar applied. Together, PC1 and PC2 accounted for 84.5% of the total variability, underscoring the significant impact of biochar on soil characteristics. Studies by Ippolito *et al.* (2017) and Wang *et al*. (2014) highlighted that raising soil pH is a crucial factor in reducing the bioavailability of heavy metals. Biochar has been shown to efficiently manage risk element availability, promote biomass growth, increase total metal uptake, and minimize leaching of these elements (Břendová *et al.* 2015). Although biochar reduced the available cadmium in acidic paddy soil, high concentrations of available cadmium may not limit hyperaccumulator uptake. Fu *et al.* (2021) suggest that biochar's improved nutrient supply and soil conditions may foster hyperaccumulator growth and boost Cd phytoextraction in polluted soil. Rees *et al.* (2020) reported that *Noccaea caerulescens*, a hyperaccumulator plant, removed over 40% of initial lead pollution from topsoil within four years by reducing the plant-available fraction of metals through biochar application. However, the effectiveness of Cd phytoextraction may decrease with successive hyperaccumulator harvests, so complete soil pollution removal through biochar application may take a long time. Biochar amendments increased the concentrations of cadmium and lead in the shoots and tended to enhance overall phytoextraction of these metals. Moreover, biochar improved soil characteristics, promoted hyperaccumulator performance, and facilitated metal movement from the topsoil into the hyperaccumulators.

Overall, biochar has shown promising effects on metal uptake and phytoextraction in *Arabidopsis helleri*, making it a valuable tool for soil remediation efforts.

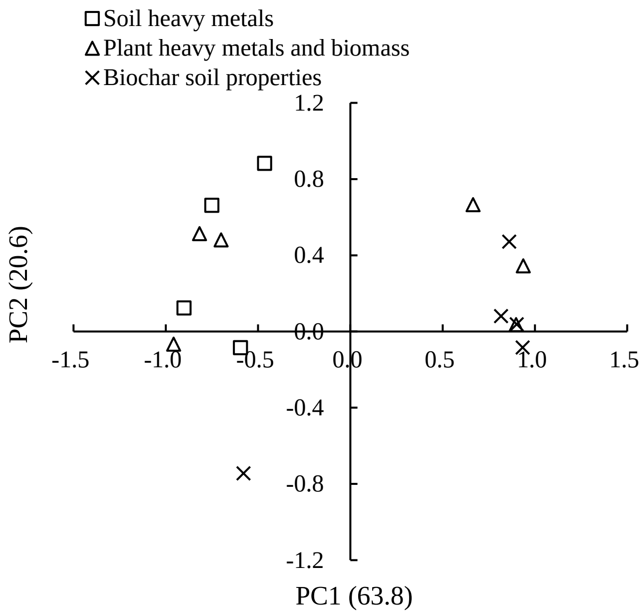

Fig. 5. Principal component analysis

CONCLUSIONS

1. The application of wheat straw-biochar had positive effects on soil properties, leading to a reduction in soil Cd and Pb bioavailability. However, it resulted in increased uptake and translocation of Cd and Pb by *Arabidopsis helleri*, particularly for Pb.
2. The biochar amendment also contributed to increased soil organic matter (SOM), and total N levels, which in turn improved the growth and vitality of *Arabidopsis helleri*. The observed increase in bioconcentration factor (BCF) and translocation factor (TF), except for Cd, within *Arabidopsis helleri* suggests that biochar amendment can be an effective strategy for remediating heavy metal-polluted soil by leveraging the unique capabilities of hyperaccumulators, while ensuring the safety of crop growth.
3. The ability of hyperaccumulators to stabilize Cd/Pb in *Arabidopsis helleri* derived-biochar highlights the potential for cyclic restoration of heavy metal-polluted soil and metal recycling, making it an environmentally sustainable approach.
4. Future research should focus on exploring intercropping techniques in heavy metal-polluted soil with the application of biochar. This approach could offer further insights and possibilities for sustainable soil remediation and metal recycling.

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APPENDIX

Table S1. Basic Paddy Soil (0 to 15 cm depth) and Biochar (g kg^{-1}) Properties

	pH (H_2O)	Organic C	Total N	Total P	Total K	CEC	Total Cd (mg kg^{-1})	Total Pb (mg kg^{-1})
Soil	6.07	20.7	3.19	0.82	11.4	18.0	22.6	713.3
Biochar	10.4	467	5.90	14.4	11.5	21.7	0.03	12.9

CEC: cation exchange capacity (cmol kg^{-1})