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Characterisation of biochar produced from two types of chestnut shells for use in remediation of cadmium- and lead-contaminated soil

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ABSTRACT

China is the major producer of chestnut, with 1.84 million tons of chestnut production, resulting in an enormous waste of chestnut shells. In the current study, shell biochar (SBC) was produced using the inside shell covering fruit, and the outside shell with thorns was used to produce thorn biochar (TBC). Both types of biochar were characterised through Brunauer–Emmett–Teller (BET) analysis, scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR). These analytical results showed a more obvious smooth surface and micro-pore structure in SBC. The vibration of C=O/C=C and C–O (phenolic) showed a significant difference between the two types of biochar. Sorption experiments indicated that the adsorption capacity of the different types of biochar for cadmium (Cd) did not differ significantly, whereas the adsorption capacity of TBC for lead was better than that of SBC. In the pakchoi cultivation experiment (28 days), the application of TBC (1.5%) promoted plant shoot weight, root weight, shoot length and root length by 465%, 143%, 109% and 97% respectively. The application of biochar effectively increased soil pH and reduced the bioavailability and migration of heavy metals. Besides, membrane integrity and chlorophyll content were enhanced because of the alleviation of oxidative stress. Noticeably, application of TBC (0.1% and 1.5%) reduced the Cd concentration in the root by 40–60%, and enhanced accumulation of Pb by 75–191%. Overall, our study demonstrated that 1.5% TBC has promising potential for remediating Cd-contaminated soil. Our study has demonstrated the remediation potential of chestnut and provided a clue for sustainable management of chestnut shell waste for further development of chestnut resources.

Keywords: adsorption, antioxidant enzymes, Cd, chestnut shell biochar, contaminated soil, pakchoi, Pb, remediation.

Introduction

Heavy metal contamination is one of the environmental issues rapidly growing because of urbanisation and industrialisation (Zhao *et al.* 2015). In China, farmland soil contaminated with heavy metals covers an area of about 2×10^7 Hm², causing an economic loss of about USD 3×10^9 (Li *et al.* 2014). In recent years, heavy metal contamination has attracted public attention because of its deleterious effects on human health (Rui *et al.* 2008a, 2008b). Besides, heavy metal contamination is a critical factor in restricting food yield (Rui *et al.* 2007a, 2007b). It is worth noting that the global population is estimated to reach 9.73 billion after 30 years, resulting in roughly doubling human food demand (Mueller *et al.* 2012; United Nations 2019). Therefore, it is urgent to give attention to controlling heavy metal pollution.

Biochar is obtained by the pyrolysis of different biomass feedstock at a particular range of temperatures under limited oxygen conditions (Gao *et al.* 2020a, 2020b). Because of the low cost and high adsorptive property of biochar, it is considered to be environmentally functional material (Zhou *et al.* 2017). Moreover, biochar has great potential to alleviate soil heavy metal stress and promote plant growth (He *et al.* 2019).

Castanea mollissima BL (Chinese chestnut), which belongs to the Fagaceae family, was once an essential food resource in the northern hemisphere (Yang et al. 2015; Zhou et al. 2021a, 2021b, 2021c). According to latest statistics of Food and Agriculture Organization of the United Nations (FAO), China is the largest producer of chestnuts globally, with an area of 3.3×10^5 hectares and an output of 1.84 million tons (FAO 2019). The surface of the chestnut is covered with two shells, one of which wraps chestnut fruit, and another is covered with thorn. There are many discarded chestnut shells around the country, especially the spiny ones, which can be harmful to people or animals in the field (Liang et al. 2013). Considering a large amount of waste, these two types of chestnut shells were used for biochar production. Pakchoi (*Brassica Chinensis*) is a common vegetable in China, which people pursue because of its unique flavour and high nutrition.

In this study, two types of biochar were synthesised and characterised. Shell biochar (SBC) was produced from the inside shell covering the fruit, and the outside shell with thorns was used to produce thorn biochar (TBC). We hypothesised that biochar exposure could positively promote pakchoi seedling growth and further alleviate cadmium (Cd) and lead (Pb) phytotoxicity under soil culture conditions. The objectives of this study were to assess the alleviation effects of biochar on pakchoi with Cd and Pb co-exposure and broaden the application of biochar in the remediation of soil. To our knowledge, this is the first report to characterise the differences between two types of biochar derived from inside and outside chestnut shell. The effects of different types of biochar on the remediation and immobilisation of Cd and Pb are shown by pakchoi pot experiment. More importantly, the work has further demonstrated the remediation potential of chestnut shell biochar and provides clues for sustainable management of chestnut shell waste.

Materials and methods

Preparation and characterisation of biochar

Two types of chestnut shells were obtained from a chestnut orchard in Huairou District, Beijing (116.505754°N, 40.3413096°E). After chopping the shells to less than 1 cm sections, they were used as feedstock for biochar preparation. Biochar was produced by pyrolysis of the chestnut shell at 600°C for 2 h in a muffle furnace (heating rate of 5°C min⁻¹ under limited oxygen supply). Then biochar was naturally cooled in the muffle furnace under a continuous nitrogen gas (N₂) supply. Finally, the first type of biochar (SBC) was produced from the fruit shell, and the second type of biochar (TBC) was produced from the shell covered with thorns. To explore the combined effect of different types of biochar, SBC and TBC were mixed at a ratio of 5:5 to make a third kind of biochar (MBC). A graphic presentation of our experimental design is given in Fig. 1.

The pH was tested by a pH meter (PH838, Smart Sensor Inc., China) at a ratio of 20 mL:1 g (deionized water:biochar). Surface and morphological features were characterised by SEM (SU-8100, Hitachi, Japan). The specific surface-area and pore-size distribution were determined by BET and Barret–Joyner–Halenda (BJH) methods with N₂ adsorption isotherms (ASAP-2020 PLUS Automatic Physisorption Analyser, Micromeritics Inc., China). Fourier transform infrared (FT-IR; Nicolet iS20, Thermo Fisher Scientific Inc., USA) analysis was finished at a range of 400–4000 cm⁻¹.

Sorption experiment

The sorption experiment was followed by Fan et al. (2020). In brief, Cd²⁺ and Pb²⁺ isotherm experiments of adsorption were conducted in 250 mL conical flasks that contained a single metal ion (Cd²⁺ or Pb²⁺, initial concentration level of 50, 75 and 150 mg L⁻¹) solution at a pH 5. Then 25 mg of biochar was added into the conical flasks with different treatments, and shaken at 180 rpm, 25°C, for 12 h. The compound solutions were filtered through 0.25 µm PTFE membrane and analysed by inductively coupled plasma–mass spectrometry (ICP–MS; Detailed methods in section *Determination of Cd and Pb contents*).

Pot experiment

The soil for the pot experiment was collected from an experimental station of Shangzhuang, Beijing. The surface soil (0–20 cm from the top) properties have been provided in Supplementary Table S1. After air-drying for 2 days and sieving with a 2-mm mesh, the soil was mixed with Cd (30 mg kg⁻¹ of Cd) and lead (500 mg kg⁻¹ of Pb) to create a co-contaminated condition of Cd and Pb. SBC, TBC and MBC were mixed with the contaminated soil to achieve 0.1%, 0.5% and 1.5% concentrations. Each pot was filled with 250 g of soil. Three biological replicates were established for each treatment. Co-contaminated soil without additional biochar was used as the control.

Seeds of pakchoi (*Brassica chinensis*) were purchased from the Chinese Academy of Agricultural Sciences, Beijing. Before sowing, seeds were sterilised with 5% H₂O₂ for 15 min and rinsed with DI thoroughly. The seeds were put in a Petri dish and germinated in a climate incubator (18–22°C, 16/8 h light and dark cycle) for 7 days (Uslu et al. 2020). Seedlings of a uniform size were selected and placed into pots containing soil. The pakchoi seedlings were carefully harvested after 28 days of soil cultivation. Then deionised water was used to thoroughly rinse seedlings, so as to remove the adhesive soil and biochar from the plant surface. Fresh weight of shoot, fresh weight of root, root length and height of pakchoi were determined separately, and the material was dried in an oven (105°C for 3 h and 80°C for 24 h).

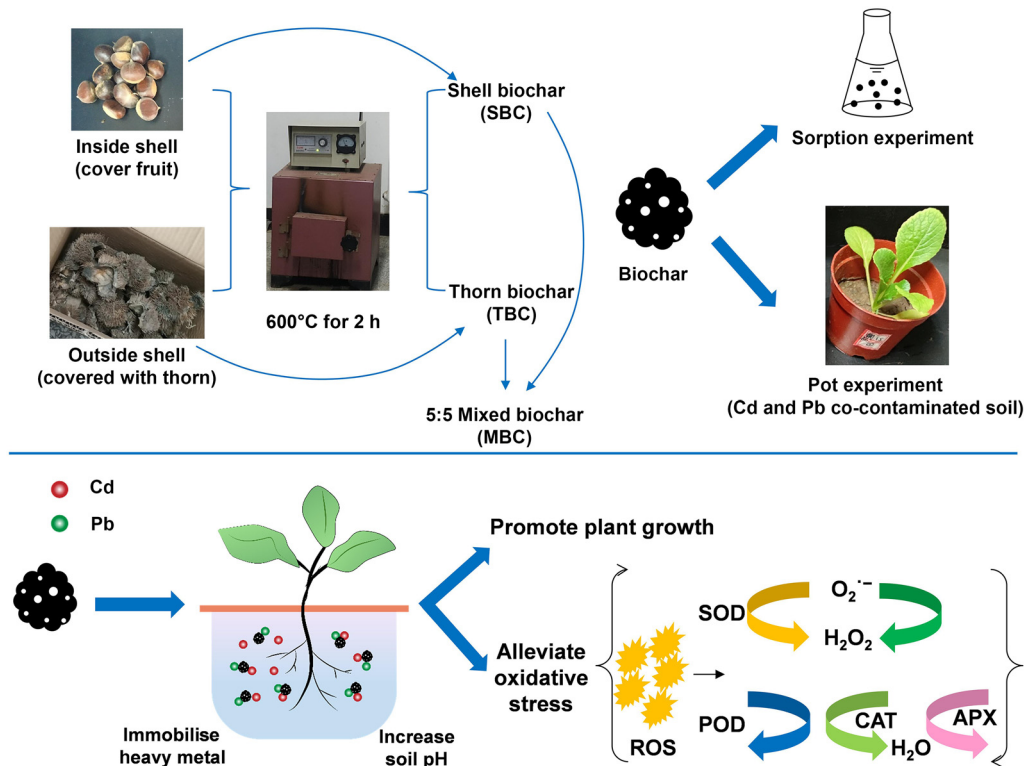


Fig. 1. Experimental design and result display.

Biochemical analysis

The assay kits purchased from Nanjing Jiancheng Bio-engineering Institute were used to test malondialdehyde (MDA) and the activity of antioxidative enzymes, including superoxide dismutase (SOD) and peroxidase (POD). Briefly, 0.2 g of the fresh pakchoi leaf was ground into powder under low temperature. The powder was mixed with 0.8 mL of PBS and centrifuged at 10 000g and 4°C for 10 min, and the supernatant was used for measurement. The SPAD value was tested by using SPAS-502 plus (Konica Minolta, Japan). The first fully expanded leaf was selected for the test, and 10 points near the main vein of each leaf were tested.

Determination of Cd and Pb concentrations

The measurement of Cd and Pb concentrations was performed as described in Rui *et al.* (2007a, 2007b). In brief, approximately 0.1 g of the pakchoi dried sample of different treatments was prepared to determine Cd and Pb. First, the pakchoi dry samples were ground to powder and thoroughly digested in a solution mixture of nitric acid–hydrofluoric acid (1:2) by using a microwave digestion system (XT-9916, Shanghai Xintuo, China; Rui *et al.* 2008a, 2008b). Then, the digested solution was diluted by ultrapure water to 10 mL and the concentrations were determined by ICP–MS (DRCII, PerkinElmer, and Norwalk, USA; Shi *et al.* 2009).

Data analysis

Statistical analyses were performed through one-way ANOVA in SPSS 20.0 (IBM, USA). The mean values for each treatment were compared using the Duncan's multiple-range test at a $P = 0.05$ confidence level. Data are expressed as means \pm s.d. ($n = 3$). The different lowercase letters indicate significant differences at $P = 0.05$.

Results and discussion

Characterisation of biochar

Our experimental design is presented graphically in Fig. 1. The skeleton of biochar is apparent, mainly in flakes and blocks. Smooth surface and micropore structures can be observed in SBC (Fig. 2c, f), whereas the surface of TBC is rough, with a lot of different sizes of ash particle (Fig. 2a, d). BJH adsorption average pore width of SBC is smaller than that of TBC, and the above result is consistent with BET surface area; more pore area results in more specific surface area (Table 1, Fig. 2d, f). The N adsorption capacity of TBC is more robust than that of SBC under the normal pressure (Fig. 3a), which is due to the bigger pore width in TBC.

Abundant additional functional groups and carbonate were produced during the pyrolysis of organic substances,

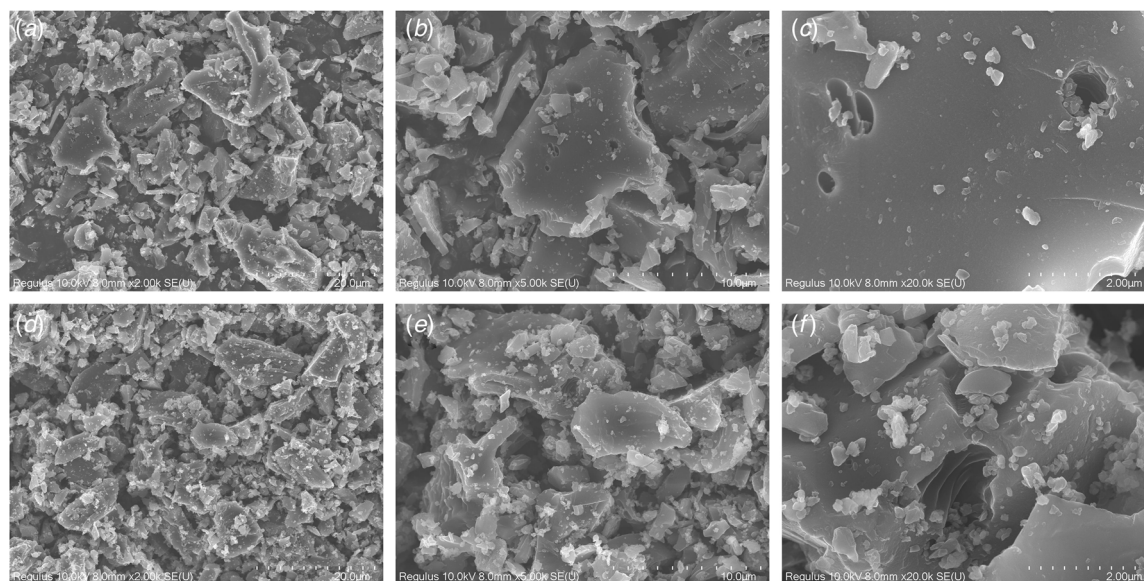


Fig. 2. SEM micrographs of (a–c) SBC and (d–f) TBC.

Table 1. Physicochemical properties of SBC and TBC.

Biochar type	pH	BET surface area ($\text{m}^2 \text{g}^{-1}$)	BJH adsorption average pore width (nm)	BJH desorption average pore width (nm)	Median pore width (nm)
SBC	9.52	18.1387	9.5938	8.4611	1.0381
TBC	9.71	14.5188	12.5131	10.5516	1.0606

which results in alkaline biochar (Yuan *et al.* 2011). In the present study, the pH of the two types of biochar was also alkaline, and there was no significant difference between TBC and SBC. The soil pH increased after applying alkaline biochar, thus reducing the bioavailability and migration of heavy metals in soil (Gao *et al.* 2020a, 2020b). Hannan *et al.* (2021) also demonstrated that biochar significantly increased the soil pH and reduced the bioavailability of nickel (Ni) concentration in soil, indicating that the alkalinity of biochar is significant for the remediation of heavy metals in soil.

Functional groups of the biochar surface were identified by FT-IR (Fig. 3b), and the adsorption peak at $3430\text{--}3432 \text{ cm}^{-1}$ was attributed to the O–H stretching vibrations (Song *et al.* 2020). The adsorption peak at $3430\text{--}3432 \text{ cm}^{-1}$ was attributed to the C–H stretching vibrations or $-\text{CH}_2$ antisymmetric stretching vibration (Song *et al.* 2020). The significant difference between SBC and TBC appears in the wavenumber range of $400\text{--}1593 \text{ cm}^{-1}$. In SBC, the peak at 1593 cm^{-1} was attributed to the vibration of $\text{C}=\text{O}/\text{C}=\text{C}$ (Tsai *et al.* 2012). In contrast, the peaks of TBC spectrum at 1423 , 1158 and 878 cm^{-1} were attributed to the vibration of C–O (phenolic), C–O (carboxylic) and $=\text{C}-\text{H}$ respectively (Wang *et al.* 2015; Mandal *et al.* 2017). The significant difference was attributed to the vibration of $\text{C}=\text{O}/\text{C}=\text{C}$ at 1593 cm^{-1} .

Surface functional groups containing oxygen, carboxyl and hydroxyl were confirmed in two types of biochar by FT-IR results. These surface functional groups have been shown to be beneficial to Cd, copper (Cu), Pb and Ni adsorption in previous studies (Yang *et al.* 2019; Silos-Llamas *et al.* 2020).

Sorption experiment of Cd or Pb

The adsorption capacity for Cd^{2+} by the three kinds of biochar was increased with an increase in the initial concentration (Fig. 4a). Besides, all types of biochar had the same change trend, meaning that adsorption of Cd^{2+} by the different types of biochar showed little difference. The adsorption amount of Pb^{2+} by SBC and MBC decreased with an increase in the initial concentration. The adsorption amount of Pb by TBC reached the maximum value of 29.23 mg L^{-1} when the initial concentration was 75 mg L^{-1} (Fig. 4b). The results showed that among the three kinds of biochar, TBC had the best adsorption effect on Pb^{2+} , which may be due to the larger median pore width of TBC biochar. In general, the adsorption effect of chestnut shell biochar on Cd^{2+} was significantly better than that on Pb^{2+} . Other researchers have reached similar conclusions, such as for the root of rose biochar and apricot atone activated carbon (Kobyay *et al.* 2005; Khare *et al.* 2017).

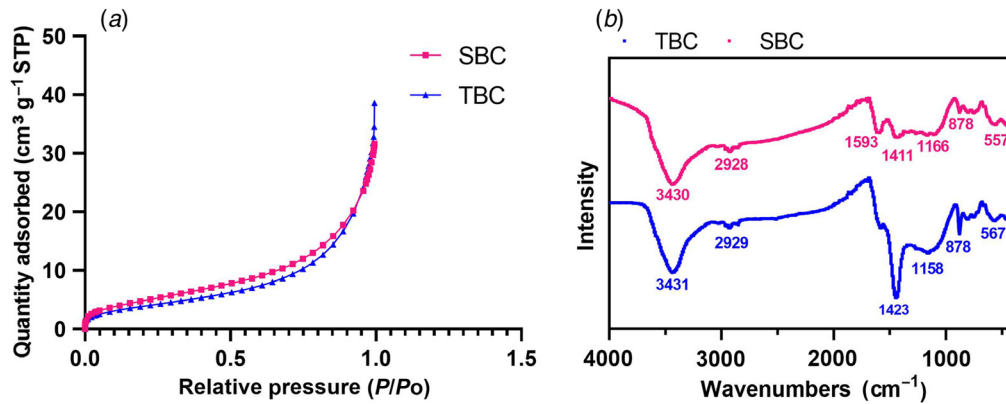


Fig. 3. (a) Adsorption isotherm line of N₂. (b) FT-IR spectra of SBC and TBC.

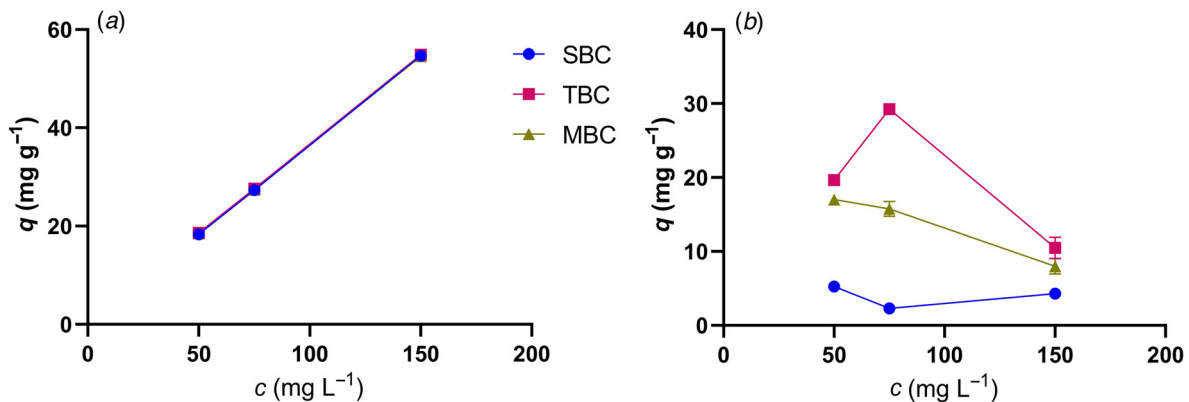


Fig. 4. Sorption of (a) Cd²⁺ and (b) Pb²⁺ on SBC, TBC and MBC in the single-metal systems.

Effects of biochar on plant phenotype under heavy metal stress

After 28 days of cultivation with different treatments, the growth of pakchoi seedlings was significantly promoted compared with the control, especially in the high concentration treatment of TBC (Supplementary Fig. S1). The application of 1.5% TBC increased plant shoot weight, root weight, shoot length and root length by 465.84%, 143.03%, 109.76% and 97.04% respectively (Fig. 5). However, all low-concentration biochar treatments (0.1%) had no apparent effect on the biomass of root, shoot length and root length. Interestingly, the middle concentration treatments (0.5%) of both SBC and TBC had an apparent effect on promoting the shoot growth and alleviating heavy metal stress, whereas the MBC treatment did not (Fig. 5a, c). Except for the treatment with 0.1% MBC and 0.5% MBC, all other treatments significantly increased shoot fresh weight at $P = 0.05$ confidence level (Fig. 5a). More importantly, the fresh weight of root and root length were significantly promoted by the high

concentration of SBC and TBC, but the high concentration of MBC had no such effect (Fig. 5b, d).

Biochar has potential to improve soil properties and plant growth, and can enhance root nutrient obtention directly as a nutrient source or indirectly by altering soil nutrient concentration. According to the root box experiment, some studies have confirmed that plant roots grow preferentially in the zone containing biochar because of the nutrition (Prendergast-Miller *et al.* 2014). Similar results have been found in field experiments. The growth rate, biomass and grain yield of maize in semi-arid farmland have been shown to be effectively enhanced by the straw biochar (Xiao *et al.* 2016). There are enough studies to prove that application of biochar can alleviate heavy metal stress. Plant growth would be negatively affected by heavy metals, but the application of biochar effectively alleviates stress and promotes plant growth (O'Connor *et al.* 2018). Moreover, the effects of biochar treatment might be related to the heavy metal adsorption ability of biochar (Table 1, Fig. 4b). In accordance with our study, it has been suggested that biochar with a high

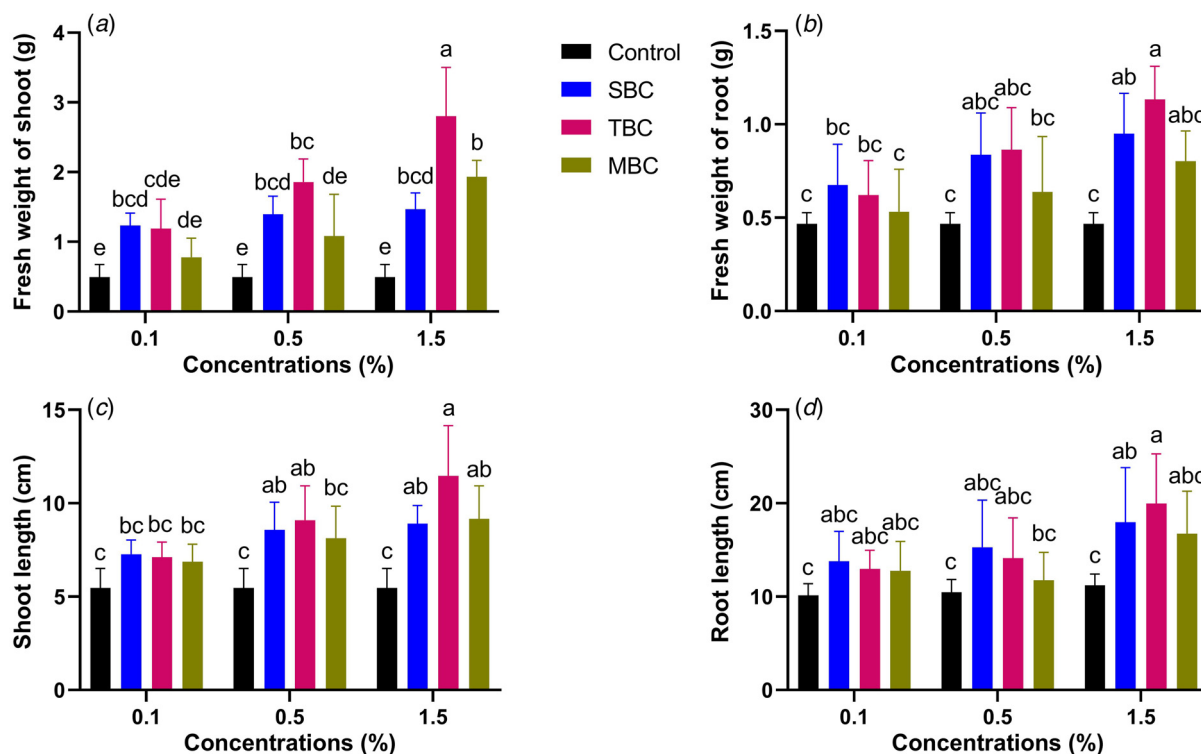


Fig. 5. Effects of biochar on (a) shoot fresh weight, (b) root fresh weight, (c) shoot length, and (d) root length of pakchoi grown in soil co-contaminated with Cd and Pb.

adsorption capacity could more effectively reduce the bioavailability of heavy metals in soil (Fan *et al.* 2020).

Effects of biochar on alleviation of oxidative stress under heavy metal stress

The oxidation defense system of the plant could be activated when the plant grows in a stressed environment, which is a crucial strategy to alleviate heavy metal stress (Wu *et al.* 2017). Reactive oxygen species (ROS) are being continuously produced in specific biochemical reactions, such as respiration and photosynthesis (Zhao *et al.* 2017). ROS are important signaling molecules involved in plant growth and defense, whereas excessive ROS would be produced and accumulated under stress (Zhang *et al.* 2019). Accumulation of excessive ROS is detrimental to plant organelle, proteins and cell membranes (Rui *et al.* 2017). ROS are mainly being scavenged in plants by antioxidant enzymes, such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and other metabolites (Adeel *et al.* 2020). Superoxide radical ($O_2^{\cdot-}$) could be transformed into molecular oxygen and hydrogen peroxide (H_2O_2) by SOD, and H_2O_2 could be decomposed into H_2O and O_2 by POD and CAT (Adeel *et al.* 2018). Besides, lipid peroxidation (MDA over-accumulation) will occur when the scavenging rate of ROS is less than the generation rate (Zhou *et al.* 2021a, 2021b, 2021c). So as to

determine the effect of biochar on alleviation of oxidative stress under heavy metal stress, malondialdehyde (MDA) and the activity of SOD and POD were measured (Fig. 6).

The MDA content in leaves decreased significantly in all biochar treatments, indicating that lipid peroxidation caused by heavy metal stress was alleviated by biochar (Fig. 6a). Interestingly, in the TBC treatment, the content of MDA in leaves decreased with an increase in the application concentration of TBC, whereas treatments with SBC and MBC showed the opposite effect. SOD activities in leaves were significantly down-regulated by approximately 23–63% after different biochar exposures (Fig. 6b). Similarly, the activity of SOD and POD in leaves decreased with an increase in the application concentration of TBC, whereas treatments with SBC and MBC showed the opposite effect. The SPAD value of pakchoi was determined to investigate the impact of biochar on the photosynthetic system under heavy metal stress (Fig. 6d). In general, the SPAD value of leaves has been significantly promoted by 39–43% with high concentration of biochar (1.5%). In contrast, the low- and medium-concentration treatments had no significant effect on the SPAD value. Similar to our study, the synthesis of chlorophyll was negatively affected under heavy metal stress, and the negative impact was alleviated by application of biochar (Wu *et al.* 2019).

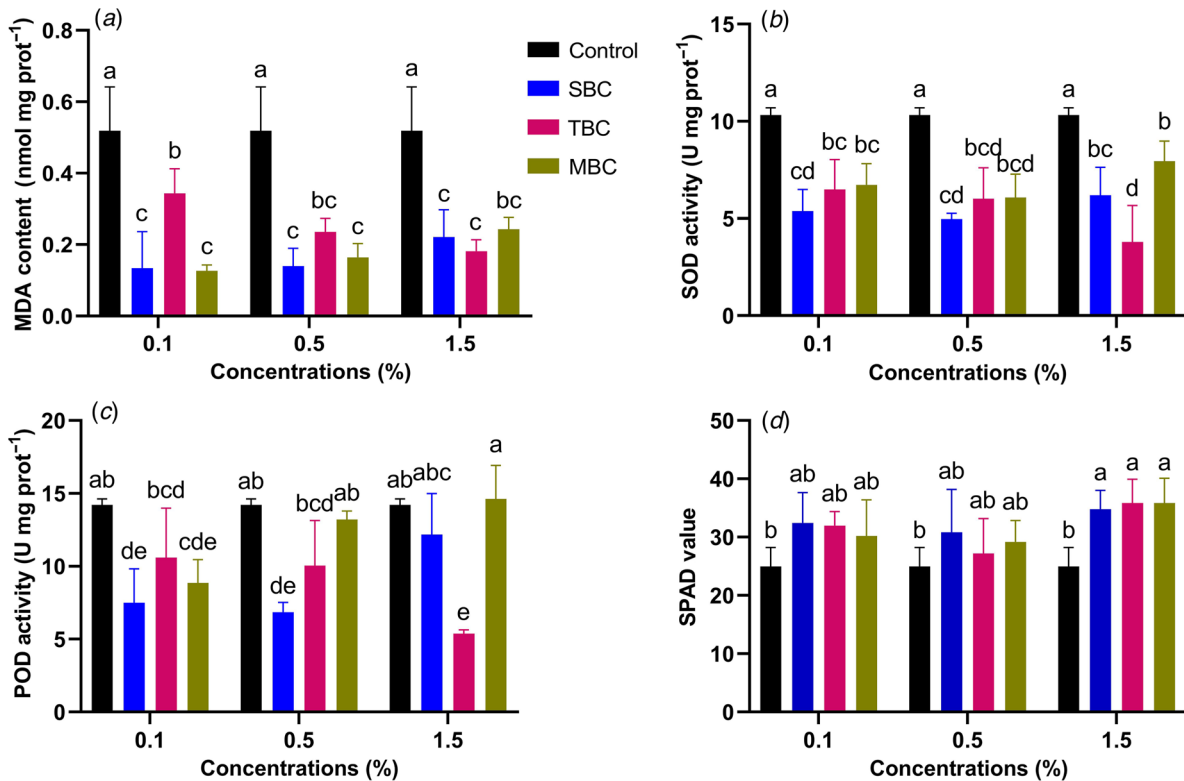


Fig. 6. Effects of biochar on (a) MDA content, (b) SOD activity, (c) POD activity and (d) SPAD value of pakchoi grown in soil co-contaminated with Cd and Pb.

Excessive ROS harmful to the membrane and cell structure would be quickly produced under biotic or abiotic stress, resulting in an increased MDA concentration, which is a significant biomarker of lipid peroxidation (Adeel *et al.* 2021). We found relatively high MDA concentrations compared with control in pakchoi grown without biochar treatments, owing to heavy metal toxicity (Fig. 6a). The results showed that the harmful effects of heavy metals on the cell membrane were alleviated owing to the biochar treatment. Coinciding with our study, the application of biochar significantly decreased the MDA concentration in plants under salt stress (Farhangi-Abriz and Torabian 2018). On one hand, catalytic disproportionation of superoxide anion radicals (O_2^-) can be accomplished by SOD and produce H_2O_2 and O_2 . On the other hand, POD can catalyse H_2O_2 to H_2O , which plays an essential role in the balance of oxidation and antioxidation (Wang *et al.* 2019). We observed that the activity of SOD and POD could be effectively decreased by the high-concentration TBC, meaning that the oxidative stress under heavy metal exposure could be alleviated by biochar (Fig. 5a, b). Our results showed a good correlation with pakchoi biomass, indicating that the regulation of the antioxidant system is the critical mechanism of biochar-induced pakchoi growth. The previous report also demonstrated that the application of biochar alleviates

oxidative stress (Bashir *et al.* 2018; Irshad *et al.* 2020). For example, Irshad *et al.* (2020) found that goethite-modified biochar promotes rice growth by alleviating oxidative stress in soil co-contaminated by Cd and As. Bashir *et al.* (2018) demonstrated that the application of biochar promoted water spinach growth and reduced the activity of SOD and POD under Cd stress.

Effects of biochar on uptake of heavy metals in pakchoi under heavy metal stress

Cadmium concentration in pakchoi root decreased significantly compared with the control (Fig. 7a). Treatment with TBC resulted in a significant reduction in the Cd concentration by almost 52–60% at the application level of 0.1% and 1.5%, whereas 0.5% TBC had no significant effect on the Cd concentration. Interestingly, for the treatment of SBC, the concentration of Cd in the roots increased with an increase in the application dose of biochar, whereas treatment with MBC had an opposite effect. Although the concentration of Cd in the roots decreased significantly, in the shoot it did not change significantly (Fig. 7b). The reason for this may have been the positive effect of biochar on plant growth, and the translocation factor of Cd being enhanced with plant growth. We found that the Pb concentration in root and shoot

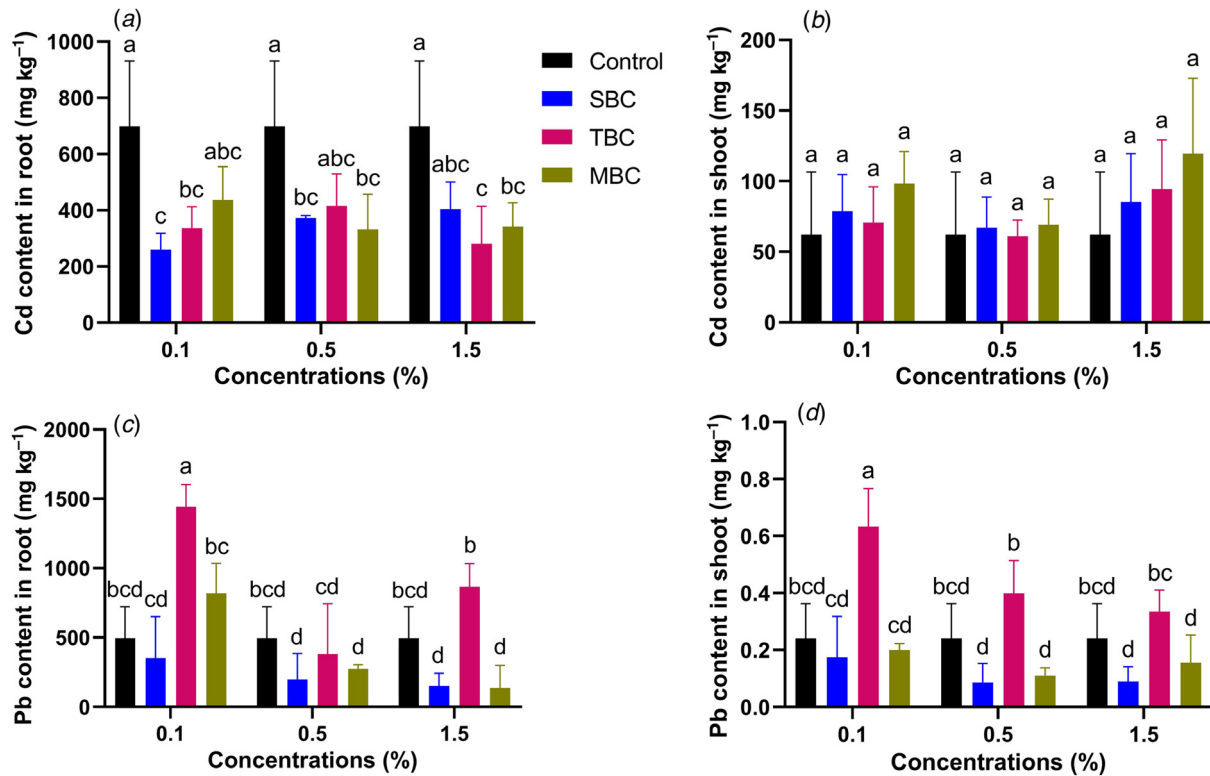


Fig. 7. Effects of biochar on Cd concentration in (a) root, (b) shoot, and Pb concentration in (c) root and (d) shoot of pakchoi grown in soil co-contaminated with Cd and Pb.

significantly increased in the low-concentration TBC treatment (0.1%). Part of the reason may be that the pakchoi root was stimulated by the low-concentration TBC treatment. At the same time, more Pb could be adsorbed by TBC at a high concentration (1.5%) to counteract this negative effect (Fig. 7c, d). In general, the positive effect of biochar increased with an increase in the biochar concentration. Our study indicated that 1.5% TBC could significantly reduce the Cd concentration in pakchoi roots during soil cultivation, whereas 0.1% TBC could effectively increase Pb concentrations in pakchoi roots and shoots.

Heavy metal pollution is a serious problem in farmland soil, limiting crop production, and is also the main reason for land abandonment (Zhou et al. 2021a, 2021b, 2021c). Biochar exhibited immense potential in treating heavy metals in soil because of its unique chemical and physical properties (such as large surface area, alkaline properties and cation exchange capacity; Beesley and Marmiroli 2011; Zhang et al. 2013). Biochar may reduce Cd bioavailability more effectively than it does Pb bioavailability. For example, biochar separately reduced the available Cd and Pb by 34.8–39.2% and 8.6–11.1% in the soil incubation experiments (28 days) (Fan et al. 2020). Besides, pakchoi, which belongs to the *Brassica*, can accumulate most of the soil heavy metals in its root (Rizwan et al. 2018). Our result clearly showed that Cd concentration in root decreased with application of biochar, whereas

low-concentration TBC promoted accumulation of Pb in pakchoi (Fig. 7). The reason may be related to the strong adsorption capacity and high pH value of TBC. Moreover, the growth dilution is probably also responsible for reduced Cd and Pb uptake in pakchoi. Pb accumulation in pakchoi was promoted by TBC, which was attributed to competitive adsorption of heavy metals and biostimulation of TBC. Similar to our study, Houben et al. (2013) also concluded that low-concentration biochar promoted heavy metal accumulation in *Brassica napus* L. because of biostimulation. Overall, the ability of pakchoi to absorb Pb can be enhanced by TBC, indicating that combining phytoremediation with biochar is promising.

Conclusions

In the current study, we compared the physiological impacts and remediation capacity of three different types of biochar on pakchoi plant growth. The growth of pakchoi seedlings was significantly promoted with biochar under heavy metal stress, especially the high concentration treatment of TBC. TBC (1.5%) promoted plant shoot weight, root weight, shoot length and root length by 465%, 143%, 109% and 97% respectively. Besides, the application of biochar effectively

decreased oxidative stress and protected membrane integrity. Interestingly, for Cd and Pb content in the root, the application of TBC decreased Cd content in the root by 40–60%, whereas application of TBC (0.1% and 1.5%) promoted the accumulation of Pb by 75–191%. This research demonstrated that 1.5% TBC can remediate Cd-contaminated soil and that combining phytoremediation of Pb-contaminated soils with 1.5% TBC is promising. Overall, the chestnut shell waste-derived biochar effectively immobilised exogenous and mobile Cd and Pb. In addition, biochar showed huge potential to improve the productivity of the plant and phytoremediation capacity under metal stress. The studies on different raw materials of biochar and soil containing different pollutants are needed for the future strategy in larger prospects.

Supplementary material

Supplementary material is available [online](#).

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare that they have no competing interest.

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