Sun *et al. Biochar* (2024) 6:45
https://doi.org/10.1007/s42773-024-00333-2

Biochar

ORIGINAL RESEARCH **Open Access**

Cupriavidus B-7 immobilized biochar: an effective solution for Cd accumulation alleviation and growth promotion in pakchoi (*Brassica Chinensis* L.)

Yefang Sun[1,2], Da Ouyang[1*], Yiming Cai[1,3], Ting Guo[1], Mei Li[1], Xinlin Zhao[4], Qichun Zhang[5], Ruihuan Chen[6], Fangzhen Li[2], Xiujuan Wen[2], Lu Xie[2] and Haibo Zhang[1*]

Abstract

Cd contamination, especially in farmland soil, can pose serious threats to human health as well as ecological security. Stabilization is an important strategy for agricultural soil Cd remediation. In this study, a Cd-resistant strain (*Cupriavidus* B-7) was isolated and loaded onto cow manure (CDB), rice straw (RSB) and pine wood biochar (PB) to investigate its effects on Cd stabilization by a 60-day pot experiment. Results indicated that the *Cupriavidus* B-7-loaded biochar (labelled as CDBB, PBB and RSBB) reduced the $CaCl_2$-extractable Cd by 43.06–59.78%, which was significantly superior to individual applications of *Cupriavidus* B-7 and biochar. Likewise, the soil physicochemical properties, urease, catalase and phosphatase activities were improved, indicating improved soil health. Consequently, dry weights of pakchoi's shoot and root were increased by 938.9–1230.9% and 149.1–281.2%, respectively, by applying CDBB, PBB and RSBB. Meanwhile, the Cd accumulation in pakchoi shoots decreased by 38.06–50.75%. Notably, the RSBB exhibited an optimal performance on pakchoi growth promotion and Cd accumulation alleviation. The structural equation model indicated the synergistic effect on pakchoi growth promotion and Cd accumulation decreased between biochar and *Cupriavidus* B-7. Our research provides some new insights into the development of strategies for green and sustainable remediation of Cd-contaminated soil.

Highlights

- A Cd resistant *Cupriavidus* B-7 (B-7) was isolated from copper mining soil
- Biochar, B-7 and their conbination were used to remediate Cd polluted soil
- Biochar loaded B-7 outperformed individual treatments for Cd stabilization
- Biochar loaded B-7 alleivated the Cd uptake and promoted pakchoi growth

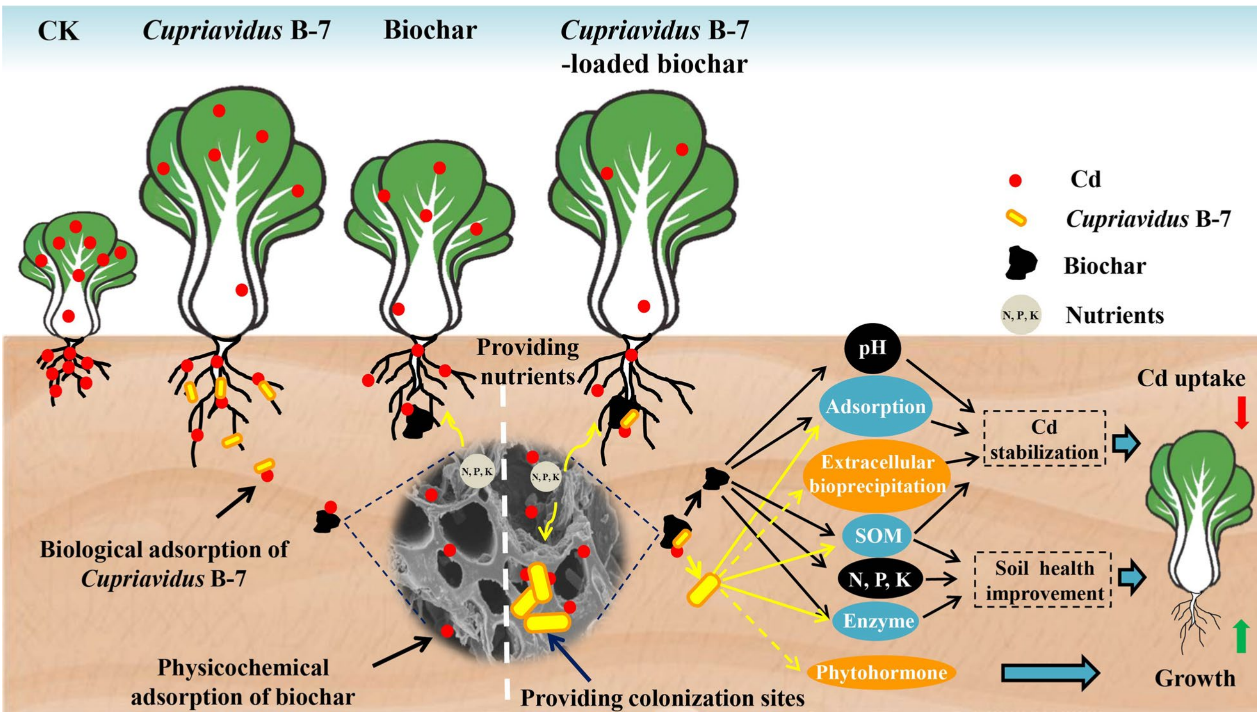
Keywords Biochar loaded bacteria, Bioremediation, Bioavailability, Cadmium, Uptake

Handling editor: Hailong Wang

*Correspondence:
Da Ouyang
ouyangda@zafu.edu.cn
Haibo Zhang
hbzhang@zafu.edu.cn
Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Graphical Abstract



1 Introduction

Soil contamination by heavy metals has received much public attention both domestically and internationally due to its toxicity, bioaccumulation and non-degradability (Zeng et al. 2019). According to the National General Survey Report on Soil Contamination, up to 16.1% of the total survey sites exceeded the soil environment quality standards, and inorganic pollutants such as Cadmium, Nickel, Arsenic and Copper were the main pollution sources (Tu et al. 2020). As one of the most toxic heavy metals, Cadmium (Cd) is identified as a priority pollutant by U.S. EPA. It can damage the liver, kidney and spleen, causing several symptoms such as osteoporosis and lung cancer (Yang et al. 2021). The main pathway of Cd entering the human body is the food chain. The bioavailable Cd in soil can be absorbed by crops and then ingested by humans. Excessive accumulation of Cd in soils not only reduces the soil quality, microbial activity and crop productivity but also threatens ecological security and human health (Liu et al. 2017; Qing et al. 2015). Therefore, it is highly crucial and urgent to develop effective, low-cost and environmentally friendly technologies for Cd polluted soil remediation.

Stabilization is a promising strategy to reduce the mobility and bioavailability of heavy metals by adding proper amendments to the polluted soil, thus alleviating the health and ecological risks of toxic heavy metals in the soil (Shen et al. 2019). Previous studies have reported that soil microorganisms can stabilize heavy metals via bio-accumulation, bio-sorption, bio-transformation and bio-mineralization (Liu et al. 2020). In the last few decades, heavy metal-tolerant bacteria and their applications to the remediation of contaminated soil have been one of the research hotspots. Many bacterial species and genera including *Cupriavidus taiwanensis* (Siripornadulsil and Siripornadulsil 2013), *Pseudomonas stutzeri* (Oh et al. 2009), *Halomonas* (Manasi et al. 2014), *Delftia* (Liu et al. 2018) and *Bacillus* (Jiang et al. 2009) were found to be capable of immobilizing Cd in soil and decreasing Cd uptake by rice.

However, some key issues still need to be resolved before the broad application of functional microbes for Cd contaminated soil remediation. Firstly, large microbial communities are hard to form in the field because high concentrations of heavy metals in soil might hinder the survival of functional microbes (Li et al. 2017). Secondly, the polluted soil generally can't support rapid growth of functional microbes, resulting from nutritional shortage and native species competition (Ma et al. 2020a, b; Shen et al. 2017). Therefore, numerous attempts have been made in the field of immobilized microbe technology. It has been observed that providing microorganisms with carrier materials can promote microbial population density and biological activity. (Ji et al. 2022; Qin et al. 2020).

Derived from pyrolysis of the plant, sludge and animal-based biomass under oxygen-limited conditions, biochar is an environmentally friendly functional material (El-Naggar et al. 2019). In recent years, biochar has been applied to many aspects of environment remediation due to its unique physicochemical characteristics (large specific surface area, abundant functional groups and mineral elements). One of the most essential biochar's environmental applications is the adsorption of heavy metals (O'Connor et al. 2018). It has been demonstrated that biochar could improve the physical and chemical properties of soils and provide nutrients to corps. Besides, biochar could decrease the mobility and bioavailability of Cd in soil by electrostatic attraction, complexation, ion exchange and precipitation (Qiu et al. 2021).

Biochar immobilization microbe usually acquired by impregnating functional microorganisms with biochar together. The contaminant enrichment of biochar increases the concentration gradient of pollution between biochar and microorganisms, which increases the speed and force of mass transfer between microorganisms and biochar. Therefore the the efficiency of contaminant degradation and removal is improved. (Bharti et al. 2019; Wu et al. 2022). In addition, Biochar could not only provide carbon sources and nutrients for the soil but also offer spaces for the growth and reproduction of microorganisms (Zhang et al. 2020). It was reported that immobilized PSB strains (*Leclercia adecarboxylata*) with biochar could promote the formation of $Pb_5(PO_4)_3Cl$ and $Pb_5(PO_4)_3OH$, which are the stable state of Pb (Teng et al. 2020). Tu et al. (2020) also found *Pseudomonas* sp. NT-2 immobilized on biochar had an excellent performance on stabilizing Cd and Cu in heavy metal polluted soil. Although there were some reports about heavy metal contaminated soil remediation using microbe immobilized on biochar, the synergetic mechanism between biochar and heavy metal (resistant) immobilizing microbes on the Cd immobilization is still not well elucidated. Furthermore, there is also a lack of knowledge about the variation of Cd bioavailability and soil properties during the heavy metal contaminated soil remediation by the biochar immobilized microbe strategy.

In this study, a heavy metal tolerant bacteria (*Cupriavidus* B-7) was first screened and isolated. Then three kinds of biochar were used as inoculum carriers for *Cupriavidus* B-7 during the remediation of Cd contaminated soil. Objectives of this research are to (1) shed light on the effects of microbial inoculation on the stabilization of Cd in soil; (2) investigate the variations of Cd bioavailability for plants and the properties impact on soil during the whole remediation period; and (3) explore the synergistic stabilization mechanism of Cd between biochar and *Cupriavidus* B-7 in soil. Results of this study are supposed to bring some insights into the green and sustainable remediation of heavy metal contaminated soil.

2 Materials and methods

2.1 Soil sampling and characterizing

The soil used in this study was collected at a depth of 0–20 cm from a lead–zinc mining area in Shangyu District, Shaoxing City, Zhejiang Province. The soil was characterized as loam. The basic physicochemical properties of the collected soil were determined, and the detailed information is presented in Additional file 1: Table S1.

2.2 Biochar preparation

The rice straw and pine wood were washed with deionized water several times and dried at 60 °C. Then the dried rice straw, pine wood and cow manure were smashed and pyrolyzed at 500 °C under nitrogen atmospheric conditions for 8 h. At last, the biochar was passed through a 0.25 mm sieve before use (Ouyang et al. 2019, 2023). The biochar derived from cow manure, rice straw and pine wood were denoted as CDB, RSB and PB, respectively.

2.3 Isolation and identification of the heavy metal strain bacteria

The bacterial strains were initially isolated from Cd contaminated soil samples collected from copper mining in Zhejiang province, China. The basic soil properties of the copper mining are presented in Additional file 1: Table S3. The Cd contaminated soil sample was firstly shaken (150 r min^{-1}, 28 °C) in 150 mL serum bottles with 90 mL sterile water and several glass beads for 30 min. Acquired solutions were added to the fresh beef extract peptone medium (BPM) with 100 mg L^{-1} Cd. After incubating for 3 days at 28 °C, the enrichment culture was spread on a fresh BPM containing Cd (100 mg L^{-1}). At last, the heavy metal tolerant strain was picked and purified after 7 times streaking method, and sixteen species of Cd resistant strains were isolated. Identification of the strain with the best cadmium passivation effect was performed by DNA extraction by a kit, and the 16S rRNA gene was amplified by PCR with primers 27F and 1492R. The PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd. The MEGA 6.0 software was used to analyze the phylogenetic tree after the multiple alignments of data by CLUSTAL X(ver.1.8). All experiment materials were sterile in our study.

2.4 Preparation of bacteria-loaded biochar and pot experiment

Immobilization of isolated Cd resistant strain (*Cupriavidus* B-7) on biochar was described as follows: the *Cupriavidus* B-7 was cultured in BPM at 28 °C and 200 rmp. The biochar was subsequently blended with the *Cupriavidus* B-7 culture for 8 h with a ratio of 1.2:1 (w:v) after the culture OD600 value reached 1.0. The incubation pot experiments were carried out in a greenhouse at 25 °C to investigate the effects of *Cupriavidus* B-7-loaded biochar on pakchoi growth (*Brassica chinensis* L.) as well as its accumulation of Cd. Eight treatments of pot experiments were included: contaminated soil only (CK), contaminated soil mixed with *Cupriavidus* B-7 (B-7), contaminated soil mixed with three types of biochar (CDB, RSB and PB), and contaminated soil mixed with *Cupriavidus* B-7-loaded biochar (CDBB, RSBB and PBB). The weight-to-weight dosages of bacteria solution, biochar and bacteria-loaded biochar towards contaminated soil were 30%, 2.5% and 2.5% (w:w), respectively. The contaminated soil was incubated for 60 days after adding *Cupriavidus* B-7, biochar and *Cupriavidus* B-7 loaded biochar. Then, the pakchoi was planted in the soil. During the incubation, the soil moisture was kept at 40% of water holding capacity to facilitate equilibrium. All treatments and controls were performed in triplicate. For further analysis, the samples were air-dried and passed through a 100-mesh sieve.

2.5 Analysis of heavy metal in the soil and plant

To monitor the dynamic change of heavy metal fractions in soil, Tessier method was applied (Tessier et al. 1979), and five different fractions specified by the Tessier sequential extraction method include water-soluble and exchangeable (Ex), carbonate bound (Carb), Fe–Mn oxides bound (Ox), organic matter bound (Org), and residual (Res) fractions. The bioavailable heavy metals in the soil were extracted by 0.01 M of $CaCl_2$ solutions, considering the fitness of DTPA-extraction for neutral soil while the soil used in our research was acid soil (Ma et al. 2020b). The heavy metal concentrations in edible parts of pakchoi were determined after its digestion by a mixture of concentrated HNO_3 and $HClO_4$ (9:1, v:v) solution. Heavy metal concentrations in extracts from the soil were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES; ICAP-7400, ThermoFisher Scientific Ltd., USA), and the heavy metal concentrations in digestion from pakchoi were measured by graphite furnace atomic absorption spectrophotometer (GFAAS).

2.6 Data analysis

Mean values with standard deviations were presented in our study. The statistical analysis was performed by SPSS Statistics 22.0 software. One-way analysis of variance (ANOVA) was applied to evaluate the effects of different amendment treatments on the analyzed parameters, and the differences between the treatment means were determined using Duncan's test at $p < 0.05$. Origin 2020 was used for Pearson's correlation analysis, and AMOS 26 was applied for the structural equation modelling (SEM).

3 Results and discussion

3.1 The isolation and identification of Cd resistant strain

We isolated 16 kinds of Cd resistant strains from the Cd polluted soil. The strains were then subjected to the fluid medium with an initial Cd concentration of 100 mg L^{-1}. After 48 h of incubation, the Cd concentrations in the fluid medium were determined and are presented in Additional file 1: Table S2, and strain k1 showed the best immobilization ability with the Cd concentration in the fluid medium significantly decreased by 33 mg L^{-1}. As seen from Fig. 1a, strain k1 formed round, white and opaque colonies with smooth edges. Scanning electron microscope images of strain k1 illustrated that the cells of bacteria were rod-like with a size of about 1.0–2.0 μm (Fig. 1b and c). In addition, We conducted sequencing of the 16S rRNA genes of strain k1 to determine its taxonomy. The NCBI BLAST search program revealed that strain k1 exhibited a sequence identity ranging from 98 to 100% with species from the genera *Cupriavidus* sp., *Ralstonia* sp., and *Arthrobacter* sp., all of which are part of the *Burkholderiaceae* family. Based on the phylogenetic analysis (Fig. 1d), the Cd resistant strain k1 is primarily similar to the genus of *Cupriavidus* sp. and thereafter denoted as *Cupriavidus* B-7. Similarly, previous studies have also reported a high heavy metal accumulating soil bacterium, *Cupriavidus* sp. W2 (Shi et al. 2020). The *Cupriavidus* sp. were previously reported as kinds of heavy metal tolerant bacteria. For instance, the *Cupriavidus taiwanensis* could grow well in the culture medium with approximately 3100 mg L^{-1}, 490 mg L^{-1}, 318 mg L^{-1} and 281 mg L^{-1} of Pb, Zn, Cu, and Cd (Chen et al. 2008). Although the the maximum inhibitory concentration of Cd was not explored in this study, it was found that the *Cupriavidus* B-7 survived well with at lest 100 mg L^{-1} of Cd.

3.2 Characterization of biochar and *Cupriavidus* B-7 loaded biochar

Table 1 shows the basic properties of biochars, and the pH values of CDB, RSB, and PB were 9.26, 10.08 and 9.68, respectively. Thus, adding biochar or *Cupriavidus* B-7-loaded biochar would effectively enhance the soil pH (Yang et al. 2021). With relatively high surface areas and porosity, CDB, RSB, and PB could serve as a safe habitat for Cd resistant bacteria immobilization (Song et al.

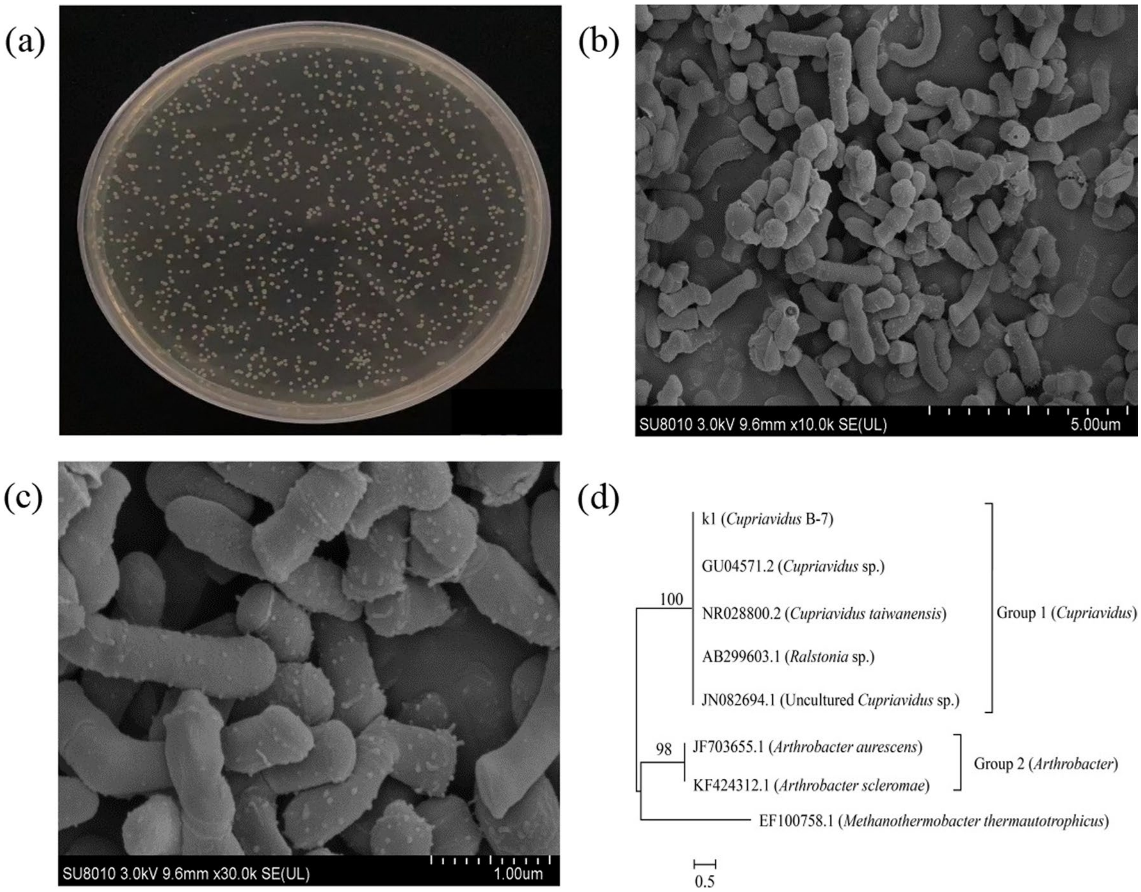


Fig. 1 Formed bacterial colonies (**a**), scanning electron microscope images (**b–c**), and phylogenetic analysis (**d**) of the isolated heavy metal resistant strain k1(*Cupriavidus* B-7)

Table 1 Basic properties of the biochar

Indicator	CDB	RSB	PB
pH	9.26 ± 0.30	10.1 ± 0.28	9.68 ± 0.37
Total N (g kg^{-1})	1.61 ± 0.12	1.12 ± 0.06	2.76 ± 0.08
Total P (g kg^{-1})	6.48 ± 0.22	1.76 ± 0.14	1.13 ± 0.07
Total K (g kg^{-1})	15.3 ± 0.25	14.5 ± 0.32	4.18 ± 0.10
Organic matter (g kg^{-1})	363 ± 1.05	518 ± 2.44	730 ± 2.76
Surface area (m^2 g^{-1})	4.81 ± 0.138	8.23 ± 0.217	6.80 ± 0.166
Porosity (m^3 kg^{-1})	0.110 ± 0.04	0.231 ± 0.03	0.163 ± 0.06

2022; Wahla et al. 2020). Besides, the three biochars contained nitrogen, phosphorus and potassium elements, which could serve as nutrient sources for the growth of Cd resistant bacteria.

The colonization of bacterium on biochar depends on the biochar properties as well as the physiological features of the bacterium (Zhu et al. 2017). Quilliam et al. (2013) found that the high mineral salt content could inhibit the colonization of microbes on biochar surfaces. However, Tu et al. (2020) also reported a heavy metal-tolerant strain *Pseudomonas* sp. NT-2 which could colonise well on the biochar surface within only 8 h. In our present study, the scanning electron microscope was used to observe the morphology of three biochars and bacteria-loaded biochars. As illustrated in Fig. 2, the *Cupriavidus* B-7 successfully colonized the surface and pores of biochars in a short time (48 h). The results suggested that the *Cupriavidus* B-7 has great potential to survive on biochar surface and synergistically immobilize Cd in soil with biochar.

3.3 Influence of *Cupriavidus* B-7 loaded biochar on soil properties

The changes in soil pH after the treatment with biochar and *Cupriavidus* B-7-loaded biochar are shown in Fig. 3a. Compared with CK (5.97), soil pH increased to 6.46, 6.5, 6.25, 6.3, 6.22, 6.37 and 6.02 after five days of incubation with RSB, RSBB, CDB, CDBB, PB, PBB and *Cupriavidus* B-7, respectively. Then, the pH of soil declined

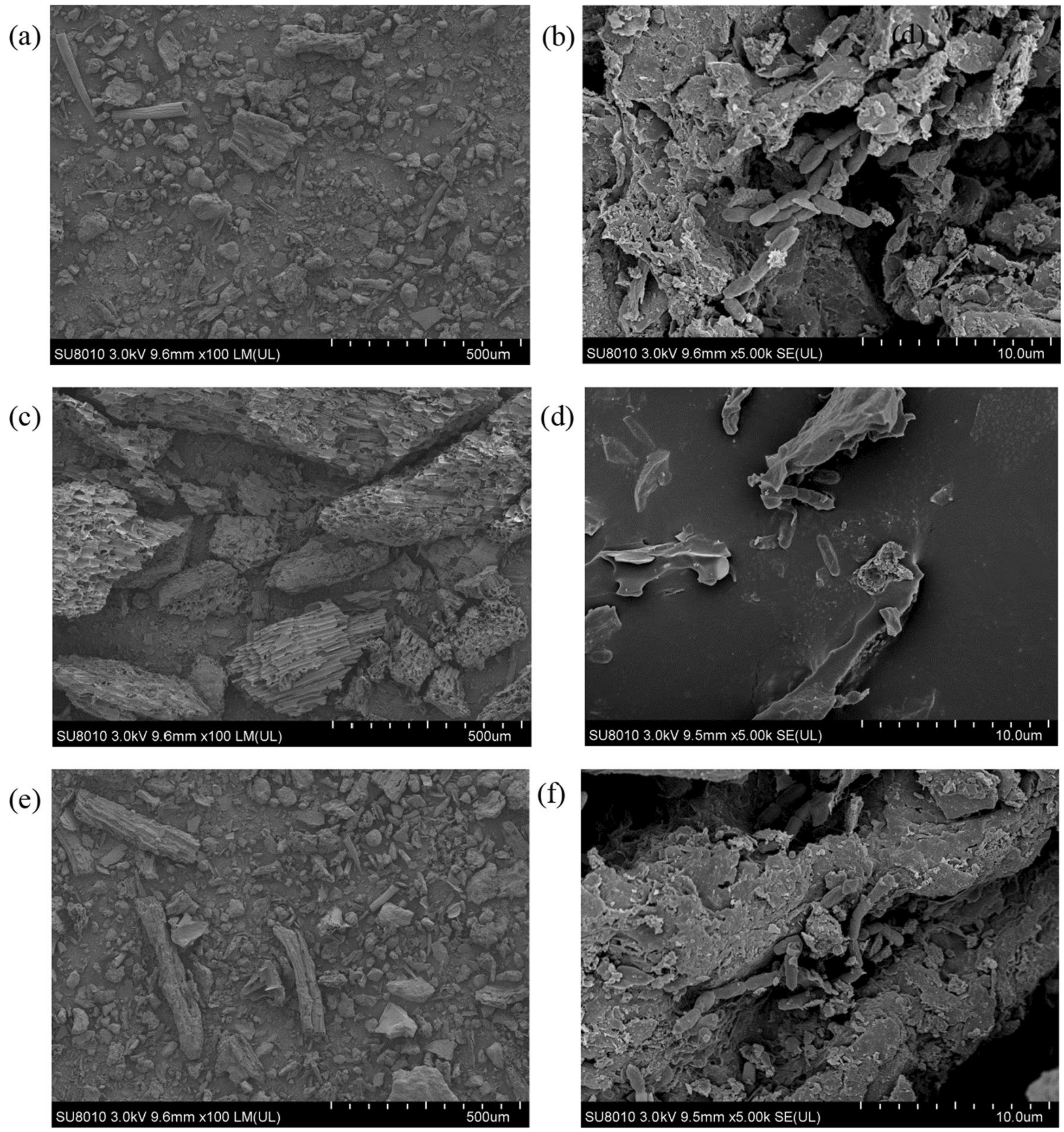


Fig. 2 Scanning electron microscope images of CDB (**a**), CDBB (**b**), PCB (**c**), PCBB (**d**), RSB (**e**) and RSBB (**f**)

slightly after 60 days of incubation. However, final pH of all treatment groups was still higher than that of the CK treatment. During the pyrolysis process, the base cations in biomass could produce alkaline substances, such as oxides, hydroxides, and carbonates. Therefore, biochar can effectively enhance soil pH (Qi et al. 2021). The soil pH of all the treatment presented a quick increase by biochar application, and then showed a slight declining trend with the increasing incubation. Tu et al. (2020) also observed a similar phenomenon and suggested that the rapid increase in soil pH was attributed to the dissolution of alkaline substances in the biochar. Subsequently, the soil pH slightly decreased once these readily released alkaline substances were depleted.

The soil organic matter, alkaline nitrogen, available phosphorus and potassium were determined after 60 days of incubation with *Cupriavidus* B-7, biochars or *Cupriavidus* B-7-loaded biochars. We have found that applying *Cupriavidus* B-7, biochar and *Cupriavidus* B-7-loaded biochar could significantly enhance the organic matter in the soil, and the biochar or *Cupriavidus* B-7-loaded biochar treatment was significantly better than

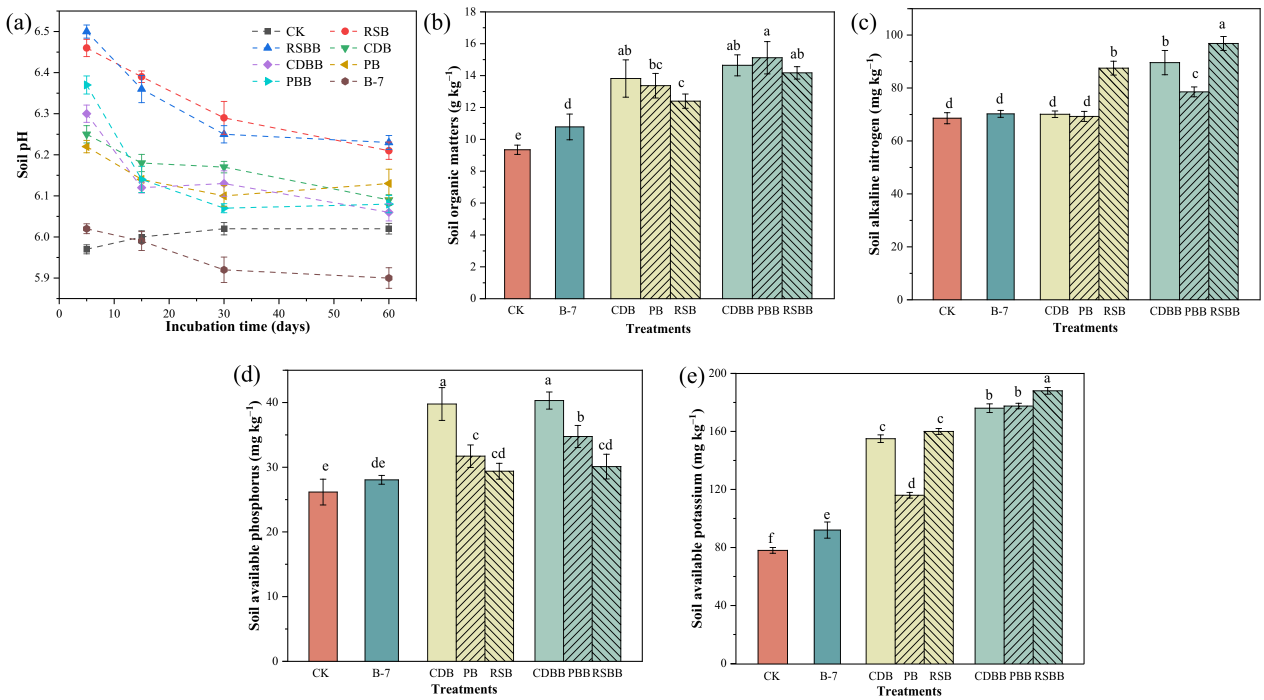


Fig. 3 The variation of soil pH during 60 days of incubation with biochar, *Cupriavidus* B-7 and *Cupriavidus* B-7-loaded biochar (**a**); The organic matter (**b**), alkaline nitrogen (**c**), available phosphorus (**d**) and potassium (**e**) values of soil after 60 days of incubation with biochar, *Cupriavidus* B-7 and *Cupriavidus* B-7-loaded biochar. Different letters indicate significant differences among treatments ($p < 0.05$; Duncan's test)

that of *Cupriavidus* B-7 alone (Fig. 3b). Similarly, applying biochar or *Cupriavidus* B-7-loaded biochar increased the alkaline nitrogen (Fig. 3c), available phosphorus (Fig. 3d) and potassium (Fig. 3e) in the soil compared with the CK treatment. In addition, according to our results, the *Cupriavidus* B-7-loaded biochar presented a better effect on organic matter, alkaline nitrogen, available phosphorus and potassium increase in the soil.

3.4 Influence of *Cupriavidus* B-7 loaded biochar on Cd bioavailability in soil

The $CaCl_2$-extractable of Cd could be used to evaluate the mobility and bioavailability of heavy metals in acidic and neutral soils. To determine the immobilization effects of different treatments on Cd, the contents of $CaCl_2$-extractable Cd in soil were analyzed after incubation with *Cupriavidus* B-7, biochars or *Cupriavidus* B-7-loaded biochars, respectively (Fig. 4a). The initial $CaCl_2$-extractable Cd was 2.34 mg kg^{-1}, and it decreased to 1.63 mg kg^{-1} after 60 days of incubation with *Cupriavidus* B-7. The stabilization of Cd by *Cupriavidus* B-7 could be mainly due to the cell surface adsorption by electrostatic attraction or ionic bonding (Li et al. 2021b). Cd^{2+} likely has the potential to engage in interactions with the surface ligands found in extracellular polymers and cell wall surface molecules, including –OH, –NH, $–SO_3$, $–HPO_3$, –C–O, –SH, and –COOH, during the process of surface biosorption (Chi et al. 2020; Qi et al. 2023). Furthermore, Li et al. (2019) also reported an available Cd reduction mechanism of *Cupriavidus* sp. through extracellular bio-precipitation of Cd^{2+} in the form of cadmium carbonate. In this study, the RSBB treatment showed the best effect on $CaCl_2$-extartable Cd decrease. It was because the *Cupriavidus* B-7 exhibits the best survival on RSB (Fig. 4b) and the Cd passivation effect by *Cupriavidus* B-7 was significantly increased.

Biochar has been proven to have immobilization effects on heavy metals in the soil, including direct adsorption via electrostatic attraction, ion exchange, precipitation and physical adsorption. Additionally, biochar can alter the soil's physicochemical properties, particularly its pH, leading to a decrease in the mobility of heavy metals in the soil (Wang et al. 2021). In this study, the $CaCl_2$-extractable Cd exhibited reductions of 39.3%, 36.3%, and 49.7%, respectively, following the addition of CDB, PB, and RSB to the soil. Among these amendments, RSB proved to be the most effective in Cd stabilization, largely due to its ability to significantly increase soil pH (Fig. 3a). Furthermore, as evidenced by BET surface area analysis (Table 1), RSB had a higher specific surface area and a porous structure compared with CDB and PB, enhancing its ability to efficiently adsorb Cd^{2+}.

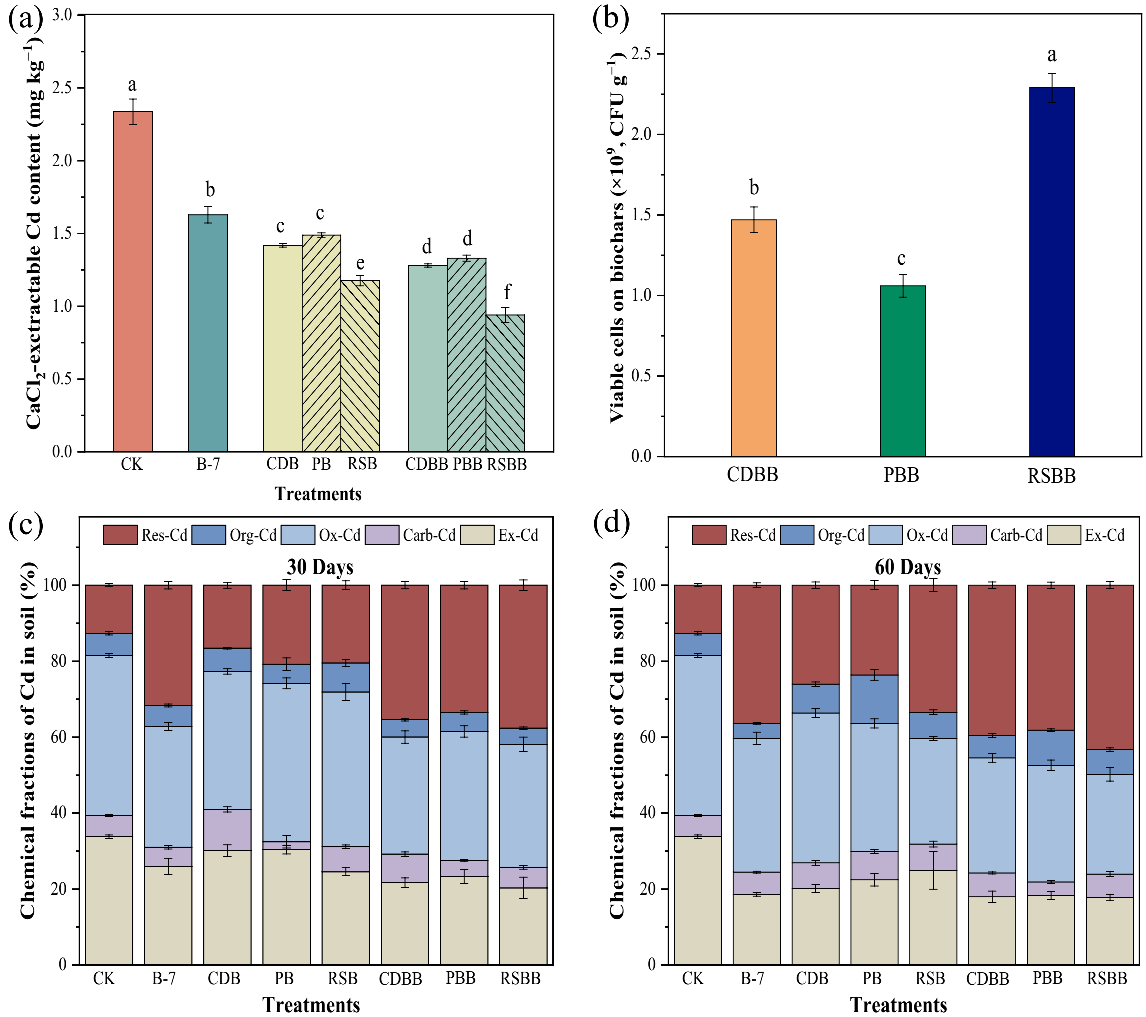


Fig. 4 Changes of Cd $CaCl_2$-exctractable in soil after 30 days of incubation (**a**), the number of viable cells on biochar (**b**) and percentage of fractions distribution in soil after 30 (**c**) and 60 days (**d**) of incubation. Different letters indicate significant differences among treatments ($p < 0.05$; Duncan's test)

As anticipated, immobilizing *Cupriavidus* B-7 onto biochar enhanced the passivation effect of biochar on Cd in the soil. The RSBB showed the best effect on Cd stabilization, with a decrease of $CaCl_2$-extractable Cd by 59.8%, compared with CK treatment. Compared with CDB, PB and RSB, the $CaCl_2$-extractable Cd in the soil after the application of CDBB, PBB and RSBB decreased by 9.7%, 10.6% and 20.1%, respectively. The extent to which *Cupriavidus* B-7 enhances Cd passivation in soil differs when immobilized on various biochars. This variation is primarily attributed to differences in the physicochemical properties of the biochars and their effectiveness in immobilizing *Cupriavidus* B-7 (Wei et al. 2022). In this study, the number of viable cells on CDBB, PBB, and RSBB was determined to be 1.47×10^9, 1.06×10^9 and 2.29×10^9 CFU g^{-1} (Fig. 4b), respectively. The viable cells on RSBB were approximately two times more than those on CDBB and PBB, suggesting that RSB is more suitable for the colonization of *Cupriavidus* B-7. Therefore, RSB exhibited the greatest enhancement in the $CaCl_2$-extractable Cd decrease in the soil after being loaded with Cupriavidus B-7.

3.5 Influence of bacteria-loaded biochar on Cd fractions in soil

The migration and biological toxicity of heavy metals are strongly affected by their chemical fractions in soil. After the introduction of *Cupriavidus* B-7, biochars, or *Cupriavidus* B-7-loaded biochars, the changes in Cd fractions within the soil were assessed through Tessier sequential extraction (Fig. 4c and d). For the original soil (CK treatment), the dominant Cd fraction was FeMn oxide bound fraction (42.16%), followed by exchangeable Cd (33.77%), while the residual, organic matter and carbonate bound Cd accounted for less (12.66%, 5.84% and 5.57%, respectively). A higher concentration of exchangeable Cd in the untreated soil indicates greater Cd effectiveness and mobility, thereby posing a higher ecological environmental risk. Besides, the FeMn oxide bound fraction of Cd could be released from the soil and be taken up by corps when the soil transforms into a reductive environment (Li et al. 2021a).

After 30 days of incubation, the proportion of exchangeable Cd decreased from 33.77% to 30.10%, 30.36% and 24.54%, respectively, with the addition of CDB, PB and RSB. The FeMn oxide bound fraction of Cd decreased from 42.16% to 36.32%, 41.73%, and 40.75%, respectively. Accordingly, the residual Cd fraction increased from 12.66% to 16.57–20.80%, and 20.47%, respectively. Compared with biochars, the *Cupriavidus* B-7 presented a much better effect on the transformation of exchangeable and FeMn oxide bound fraction Cd into residual fraction, resulting in an increase in the proportion of residual Cd from 12.66% to 31.66%. As reported by previous study, biochar could release alkaine substance, facilitating the formation of (oxy)hydroxide and carbonate bound Cd (Cui et al. 2019). However, microbial cells could tend to transport heavy metal ions into the cell and change the heavy metals from exchangeable fraction to residual fraction through respiration (Ji et al. 2022). As the incubation period extended to 60 days, the residual fraction of Cd in the soil showed a slight increase, rising from 31.66% to 36.40% in the *Cupriavidus* B-7 treatment. However, the residual Cd increased pronouncedly from 16.57% to 20.80% (30 days of incubation) to 23.64–33.46% in the biochar treatments. Remarkably, the RSB treatment demonstrated the most effective transformation of Cd from the exchangeable and FeMn oxide-bound fractions to the residual fraction.

Upon *Cupriavidus* B-7 was immobilized onto biochar, the CDBB, PBB and RSBB increased the soil residual Cd fraction to 35.38%, 33.51%, 37.62% and 39.63%, 38.17%, 43.29% within 30 and 60 days, respectively. As a result, the exchangeable in the soil was decreased to 21.67%, 23.29%, 20.29% and 17.96%, 18.26%, 17.78% within 30 and 60 days, respectively. As compared to their separate applications, the *Cupriavidus* B-7-loaded biochars showed an improvement in reducing the exchangeable Cd and increasing the residual Cd fractions in the soil. These results were consistent with previous studies (Ji et al. 2022; Song et al. 2022; Zhang et al. 2023), demonstrating that immobilizing heavy metal-tolerant (or immobilizing) bacteria onto biochar can effectively enhance the stabilization and immobilization of heavy metals in soil, both in terms of effectiveness and rate. This may result not only from the immobilizing mechanisms by the biochar and bacteria as discussed above, but also from their synergistic effects. For instance, the larger specific surface area of biochar increased the contact area between *Cupriavidus* B-7 and soil, leading to the exposure of more metal ion binding sites (Zhang et al. 2023) and thus enhanced the immobilizing effect on heavy metals of *Cupriavidus* B-7-loaded biochars.

3.6 The growth promotion and Cd uptake alleviation of pakchoi (*Brassica chinensis* L.) by bacteria-loaded biochar

To evaluate the effectiveness of *Cupriavidus* B-7, biochar, and *Cupriavidus* B-7 loaded biochar on pakchoi growth improvement, the dry weight of pakchoi was determined by the end of incubation (Fig. 5). As shown in Fig. 5a, the growth of pakchoi was inhibited in the CK treatment with only 0.30 g and 0.055 g dry weight of shoots (edible parts) and roots, respectively. Prior studies also documented growth inhibition of pakchoi in soil contaminated with Cd (II) (Huang et al. 2021; Qi et al. 2023). However, many studies reported that, under the heavy metal stress, certain heavy metal-resistant bacteria would produce amino acids and amines such as polyamines, which could facilitate plant growth (Rady and Hemida 2015; Sang et al. 2017; Wang et al. 2022). Additionally, Zheng et al. (2023) discovered that a heavy metal-resistant strain identified as *Cupriavidus* sp. could produce phytohormones, such as indole acetic acid (IAA), when exposed to heavy metal stress, thereby promoting the growth of pakchoi. In our present study, the dry weight of shoots and roots of pakchoi significantly increased to 4.75 g and 0.22 g, respectively, with the addition of *Cupriavidus* B-7. Likewise, all three types of biochar could effectively improve the growth of pakchoi, with an increase in the dry weight of shoots and roots to 1.32–1.76 g and 0.065–0.126 g, respectively. Furthermore, loading the *Cupriavidus* B-7 on biochar could significantly strengthen the effectiveness of biochar on plant growth promotion. Specifically, the dry weight of shoots of pakchoi in the CDBB, PBB, and RSBB treatments were 1.73, 2.96, and 1.99 times that of the CBD, PB, and RSB treatments, respectively. However, the plant growth promotion by *Cupriavidus* B-7 immobilized biochars was less than that of *Cupriavidus* B-7 alone. It was because when immobilized on biochars, the toxic effect of heavy metal on *Cupriavidus* B-7 was siginificantly alleviated, and less phytohormones would be secreted by *Cupriavidus* B-7 to enhance the growth of pakchoi.

The application of *Cupriavidus* B-7 and biochar could improve the physicochemical properties of soil as

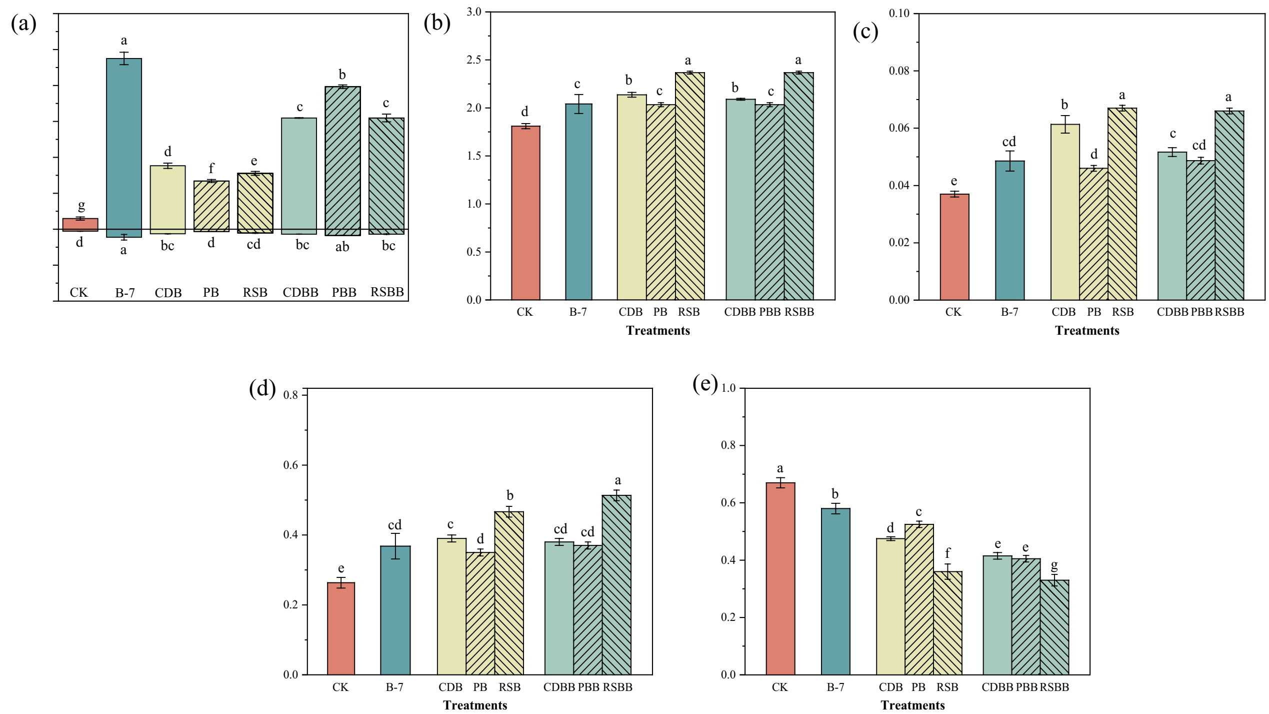


Fig. 5 The effect of biochars and Cupriavidus B-7-loaded biochars on pakchoi growth (**a**), soil enzyme (**b**–**d**) and the Cd accumulations in shoots of pakchoi (**e**). Different letters indicate significant differences among treatments ($p < 0.05$; Duncan's test)

discussed in Sect. 3.3, thereby improving the soil health and pakchoi growth (Ma et al. 2020a). Soil enzyme activity is a sensitive indicator of environmental stress and is widely recognized as an integrated bio-indicator of soil health (Gao et al. 2010). As presented in Fig. 5b–d, all applied *Cupriavidus* B-7 and biochar treatments increased the acid phosphatase, urease and catalase activities. The maximum of catalase activities were found in RSBB treatment while there was no significant difference in the phosphatase and urease activities between RSBB and RSB treatments, which were higher than those in the other treatments. In comparison to CK treatment, the activities of phosphatase, urease, and catalase in the RSBB treatment increased by 30.76%, 78.38%, and 94.93%, respectively. The results of soil enzyme activity improvement by *Cupriavidus* B-7, biochar and *Cupriavidus* B-7-loaded biochar were in accordance with the available soil nutrient elements and organic matters (Fig. 3). As shown in Fig. 6, the enzyme activities presented a significant correlation with available nutrient elements, pH, organic matter and the residual Cd in soil. Biochar mainly consists of carbon (C), which could partly serve as a carbon source for microorganisms. At the same time, the macronutrients (N, P, and K) in biochar supply extra nutrients for microbe growth (Ali et al. 2019). In addition, biochar with many porous structures can offer a habitat for microorganisms, facilitating their proliferation and enhancing soil enzyme activities (Nie et al. 2018).

Available heavy metals in the soil are readily taken up by crops, and our research revealed that application of *Cupriavidus* B-7, biochar and *Cupriavidus* B-7-loaded biochar significantly decreased the Cd content in shoots of pakchoi by the end of incubation (Fig. 5e). Among them, the RSBB treatment was optimal, decreasing the Cd content in shoots by 50.75% compared with the CK treatment. In general, the application of *Cupriavidus* B-7 loaded biochar (CDBB, PBB and RSBB) exhibited a more favourable effect compared to the individual applications of *Cupriavidus* B-7 and biochar, indicating a synergistic effect between *Cupriavidus* B-7 and biochar on pakchoi Cd accumulation reduction. Similar to the present work, Chen et al. (2023) discovered that biochar, on one hand, could alleviate the toxic effects of heavy metals on microbes. On the other hand, it could assist the microbes in secreting organic substances and facilitating extracellular electron transfer, thereby enhancing the Cd passivation of microbes.

The structural equation model (SEM) was used to evaluate the impact of biochar and *Cupriavidus* B-7 on pakchoi Cd uptake (Fig. 6b). The SEM results suggested that the bioavailable Cd and soil nutrients were two critical factors for the Cd accumulation of pakchoi. Biochar had a significant positive correlation with the soil pH,

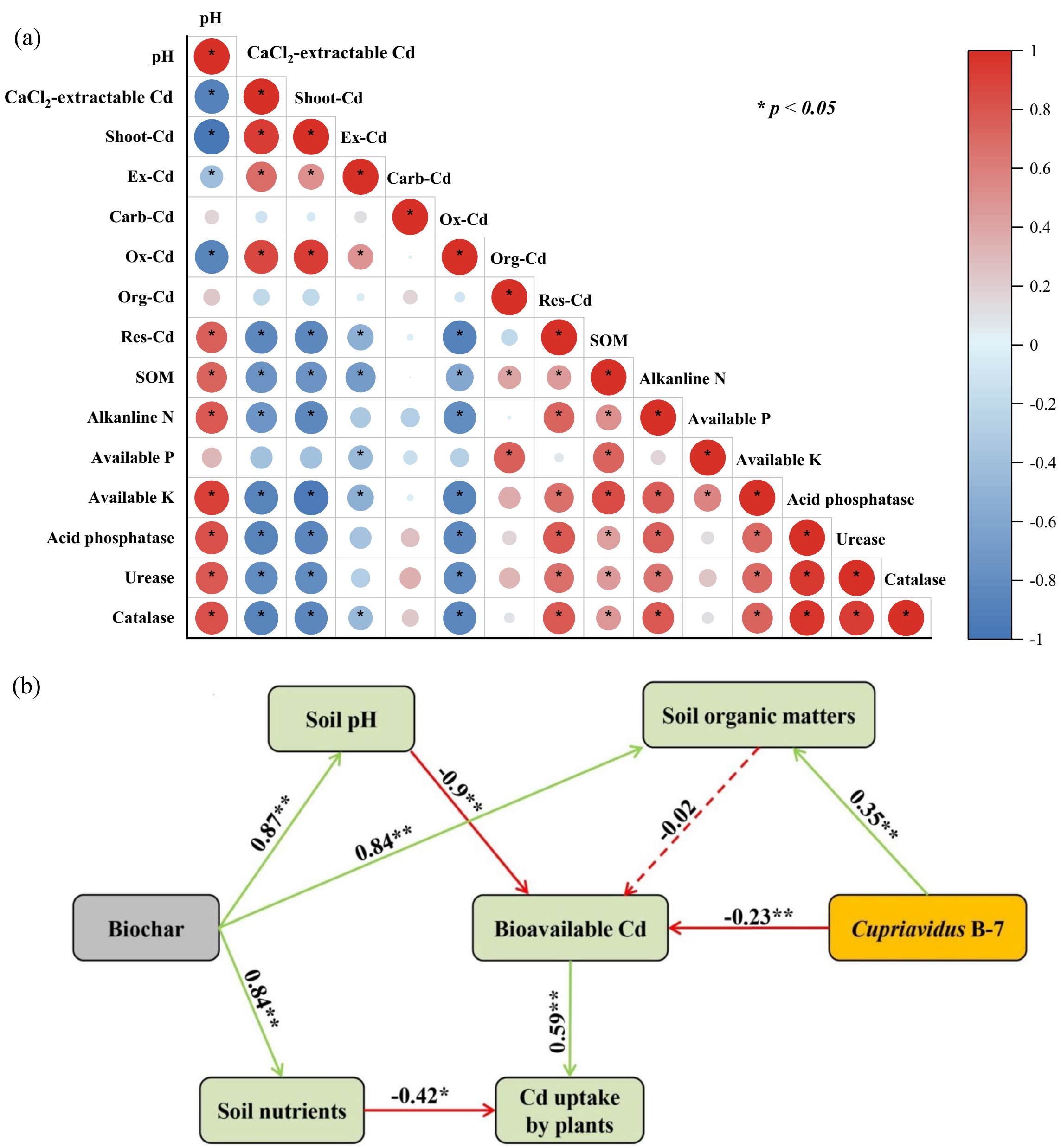


Fig. 6 Correlation analysis among soil physiochemical properties, Cd fractions, enzymes and Cd in the shoot of pakchoi (**a**), and the structural equation model of Cd accumulation of the pakchoi under *Cupriavidus* B-7 immobilized biochar application condition (**b**). Green and red arrows indicate positive and negative relationships, respectively. Continuous and dashed arrows indicate significant and nonsignificant relationships, respectively (*$p < 0.05$ and **$p < 0.001$)

organic matter and nutrients ($p < 0.001$). Among these, the soil pH ($p < 0.001$) and organic matter could decrease the bioavailable Cd concentration and therefore decrease the Cd uptake of pakchoi. At the same time, biochar would promote the growth of pakchoi by increasing the soil nutrients, and the larger biomass could decrease the Cd concentration in plants. As for *Cupriavidus* B-7, it could directly decrease the bioavailable Cd concentration ($p < 0.001$) and indirectly decrease the bioavailable Cd concentration by increasing the soil organic matter ($p < 0.001$).

Although the application of *Cupriavidus* B-7 alone showed a more pronounced effect on enhancing the pakchoi growth compared to CDBB, PBB, and RSBB, the Cd accumulation in pakchoi shoot was reduced by 28.44%, 30.17%, and 43.1% in the CDBB, PBB, and

RSBB treatments, respectively. This was significantly better than the application of *Cupriavidus* B-7 alone. Based on the above results, the *Cupriavidus* B-7-loaded biochar treatment, especially, the RSBB treatment not only improved soil health and increased crop yields but also significantly alleviated the accumulation of Cd in crops. It is a promising technology for the safe utilization of heavy metal-contaminated farmland.

3.7 Environmental implication

The farmland Cadmium (Cd) contamination poses a great threat to human health and ecosystem function due to its carcinogenic, teratogenic and mutagenic properties. In this study, we screened a Cd resistant strain (*Cupriavidus* B-7) and successfully loaded the *Cupriavidus* B-7 onto biochar. The effectiveness of *Cupriavidus* B-7-loaded biochar in reducing Cd uptake by pakchoi was demonstrated. Additionally, the growth of pakchoi was significantly improved due to the enhancement of soil health by *Cupriavidus* B-7-loaded biochar. Furthermore, the *Cupriavidus* B-7-loaded biochar is a low-cost and environmentally friendly strategy for farmland in-situ remediation. Therefore, it meets the requirement of pollution reduction and carbon reduction when addressing environmental issues. However, it is necessary to carry out field experiments in further steps to evaluate the efficiency and its long-term effectiveness before practice application.

4 Conclusions

The *Cupriavidus* B-7 and biochar effectively converted the exchangeable Cd to a residual fraction in the soil and reduced the bioavailability of Cd in the soil. *Cupriavidus* B-7 and biochar could significantly improve the soil's physicochemical properties and provide nutrients for microbes and plants, enhancing the soil health, Therefore, the *Cupriavidus* B-7 and biochar could not only alleviate the accumulation of Cd in pakchoi but also increased its growth. In addition, *Cupriavidus* B-7-loaded biochar showed a better effect on Cd immobilization and pakchoi growth increase, indicating a synergistic effect between *Cupriavidus* B-7 and biochar. This research suggested that the *Cupriavidus* B-7-loaded biochar is a promising and sustainable technology for in-situ Cd contaminated farmland soil.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00333-2.

Additional file 1. Supplementary Material.

Acknowledgements

Funding from the National Natural Science Foundation of China, the Special Support Program for high-level Talents of Zhejiang Province, Natural Science Foundation of Zhejiang Province and Key Laboratory of Environmental Pollution Control Technology Research of Zhejiang Province are gratefully acknowledged.

Author contributions

Yefang Sun: Investigation, Writing—Original Draft; Da Ouyang: Conceptualization, Writing-Review & Editing, Funding acquisition;Yiming Cai: Review & Editing, Funding acquisition; Guo Ting: Review & Editing, Funding acquisition; Mei Li: Review & Editing; Xinlin Zhao: Review & Editing; Qichun Zhang: Investigation, Review & Editing; Ruihuan Chen: Review & Editing; Fangzhen Li: Review & Editing; Xiujuan Wen: Review & Editing; Lu Xie: Review & Editing; Haibo Zhang: Supervision, Conceptualization, Writing-Review & Editing, Funding acquisition.

Funding

This work was financially supported by the National Natural Science Foundation of China [Grant Numbers: 42177021 and 42207012]; the Special Support Program for high-level Talents of Zhejiang Province [Grant Numbers: 2022R52015]; Natural Science Foundation of Zhejiang Province [Grant Numbers: LZ24D010001 and LQ24D030002]; Key Laboratory of Environmental Pollution Control Technology Research of Zhejiang Province [Grant Numbers: 2021ZEKL09].

Availability of data and materials

The data that support the finding of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

[1]Sino-Spain Joint Laboratory for Agricultural Environment Emerging Contaminants of Zhejiang Province, School of Environment and Resources, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China. [2]Shaoxing Academy of Agricultural Sciences, Shaoxing 312003, China. [3]Key Laboratory of Environmental Pollution Control Technology Research of Zhejiang Province, Eco-Environmental Science Research and Design Institute of Zhejiang Province, Hangzhou 310007, China. [4]Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha 410205, China. [5]School of Environment and Resources, Zhejiang University, Hangzhou 310007, China. [6]College of Life and Environmental Science, Wenzhou University, Wenzhou 325035, China.

Received: 15 January 2024 Revised: 10 April 2024 Accepted: 16 April 2024
Published online: 09 May 2024

References

Ali A, Guo D, Jeyasundar PGSA, Li Y, Xiao R, Du J et al (2019) Application of wood biochar in polluted soils stabilized the toxic metals and enhanced wheat (*Triticum aestivum*) growth and soil enzymatic activity. Ecotoxicol Environ Saf 184:109635. https://doi.org/10.1016/j.ecoenv.2019.109635

Bharti V, Vikrant K, Goswami M, Tiwari H, Sonwani RK, Lee J et al (2019) Biodegradation of methylene blue dye in a batch and continuous mode using biochar as packing media. Environ Res 171:356–364. https://doi.org/10.1016/j.envres.2019.01.051

Chen WM, Wu CH, James EK, Chang JS (2008) Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*. J Hazard Mater 151:364–371. https://doi.org/10.1016/j.jhazmat.2007.05.082

Chen HM, Min FF, Hu X, Ma DH, Huo ZL (2023) Biochar assists phosphate solubilizing bacteria to resist combined Pb and Cd stress by promoting acid secretion and extracellular electron transfer. J Hazard Mater 452:131176. https://doi.org/10.1016/j.jhazmat.2023.131176

Chi Y, Huang Y, Wang J, Chen X, Chu S, Hayat K et al (2020) Two plant growth promoting bacterial Bacillus strains possess different mechanisms in adsorption and resistance to cadmium. Sci Total Environ 741:140422. https://doi.org/10.1016/j.scitotenv.2020.140422

Cui L, Noerpel MR, Scheckel KG, Ippolito JA (2019) Wheat straw biochar reduces environmental cadmium bioavailability. Environ Int 126:69–75. https://doi.org/10.1016/j.envint.2019.02.022

El-Naggar A, Lee SS, Rinklebe J, Farooq M, Song H, Sarmah AK et al (2019) Biochar application to low fertility soils: a review of current status, and future prospects. Geoderma 337:536–554. https://doi.org/10.1016/j.geoderma.2018.09.034

Gao Y, Mao L, Miao CY, Zhou P, Cao JJ, Zhi YE et al (2010) Spatial characteristics of soil enzyme activities and microbial community structure under different land uses in Chongming Island, China: geostatistical modelling and PCR-RAPD method. Sci Total Environ 408:3251–3260. https://doi.org/10.1016/j.scitotenv.2010.04.007

Huang Y, Chen J, Zhang D, Fang B, YangJin T, Zou J et al (2021) Enhanced vacuole compartmentalization of cadmium in root cells contributes to glutathione-induced reduction of cadmium translocation from roots to shoots in pakchoi (*Brassica chinensis* L.). Ecotoxicol Environ Saf 208:111616. https://doi.org/10.1016/j.ecoenv.2020.111616

Ji X, Wan J, Wang X, Peng C, Wang G, Liang W et al (2022) Mixed bacteria-loaded biochar for the immobilization of arsenic, lead, and cadmium in a polluted soil system: Effects and mechanisms. Sci Total Environ 811:152112. https://doi.org/10.1016/j.scitotenv.2021.152112

Jiang C, Sun H, Sun T, Zhang Q, Zhang Y (2009) Immobilization of cadmium in soils by UV-mutated *Bacillus subtilis* 38 bioaugmentation and NovoGro amendment. J Hazard Mater 167:1170–1177. https://doi.org/10.1016/j.jhazmat.2009.01.107

Li X, Meng D, Li J, Yin H, Liu H, Liu X et al (2017) Response of soil microbial communities and microbial interactions to long-term heavy metal contamination. Environ Pollut 231:908–917. https://doi.org/10.1016/j.envpol.2017.08.057

Li F, Zheng Y, Tian J, Ge F, Liu X, Tang Y et al (2019) *Cupriavidus* sp. strain Cd02-mediated pH increase favoring bioprecipitation of Cd2+ in medium and reduction of cadmium bioavailability in paddy soil. Ecotoxicol Environ Saf 184:109655. https://doi.org/10.1016/j.ecoenv.2019.109655

Li S, Chen S, Wang M, Lei X, Zheng H, Sun X et al (2021a) Redistribution of iron oxides in aggregates induced by pe + pH variation alters Cd availability in paddy soils. Sci Total Environ 752:142164. https://doi.org/10.1016/j.scitotenv.2020.142164

Li W, Chen Y, Wang T (2021b) Cadmium biosorption by lactic acid bacteria *Weissella viridescens* ZY-6. Food Control 123:107747. https://doi.org/10.1016/j.foodcont.2020.107747

Liu B, Ai S, Zhang W, Huang D, Zhang Y (2017) Assessment of the bioavailability, bioaccessibility and transfer of heavy metals in the soil-grain-human systems near a mining and smelting area in NW China. Sci Total Environ 609:822–829. https://doi.org/10.1016/j.scitotenv.2017.07.215

Liu Y, Tie B, Li Y, Lei M, Wei X, Liu X et al (2018) Inoculation of soil with cadmium-resistant bacterium *Delftia* sp. B9 reduces cadmium accumulation in rice (*Oryza sativa* L.) grains. Ecotoxicol Environ Saf 163:223–229. https://doi.org/10.1016/j.ecoenv.2018.07.081

Liu Y, Tie B, Peng O, Luo H, Li D, Liu S et al (2020) Inoculation of Cd-contaminated paddy soil with biochar-supported microbial cell composite: a novel approach to reducing cadmium accumulation in rice grains. Chemosphere 247:125850. https://doi.org/10.1016/j.chemosphere.2020.125850

Ma H, Wei M, Wang Z, Hou S, Li X, Xu H (2020a) Bioremediation of cadmium polluted soil using a novel cadmium immobilizing plant growth promotion strain *Bacillus* sp. TZ5 loaded on biochar. J Hazard Mater 388:122065. https://doi.org/10.1016/j.jhazmat.2020.122065

Ma Q, Zhao W, Guan D, Teng HH, Ji J, Ma LQ (2020b) Comparing $CaCl_2$, EDTA and DGT methods to predict Cd and Ni accumulation in rice grains from contaminated soils. Environ Pollut 260:114042. https://doi.org/10.1016/j.envpol.2020.114042

Manasi RV, Santhana Krishna Kumar A, Rajesh N (2014) Biosorption of cadmium using a novel bacterium isolated from an electronic industry effluent. Chem Eng J 235:176–185. https://doi.org/10.1016/j.cej.2013.09.016

Nie C, Yang X, Niazi NK, Xu X, Wen Y, Rinklebe J et al (2018) Impact of sugarcane bagasse-derived biochar on heavy metal availability and microbial activity: a field study. Chemosphere 200:274–282. https://doi.org/10.1016/j.chemosphere.2018.02.134

O'Connor D, Peng T, Zhang J, Tsang DCW, Alessi DS, Shen Z et al (2018) Biochar application for the remediation of heavy metal polluted land: a review of in situ field trials. Sci Total Environ 619–620:815–826. https://doi.org/10.1016/j.scitotenv.2017.11.132

Oh SE, Hassan SHA, Joo JH (2009) Biosorption of heavy metals by lyophilized cells of *Pseudomonas stutzeri*. World J Microb Biot 25:1771–1778. https://doi.org/10.1007/s11274-009-0075-6

Ouyang D, Chen Y, Yan JC, Qian LB, Han L, Chen M (2019) Activation mechanism of peroxymonosulfate by biochar for catalytic degradation of 1,4-dioxane: important role of biochar defect structures. Chem Eng J 370:614–624. https://doi.org/10.1016/j.cej.2019.03.235

Ouyang D, Wu R, Xu Z, Zhu X, Cai Y, Chen R et al (2023) Efficient degradation of Bisphenol A by Fe^{3+}/Fe^{2+} cycle activating persulfate with the assistance of biochar-supported MoO_2. Chem Eng J 455:140381. https://doi.org/10.1016/j.cej.2022.140381

Qi X, Gou J, Chen X, Xiao S, Ali I, Shang R et al (2021) Application of mixed bacteria-loaded biochar to enhance uranium and cadmium immobilization in a co-contaminated soil. J Hazard Mater 401:123823. https://doi.org/10.1016/j.jhazmat.2020.123823

Qi WY, Chen H, Wang Z, Xing SF, Song C, Yan Z et al (2023) Biochar-immobilized *Bacillus megaterium* enhances Cd immobilization in soil and promotes *Brassica chinensis* growth. J Hazard Mater 458:131921. https://doi.org/10.1016/j.jhazmat.2023.131921

Qin C, Yuan X, Xiong T, Tan YZ, Wang H (2020) Physicochemical properties, metal availability and bacterial community structure in heavy metal-polluted soil remediated by montmorillonite-based amendments. Chemosphere 261:128010. https://doi.org/10.1016/j.chemosphere.2020.128010

Qing X, Yutong Z, Shenggao L (2015) Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicol Environ Saf 120:377–385. https://doi.org/10.1016/j.ecoenv.2015.06.019

Qiu B, Tao X, Wang H, Li W, Ding X, Chu H (2021) Biochar as a low-cost adsorbent for aqueous heavy metal removal: a review. J Anal Appl Pyrol 155:105081. https://doi.org/10.1016/j.jaap.2021.105081

Quilliam RS, Glanville HC, Wade SC, Jones DL (2013) Life in the 'charosphere'—does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem 65:287–293. https://doi.org/10.1016/j.soilbio.2013.06.004

Rady MM, Hemida KA (2015) Modulation of cadmium toxicity and enhancing cadmium tolerance in wheat seedlings by exogenous application of polyamines. Ecotoxicol Environ Saf 119:178–185. https://doi.org/10.1016/j.ecoenv.2015.05.008

Sang Q, Shan X, An Y, Shu S, Sun J, Guo S (2017) Proteomic analysis reveals the positive effect of exogenous spermidine in tomato seedlings' response to high-temperature stress. Front Plant Sci 8:120. https://doi.org/10.3389/fpls.2017.00120

Shen Y, Li H, Zhu W, Ho SH, Yuan W, Chen J et al (2017) Microalgal-biochar immobilized complex: a novel efficient biosorbent for cadmium removal from aqueous solution. Bioresource Technol 244:1031–1038. https://doi.org/10.1016/j.biortech.2017.08.085

Shen Z, Jin F, O'Connor D, Hou D (2019) Solidification/Stabilization for soil remediation: an old technology with new vitality. Environ Sci Technol 53:11615–11617. https://doi.org/10.1021/acs.est.9b04990

Shi Z, Zhang Z, Yuan M, Wang S, Yang M, Yao Q et al (2020) Characterization of a high cadmium accumulating soil bacterium, *Cupriavidus* sp. WS2. Chemosphere 247:125834. https://doi.org/10.1016/j.chemosphere.2020.125834

Siripornadulsil S, Siripornadulsil W (2013) Cadmium-tolerant bacteria reduce the uptake of cadmium in rice: potential for microbial bioremediation. Ecotoxicol Environ Saf 94:94–103. https://doi.org/10.1016/j.ecoenv.2013.05.002

Song L, Niu X, Zhou B, Xiao Y, Zou H (2022) Application of biochar-immobilized *Bacillus* sp. KSB7 to enhance the phytoremediation of PAHs and heavy metals in a coking plant. Chemosphere 307:136084. https://doi.org/10.1016/j.chemosphere.2022.136084

Teng Z, Shao W, Zhang K, Yu F, Huo Y, Li M (2020) Enhanced passivation of lead with immobilized phosphate solubilizing bacteria beads loaded with biochar/ nanoscale zero valent iron composite. J Hazard Mater 384:121505. https://doi.org/10.1016/j.jhazmat.2019.121505

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844–851. https://doi.org/10.1021/ac50043a017
Tu C, Wei J, Guan F, Liu Y, Sun Y, Luo Y (2020) Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environ Int 137:105576. https://doi.org/10.1016/j.envint.2020.105576
Wahla AQ, Anwar S, Mueller JA, Arslan M, Iqbal S (2020) Immobilization of metribuzin degrading bacterial consortium MB3R on biochar enhances bioremediation of potato vegetated soil and restores bacterial community structure. J Hazard Mater 390:121493. https://doi.org/10.1016/j.jhazmat.2019.121493
Wang J, Shi L, Zhai L, Zhang H, Wang S, Zou J et al (2021) Analysis of the long-term effectiveness of biochar immobilization remediation on heavy metal contaminated soil and the potential environmental factors weakening the remediation effect: a review. Ecotoxicol Environ Saf 207:111261. https://doi.org/10.1016/j.ecoenv.2020.111261
Wang X, Cai D, Ji M, Chen Z, Yao L, Han H (2022) Isolation of heavy metal-immobilizing and plant growth-promoting bacteria and their potential in reducing Cd and Pb uptake in water spinach. Sci Total Environ 819:153242. https://doi.org/10.1016/j.scitotenv.2022.153242
Wei T, Li X, Li H, Gao H, Guo J, Li Y et al (2022) The potential effectiveness of mixed bacteria-loaded biochar/activated carbon to remediate Cd, Pb co-contaminated soil and improve the performance of pakchoi plants. J Hazard Mater 435:129006. https://doi.org/10.1016/j.jhazmat.2022.129006
Wu C, Zhi D, Yao B, Zhou Y, Yang Y, Zhou Y (2022) Immobilization of microbes on biochar for water and soil remediation: a review. Environ Res 212:113226. https://doi.org/10.1016/j.envres.2022.113226
Yang T, Xu Y, Huang Q, Sun Y, Liang X, Wang L et al (2021) An efficient biochar synthesized by iron-zinc modified corn straw for simultaneously immobilization Cd in acidic and alkaline soils. Environ Pollut 291:118129. https://doi.org/10.1016/j.envpol.2021.118129
Zeng P, Guo Z, Xiao X, Peng C, Feng W, Xin L et al (2019) Phytoextraction potential of *Pteris vittata* L. co-planted with woody species for As, Cd, Pb and Zn in contaminated soil. Sci Total Environ 650:594–603. https://doi.org/10.1016/j.envpol.2021.118129
Zhang C, Li J, Wu X, Long Y, An H, Pan X et al (2020) Rapid degradation of dimethomorph in polluted water and soil by *Bacillus cereus* WL08 immobilized on bamboo charcoal-sodium alginate. J Hazard Mater 398:122806. https://doi.org/10.1016/j.jhazmat.2020.122806
Zhang T, Li T, Zhou Z, Li Z, Zhang S, Wang G et al (2023) Cadmium-resistant phosphate-solubilizing bacteria immobilized on phosphoric acid-ball milling modified biochar enhances soil cadmium passivation and phosphorus bioavailability. Sci Total Environ 877:162812. https://doi.org/10.1016/j.scitotenv.2023.162812
Zheng Y, Tang J, Liu C, Liu X, Luo Z, Zou D et al (2023) Alleviation of metal stress in rape seedlings (*Brassica napus* L.) using the antimony-resistant plant growth-promoting rhizobacteria *Cupriavidus* sp. S-8–2. Sci Total Environ 858:159955. https://doi.org/10.1016/j.scitotenv.2022.159955
Zhu X, Chen B, Zhu L, Xing B (2017) Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: a review. Environ Pollut 227:98–115. https://doi.org/10.1016/j.envpol.2017.04.032