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Synergistic remediation of electroplating wastewater contaminated soil and reduction of risk of groundwater contamination by biochar and *Pseudomonas hibiscicola* strain L1

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A R T I C L E I N F O

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A B S T R A C T

With the continuous development of electroplating industry, a large amount of electroplating wastewater is generated, which can be harmful to soil and basement. Introducing microorganisms into polluted soil can improve the soil environment, but it has the disadvantages of easy loss and low activity. In this study, the synergistic effect of biochar and strain L1 (BL1) was utilized to effectively reduce the risk of groundwater contamination by Ni(II), Cu(II), Cr(VI), and Zn(II), which are common heavy metals in electroplating wastewater. And the mechanism was found as BL1 was found to increase the porosity and water retention of the soil by specific surface area determination (BET) and scanning electron microscopy (SEM), favored the growth of soil microorganisms. It was found that BL1 could improve soil pH, enzyme activity, total organic carbon and other indicators by measuring soil physical and chemical properties. The results of microbial community analysis showed that BL1 increased the diversity of soil community and enriched microorganisms with nitrification and denitrification functions, thus promoting the removal of NH_4^+-N and NO_3-N. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) diffraction results showed that -OH, -CH, C=O, Si-O-Si, CO_3^{2-} and PO_4^{3-} of BL1 complexed with heavy metals to form precipitates. Thus, the conversion of heavy metals to the stable state was promoted. These results show that the addition of BL1 can effectively improve the soil environment and promote the self-recovery of soil function.

1. Introduction

With the continuous development of electroplating industry, electroplating wastewater has become one of the major sources of pollution. Among them, heavy metals such as nickel, chromium, copper and zinc, which are widely used in the electroplating process, can seriously threaten the ecological environment and human health (John et al., 2016). Some electroplating enterprises lose electroplating wastewater due to aging equipment or mismanagement, which in turn contaminates soil and groundwater (Baveye and Laba, 2015). Heavy metals in soil are characterized by high and stable toxicity and difficult to degrade and recover (Lan et al., 2021a; Gao et al., 2023). Therefore, the problem of heavy metal contamination of soil and groundwater by electroplating wastewater is receiving increasing attention and requires environmentally effective treatment methods (Anae et al., 2021).

Common soil heavy metal treatment methods currently available include physical (Dhaliwal et al., 2020), chemical and biological (Rehman et al., 2023) treatments. Physical methods mainly involve replacing or sweeping the contaminated soil, which is costly and requires long-term treatment (Dhaliwal et al., 2020). Chemical remediation includes vitrification techniques (Shu et al., 2021), chemical leaching (Ma et al., 2019), chemical immobilization, and electrokinetic remediation (Li et al., 2020). However, these methods not only change the basic shape of the soil but may also release toxic gases (Qi et al., 2023). It has been shown that certain functional microorganisms can minimize the heavy metals available by means of bio-absorption and bio-mineralization (Yang et al., 2021). Microorganisms possess numerous anionic groups on their surface that promote the immobilization of heavy metal cations (Lan et al., 2021). However, it is difficult for microorganisms in contaminated soils to maintain their activity and

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effectiveness, so their remediation capacity is often inadequate.

Biochar is a multi-porous material with high carbon content, high alkalinity, capacity for cation exchange and adsorption capacity (An et al., 2021a,b; Senthilkumar et al., 2022). In addition, biochar not only serves as a carrier for microorganisms, but also provides nutrients for microorganisms, solving the problems of easy loss of microorganisms and limited conditions of use (Abdin et al., 2020; Rajendran et al., 2022; Lan et al., 2021ab; Huang et al., 2023). In addition, biochar not only serves as a carrier for microorganisms, but also provides nutrients to microorganisms (Chen et al., 2023). Thereby, the coupled synergistic and regulatory effects of biochar on microorganisms are of increasing interest (Wang et al., 2024; Zhou et al., 2023 showed that the combination of Pb(II)-resistant phosphorus-solubilizing bacteria (PSB) and straw biochar removed up to 71.30% of Pb(II). It was found that composite application of tea tree biomass charcoal with *Ralstonia Bcul*-1 could significantly improve soil Cd pollution; meanwhile, it could also increase denitrifying microbial activity, strengthen NH_4^+-N fixation efficacy, and reduce soil ammonium nitrogen by 23.89% (Huang et al., 2023). However, It was also found that the addition of external strains and biochar reduced the diversity of soil microorganisms (Wang et al., 2021). Therefore, it is necessary to systematically assess the possible positive or negative impacts of carbon and bacterial coupling systems in the application environment.

Much of the published literature focuses on aqueous solutions and few studies have focused on mechanisms in complex soil environments. Also, the respective contributions of biochar and heavy metal resistant microorganisms to soil remediation are unclear. In particular, the mechanisms for soil remediation of electroplating wastewater contaminated soils containing multiple pollutants are unclear. In addition, soil peroxidase and urease activities are sensitive to heavy metals, and assessment of their activities can be considered as effective bioindicators of soil health, but their dynamics have been less studied (Sun et al., 2016). *Pseudomonas hibiscicola* strain L1, previously isolated from activated sludge obtained from MBR, was found to have the ability to remove Ni(II), Cu(II), Cr(VI) and Zn(II), and ammonia nitrogen from water (An et al., 2023). On this basis, the purposes of this research were to (1) evaluate the stabilization efficiency of peanut shell biochar-loaded strain L1(BL1) against multiple metals (Ni(II), Cu(II), Cr(VI) and Zn(II)) contaminated soil and the removal of ammonia nitrogen; (2) study the effect of soil heavy metal morphology by the sequential extraction method of the Community Bureau of Reference (BCR); (3) examined changes in soil physicochemical properties and enzyme activities over time; (4) reveal the mechanism by SEM, FTIR, XRD and microbial community analysis. The aim of this study is to provide theoretical and practical basis for remediation of heavy metal pollution by microbial material remediation.

2. Materials and methods

2.1. Materials

Chengkou County, Chongqing City, soil samples were taken from a non-contaminated location (Deng et al., 2022). Experimental soil details are shown in Table S3. A total of five sampling locations were uniformly dispersed, spanning depths ranging from 0 to 20 cm. The collection of samples followed a sequential order, starting from the subsoil, followed by the mesocosm, and concluding with the topsoil. About 1 kg of soil sample was collected from each sampling point, and the collection information of each sample was recorded. All soil samples were mixed well and brought back to the laboratory, where impurities such as debris and plant residues were removed, and the samples were spread into 2–3 cm layers and left to air-dry in a cool, ventilated place for 1 week. Following the process of air-drying, the specimens underwent crushing and grinding procedures, subsequently being subjected to a sieving process utilizing a mesh size of 2 mm. This sieving step was implemented to exclude any unwanted constituents, such as stones and organic residues, from the samples. Then, a solution of 300 mg L^{-1} each of Ni $(NO_3)_2$, KCr_2O_7, $ZnCl_2$, NH_4Cl and $CuSO_4$ was added to the soil. Ni(II), Zn(II), Cu(II), and Cr(VI) were analyzed using inductively coupled plasma photoemission spectrometers (iCAP 7200, USA). Nessler's reagent was utilized for spectrophotometric determination of NH_4^+-N (An et al., 2023). All reagents from Chengdu Cologne Chemical Reagent Factory.

2.2. Preparation of BC and BL1

The detailed method for the preparation of peanut shell biochar (BC) was recorded in Text S1. In our previous study we explored the basic elements of BC(An et al., 2021). *Pseudomonas hibiscicola* strain L1 (strain L1) was precultured in LB at 30 °C, 120 rpm for 24 h before use. After that, 1 mL of strain L1 was added to 100 mL of NM medium and incubated for 24 h. The composition of the medium is shown in Text S2. In order to immobilize the strain L1 on BC (BL1), 1% (v/v) of strain L1 and 1 g L^{-1} of BC were incubated in a shaker for 12 h (30 °C, 120 rpm) (Schommer et al., 2023). The successful immobilization of strain L1 in BC was confirmed by scanning electron microscopy in Fig. S2.

2.3. Soil column experiment

To determine how BC and strain L1 reduce soil and groundwater heavy metal pollution and their remediation effects. As shown in Fig. S1, soil column experiments were conducted. Four experimental groups were set up: blank control group (CK), addition of 1 g BC (BC), addition of 1 g BC and 10 mL of strain L1 group (BL1) and addition of 10 mL of strain L1 group (L1). The treated soil was then separated by suction filtration and dried in a desiccator at room temperature (25 °C). The dried soil sieved (10 mesh) and stored. The prepared contaminated soil (40 g) was then mixed with BC and strain L1 and spread into columns (The dimensions of the cylinder are 25 mm outer diameter and 400 mm total length.). The soil columns were filled by shaking the soil in the middle of the columns in order to disperse it uniformly and to prevent porosity during the filling process. In order to mitigate soil loss during leaching, a layered approach was used by introducing a 1 cm layer of quartz sand in the upper and lower parts of the column, and in order to avoid fluid loss, the lower end of the soil column was effectively sealed with Parafilm and the level was made to remain at a height of 2 cm above the soil column. In order to minimize the effects caused by the disturbance of soil sampling, 12 flat rows of columns were set up in each group. To ensure that the temperature in the experiment remained consistent over the 28 days, the soil columns were placed in a thermostat and the temperature was set at 25 °C. Samples were taken every 7 d for leaching analysis. The collected samples were centrifuged and filtered (8000 rpm, 0.22 μm) to determine Cr(VI), Cu(II), Ni(II), Zn(II), NH_4^+-N, NO_3^--N and NO_2^--N. Data are averaged for each of the 3 groups.

2.4. Metal fractions

The heavy metal fractions in soil were analyzed by Bureau Communautaire de R'ef'erence (BCR) sequential extraction method and were categorized into four fractions: exchangeable fraction (C1), reducible fraction (C2), oxidizable fraction (C3) and residual fraction (C4) (Wu et al., 2019; Wei et al., 2022). Heavy metal biotoxicity follows the following correlation: C1>C2>C3>C4. C1: This fraction can be extracted by acid, measured by adding 20 ml of CH_3COOH (0.11 mol L^{-1}) and shaking for 16 h; C2: This fraction can be reduced, measured by adding 20 ml of NH_2OH-HCl (0.10 mol L^{-1}, pH 2.0) and shaking for 16 h; C3: This fraction of metal can be oxidized, measured by adding 20 mL of 30% H_2O_2 twice and stirring occasionally in a water bath at 85 ± 2 °C until dry. After waiting for cooling, 25 mL of NH_4OAc (1 mol L^{-1}, pH 5.0), was added oxidative-reductive potential to the dried residue and shaken for 16 h; C4: In a PTFE beaker, the HNO_3-HF-$HClO_4$ mixture was heated repeatedly to eliminate the white fumes and the contents of the crucible became viscous, then 1 ml of a solution of V (HNO_3): V (H_2O) = 1:1 was added and the residue was dissolved by heating at low temperature.

2.5. Soil physicochemical properties analysis

In order to examine the impact of BC and strain L1 on soil physicochemical parameters, measurements of pH, oxidative-reductive potential (Eh), cation exchange capacity (CEC), and total organic carbon (TOC) were conducted at 7-day intervals (Mujtaba Munir et al., 2020). Soil samples were taken from 3 replicates of each group every 7 d. Samples were taken from 1 to 2 cm of the surface layer, 9–11 cm of the middle and 1–2 cm of the bottom layer, air-dried and mixed homogeneously. Then, metal fractions of the soil and soil properties were determined. The soil-water ratio was 1:2.5, stirred for 2 min and left to stand for 30 min, then the pH and Eh values of the solution were measured using a bench-top acidimeter (PHSJ-3F). TOC was determined by low-temperature exothermic method using potassium dichromate-sulphuric acid. Determination of CEC by hexaammine cobalt trichloride leaching-spectrophotometric method (HJ 889–2017). Dtailed assays are available in Text S3.

2.6. Soil enzymatic activity analysis

The effects of BC and strain L1 on the enzymatic activities (urease and catalase activities) of the soil were examined at 7 d intervals. Urease activity was assayed according to (Tu et al., 2018). Catalase activity was in accordance with (Deng et al., 2022). Detailed measurements are in Text S7 and S8.

2.7. Microbial community diversity

Following a 28-day incubation period, the soil that had undergone reaction was subsequently sent to Beijing Qingke Xinye Biotechnology Co., Ltd. for the purpose of conducting microbial community diversity testing. The extraction of total genomic DNA from soil samples was performed using the Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) in accordance with the instructions provided by the manufacturer. The primer pairs 338 F (5′- ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the hypervariable region V3-V4 of the bacterial 16 S rRNA gene. The PCR results underwent analysis on an agarose gel and were subsequently purified using the Omega DNA purification kit, manufactured by Omega Inc. in Norcross, GA, USA. The PCR products that had undergone purification were gathered, and afterwards subjected to paired-end sequencing with a read length of 2 × 250 bp using the Illumina Novaseq 6000 platform.

2.8. Characterization

Before and 28 d after the reaction, changes in soil surface morphology were observed by SEM (FEI Nova NanoSEM, Shanghai); the composition of the mineral phases (2θ range from 10° to 80°) was detected using XRD (X′ Pert Powder, PANalytical, Holland); and changes in soil surface functional groups were detected by FTIR (Nicolet iS5,

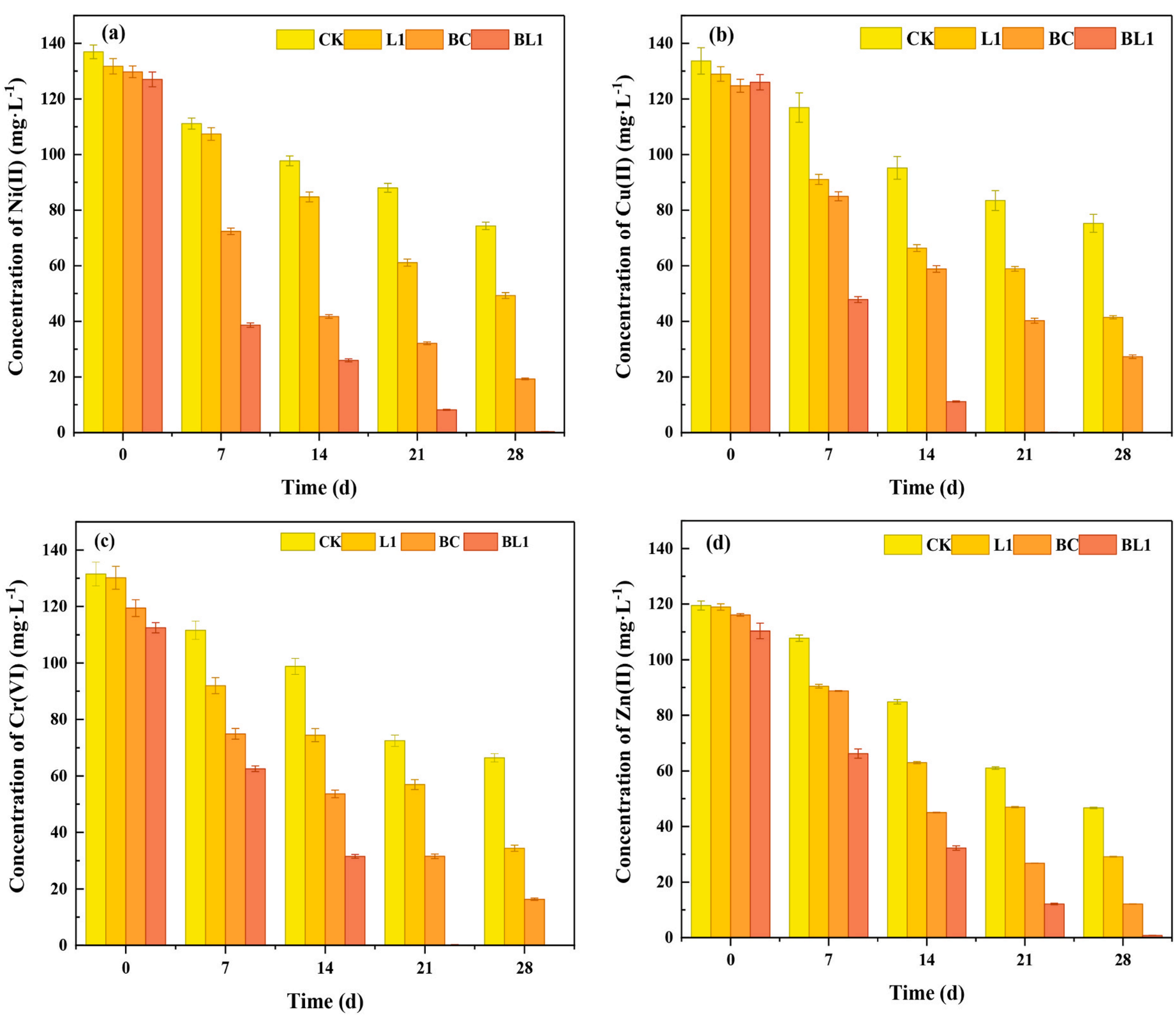

Fig. 1. Performance of BC and strain L1 in soil. The removal efficiency of (a) Ni(II), (b) Cr(VI), (c) Cu(II), (d) Zn(II).

Thermo, USA) in the range of 400–4000 cm^{-1}.

3. Results and discussion

3.1. Immobilization performance of BC and strain L1 to Ni(II), Zn(II), Cu (II), Cr(VI) in soil

In order to assess the effect of strains L1 and BC on soil heavy metal stabilization, fluctuations in heavy metal concentrations in the drench solution were analyzed in four different systems (Fig. 1, Table S1). Due to the adsorption effect of the soil itself, the initial concentrations of heavy metals Ni(II), Cu(II), Zn(II), Cr(VI) and NH_4^+-N in the drench solution were measured to be 136.93, 121.36, 116.10, 112.46 and 126.67 mg L^{-1}, respectively. The concentrations of Ni(II), Cu(II), Cr(VI) and Zn (II) in the drench solution of the CK group were 74.34, 75.26, 66.44 and 46.69 mg L^{-1}, respectively, after 28 d. This is due to the fact that the leaching itself reduces the concentration of pollutants in the soil and the soil has a certain degree of adsorption of heavy metals. But the concentration of leaching heavy metals is still higher, which requires an effective methods to reduce the concentration of heavy metals in soil.

The concentrations of Ni(II), Cu(II), Cr(VI) and Zn(II) in the drench solution of L1 group were 49.27, 37.46, 34.38 and 29.14 mg L^{-1} after 28 d of incubation, respectively. Compared with the CK group, the concentrations of Ni(II), Cu(II), Cr(VI) and Zn(II) were reduced by 25.07, 37.80, 32.06 and 17.29 mg L^{-1}, respectively. This indicates that strain L1 is effective in reducing the concentration of heavy metals. The results of previous studies showed that the presence of carboxyl, hydroxyl, amino, amide, alcohol, aldehyde and ether functional groups on the surface of strain L1 not only creates adsorption sites for the formation of heavy metal complexes, but also transforms heavy metals from toxic to less hazardous forms through oxidative reduction (An et al., 2021a,b).

In the BC group, the concentrations of Ni(II), Cu(II), Cr(VI) and Zn(II) in the drench solution were 19.27, 27.26, 16.35 and 12.13 mg L^{-1} after 28 d of incubation, respectively. Compared to the CK group, the concentrations of Ni(II), Cu(II), Cr(VI) and Zn(II) were reduced by 55.07, 48.00, 50.09 and 34.56 mg L^{-1}, respectively. Comparing the removal of heavy metals by biochar and strain L1, biochar was more effective than strain L1. This may be due to the fact that the growth of single strain L1 was affected by the pollutants and was not active in the soil.

The concentrations of Ni(II), Cu(II), Cr(VI) and Zn(II) in the drench solution after 28 d of incubation in BL1 group were 0.35, 0, 0 and 0.83 mg L^{-1}, respectively. The concentrations of Ni(II), Cu(II), Cr(VI), and Zn (II) were reduced by 48.92, 37.46, 34.38, and 28.31 mg L^{-1}, respectively, compared with the L1 group. The concentrations of Ni(II), Cu(II), Cr(VI), and Zn(II) were reduced by 18.97, 27.26, 16.35, and 11.30 mg L^{-1}, respectively, compared to the BC group. Heavy metal concentrations of the BL1 group decreased significantly and Cu(II) and Cr(VI) were no longer detected in the BL1 group at 21 d, suggesting that the coupling of biochar and strain L1 significantly enhanced the removal efficiency of heavy metals. This is due to the fact that BC has a well-developed porous structure that provides shelter for soil microorganisms. BC can also provide nutrients and ions for the cultivation of soil microorganisms. In summary, BL1 reduced the heavy metal concentration in the leachate and was effective in remediation of heavy metal contaminated soil.

3.2. Effect of BC and strain L1 on the immobilization efficiency of heavy metals

The bioavailability of heavy metals is a key determinant of their biotoxicity. Consequently, the investigation focused on the examination of the alteration of heavy metal compositions in soil polluted with pollutants by the utilization of the BCR sequential extraction method (Zhang et al., 2022). The magnitude of biotoxicity of heavy metal forms was C1 > C2 > C3 > C4 (Qureshi et al., 2020). The chemical forms of the four toxic heavy metal elements were predominantly unstable acid-soluble in the initial soil (Cu(II) 38.53%, Zn(II) 54.43%, Cr(VI) 21.46%, Ni(II) 49.23%) and reducible (Cu(II) 17.11%, Zn(II) 26.20%, Cr (VI) 22.65%, Ni(II) 28.32%). This means that heavy metals were much more biotoxic in untreated soils. And the proportion of C4 of Cr(VI) in the initial soil was larger at 44.73% compared to the other three heavy metals, probably because the initial soil Eh was lower in the reduced state, which facilitated the conversion of Cr(VI) to Cr(III). With the addition of BC and strain L1, the bioavailability of heavy metals began to decrease, as shown in Fig. 2. The percentage of C1 and C2 decreased, while the percentage of C3 and C4 increased. As shown in Fig. 2a, the C4 of Zn(II) increased to 9.75%, 16.64%, 32.86% and 56.36% in CK, L1, BC and BL1 treatments, respectively, after 28 d of reaction. Meanwhile, the proportion of acid-soluble heavy metals decreased from 54.43% to: 46.37%, 37.36%, 29.36% and 8.57%, respectively. As shown in Fig. 2b, after 28 d, C4 of Cu(II) increased from 25.78% to 35.37%, 39.64%, 52.86% and 68.16% in CK, L1, BC and BL1 treatments, respectively. While the percentage of C1 decreased from 38.53% to 24.62%, 21.26%, 9.36% and 3.57%, respectively. It is noteworthy that the proportion of C4 in Cu(II) increased more in the CK group compared to the other three heavy metals. Similar results were found in (Tu et al., 2020), which was attributed to the strong affinity of Cu(II) for soil organic matter (Sellaoui et al., 2018). Fig. 2c shows that the C4 of Cr(VI) increased to 50.54%, 54.78%, 66.45% and 70.25% and the percentage of C1 decreased from 21.46% to 15.23%, 9.23%, 4.66% and 2.38% in comparison with CK, L1, BC and BL1 treatments, respectively. As shown in Fig. 2d, C4 of Ni(II) increased by 11.09%, 10.73% and 21.29% in L1, BC and BL1 treatments, respectively, compared to CK. While the percentage of C1 decreased from 49.23% to 32.42%, 20.52% and 4.27%, respectively. Microorganisms can transport heavy metal ions into cells through respiration, thereby immobilizing and transforming heavy metals (Priya et al., 2022). However, a comparison between the L1 and BC groups could reveal that biochar contributed more to heavy metal immobilization. The results in section 3.4.2 show that BC increases the pH of the soil, which can contribute to the formation of insoluble heavy metal hydroxides, carbonates and silicates, thus reducing exchangeable heavy metals. This was substantiated by the XRD analyzes. Furthermore, the introduction of BC has the potential to enhance the overall organic carbon concentration, so facilitating the conversion of heavy metals into a more resilient and secure form (Xiong et al., 2019; Mujtaba Munir et al., 2020). Overall, these four heavy metals could be passivated by BC and strain L1, thereby reducing their leaching, with Cu(II) having a greater affinity for the soil and biochar immobilizing more soil heavy metals. Furthermore, the combination of BC synergized with strain L1 was more efficient than the stabilization of single components.

3.3. Effect of BC and strain L1 on NH_4^+-N in soil

The results in Fig. 3 show that after 28 d, NH_4^+-N in the strain L1 group decreased from 126.67 mg L^{-1} to 16.67 mg L^{-1}, which was 24.19 mg L^{-1} less NH_4^+-N leaching than the CK group. This demonstrated that strain L1 had good NH_4^+-N removal ability. The NH_4^+-N content of the single biochar group decreased from 124.67 mg L^{-1} to 24.33 mg L^{-1}, which reduced the NH_4^+-N leaching by 16.53 mg L^{-1} compared to the CK group. The results indicated that strain L1 had a better NH_4^+-N removal effect than BC. The BL1 group showed excellent synergistic effects as no NH_4^+-N leaching was detected after 21 d. It was found that tea biochar-Ralstonia Bcul)-1 reduced NH_4^+-N by 35.17% at a level of 33.58 mg/kg (Huang et al., 2023). It was found that the treatment of soil with biogas biochar resulted in no detectable NH_4^+-N in the leachate collected after 67 d (Novak et al., 2010). Comparison showed that BL1 has good performance in removing ammonia nitrogen.

The results showed that no_3^--N was detected in the BL1 group at 21 d. The removal ratio was increased by 26.70%, 8.59% and 2.84% compared with the CK group, BC group and strain L1, respectively. The surface of BC was found to contain functional groups, specifically phenolic and quinone groups, as shown by FTIR analysis. These

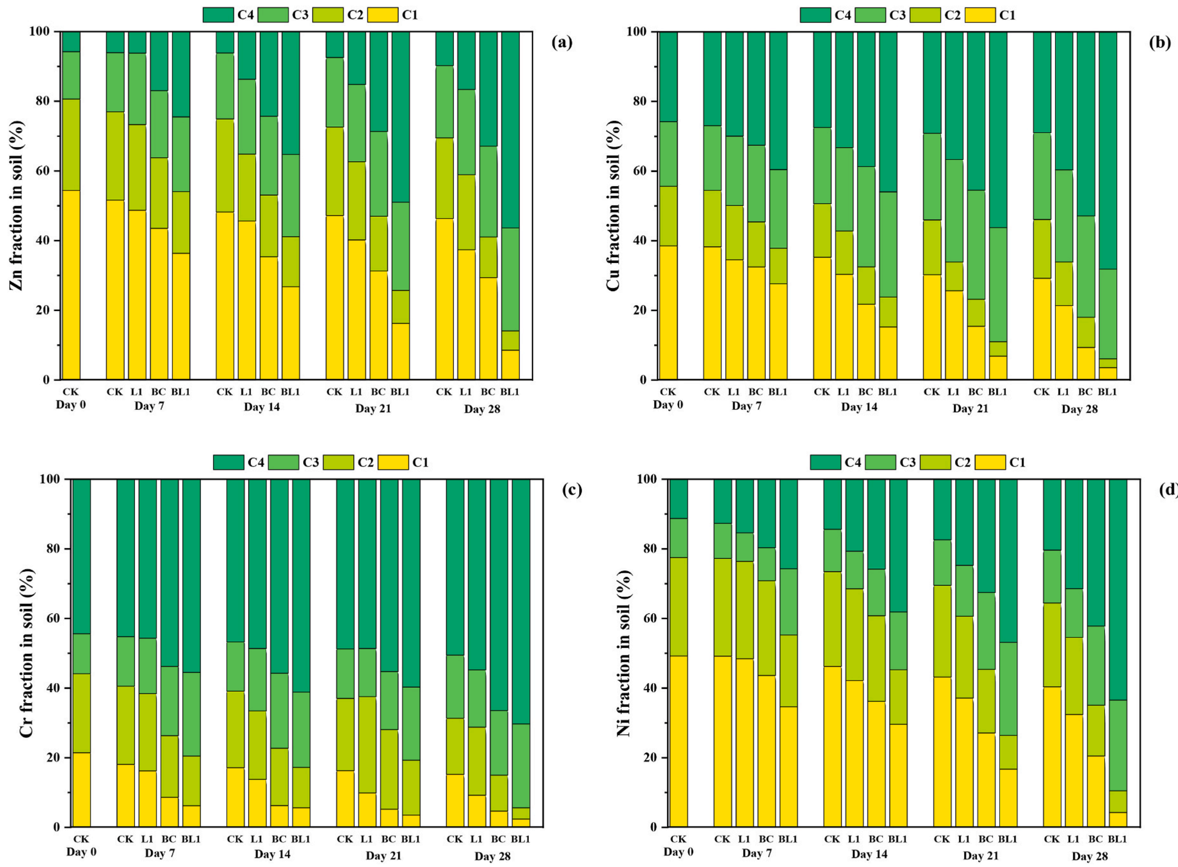

Fig. 2. Proportions of each chemical form of heavy metals in contaminated soil after 7, 14, 21 and 28 d of incubation: (a) Zn(II), (b) Cu(II), (c) Cr(VI), and (d) Ni(II).

functional groups have the potential to facilitate electron transport and potentially enhance the process of denitrification (Saquing et al., 2016). In addition, it has been shown that biochar releases some soluble organic substances and nutrients that stimulate and increase the expression of denitrification genes (Xiao et al., 2018). In conclusion, BC and strain L1 were effective in controlling the leakage of NH_4^+-N and NO_3^--N from the soil.

3.4. Effect of BC and strain L1 on soil properties

3.4.1. Enzyme activities in soil

Soil peroxidase and urease activities are metal-sensitive biomarkers for assessing the impact of biogenesis on microbial metabolism (He et al., 2019). Fig. 4a shows that the activities of catalase and urease in CK group firstly decreased and then rebounded. The early decline may be the result of inhibition by high concentration of heavy metals. While the peroxidase activity in BL1 group increased significantly from 0.90 to 6.73 (0.02 mol L^{-1} $KMnO_4$) $h^{-1}g^{-1}$. Urease activity increased from 0.99 to 5.15 (NH_4^+-N g^{-1} 24 h^{-1}). Urease breaks down urea, which raises the pH of the soil and thus promotes the fixation of heavy metals. In addition, urease induces carbonate precipitation, which reduces metal availability through biomineralization. A similar trend can be found in (Cheng et al., 2020). This suggests that the porous structure of biochar protected against soil enzyme activity and promoted self-restoration of soil function. This may be due to the release of metabolites such as proteins from the interaction of biochar with microorganisms, thus increasing the activity of soil microorganisms. Meanwhile, biochar has the potential to contribute carbon resources to support microbial metabolism, hence augmenting the microbial requirement for nitrogen resources and promoting urease activity (Li et al., 2019). The urease activity was increased in the L1 group of the added strain only, from 0.99 to 2.11 (NH_4^+-N g^{-1} 24 h^{-1}). This suggests that the addition of strain L1 to contaminated soils may have a remedial effect as this microorganism is able to improve soil properties by mineralizing heavy metals (Lipińska et al., 2021). In summary, the employing of BC and strain L1 demonstrated a positive impact on the restoration of the soil microenvironment, leading to accelerated healing and better effectiveness.

3.4.2. Changes in physicochemical properties of soil

The adverse impacts of certain metals in soil are contingent upon their accessibility and movement, which are primarily determined by the physicochemical features of the soil. Fig. 5 illustrates the alterations seen in the physicochemical characteristics of the soil. pH can affect the morphology of heavy metals in soil. Elevated pH levels have been found to be useful in mitigating the presence of exchangeable heavy metal forms within soil, hence minimizing potential environmental damage. After 28 d of incubation, the pH of each group increased to: 7.2 (CK), 7.48 (L1), 7.78 (BC), and 8.2 (BL1). One reason for the increase in pH after adding BC may be the high proportion of ash and alkaline cations in the biochar (Li et al., 2018). The higher soil pH in the BL1 group compared to the BC group probably resulted from the improved microbial attack on the organic anions, which depleted the H^+ in the soil solution. And the addition of BC triggered the decarboxylation of organic anions, which raised the soil pH. The transition of soil pH from an acidic to a slightly alkaline state further promoted the proliferation and bioremediation capabilities of the strains within the soil. CEC is a measure of a soil's capability to retain fertility, buffer capacity and adsorb cations. After 28 d of incubation, the CEC increased in each group from 6.91 to 11.62 (CK), 20.62 (L1), 36.73 (BC), and 41.87 cmol kg^{-1} (BL1), respectively. The utilization of BL1 on the soil led to a notable enhancement in the CEC, hence enhancing the electrostatic attraction of heavy metals on the soil's surface. The mobility of these metals was diminished as a result of this (Chao et al., 2018). Soil Eh was a key factor

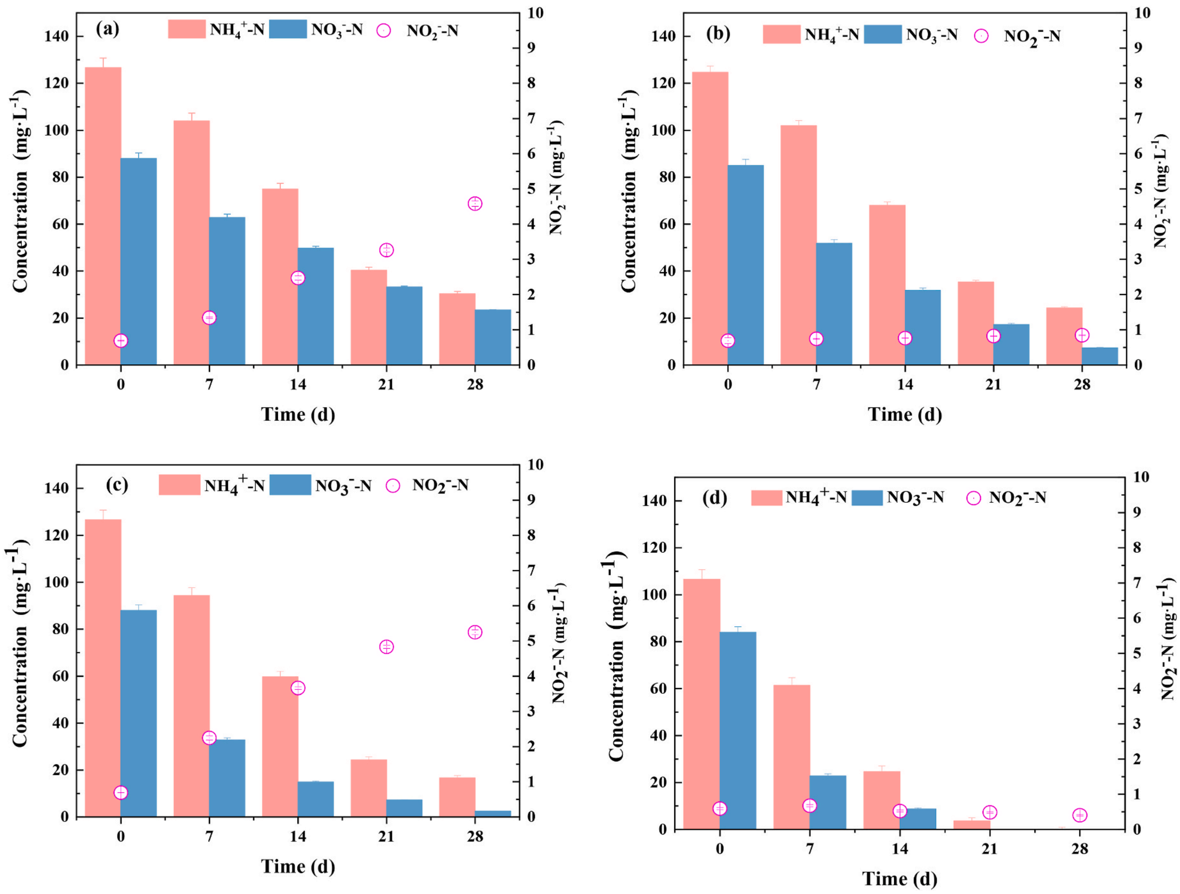

Fig. 3. Performance of BC and strain L1 to NH_4^+-N in soil after 7, 14, 21 and 28 d of incubation: (a) CK, (b) BC, (c) strain L1, (d) BL1.

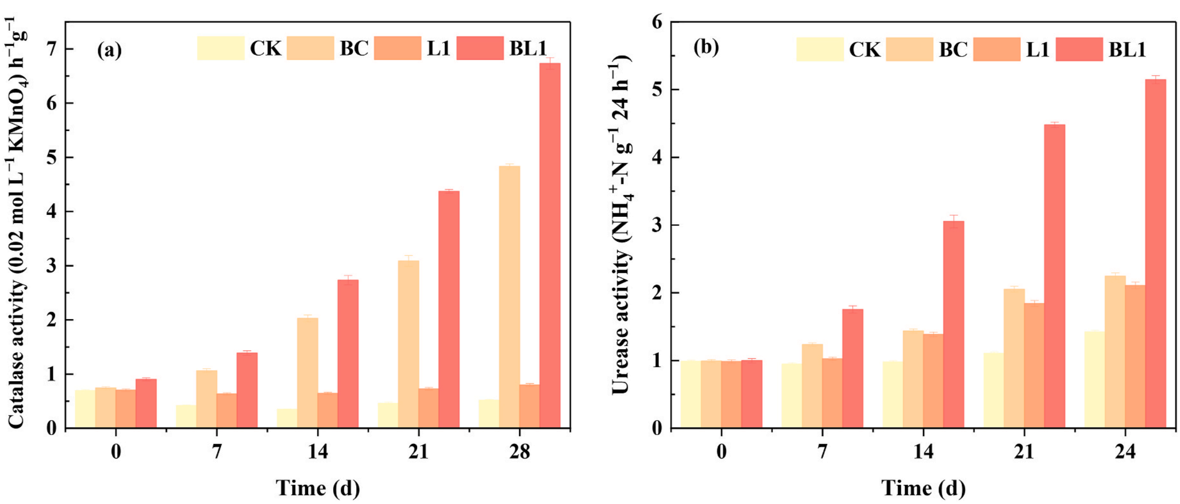

Fig. 4. The changes of (a) catalase and (b) urease activities in the soil.

influencing the morphology of heavy metals, and the Eh in the BL1 group decreased to −76 mv, which was significantly lower than that in all other groups. In addition, the lower Eh in the environment not only promoted the microbial reduction of Cr(VI), but also stimulated the formation of complexes and the precipitation of heavy metals through the interaction with organic functional groups such as -OH and -COOH (An et al., 2022). TOC increased from 15.74 g kg^{-1} to 56.72 g kg^{-1} after addition of BC and strain L1. This indicated that BL1 provided a large quantity of organic matter to the soil and promoted the growth of microorganisms (Yuan et al., 2022). In conclusion, the BL1 improved buffering capacity, enhanced soil fertility and facilitated the stabilization of heavy metals.

3.5. *Remediation mechanism*

3.5.1. *SEM*

Fig. S2 shows that a large number of strain L1 was immobilized inside the pores of BC. Fig. S3 shows the SEM images of the two groups of soils after 28 d of incubation. The images show that the soil with added biochar showed a loose porous structure, while the soil in the blank

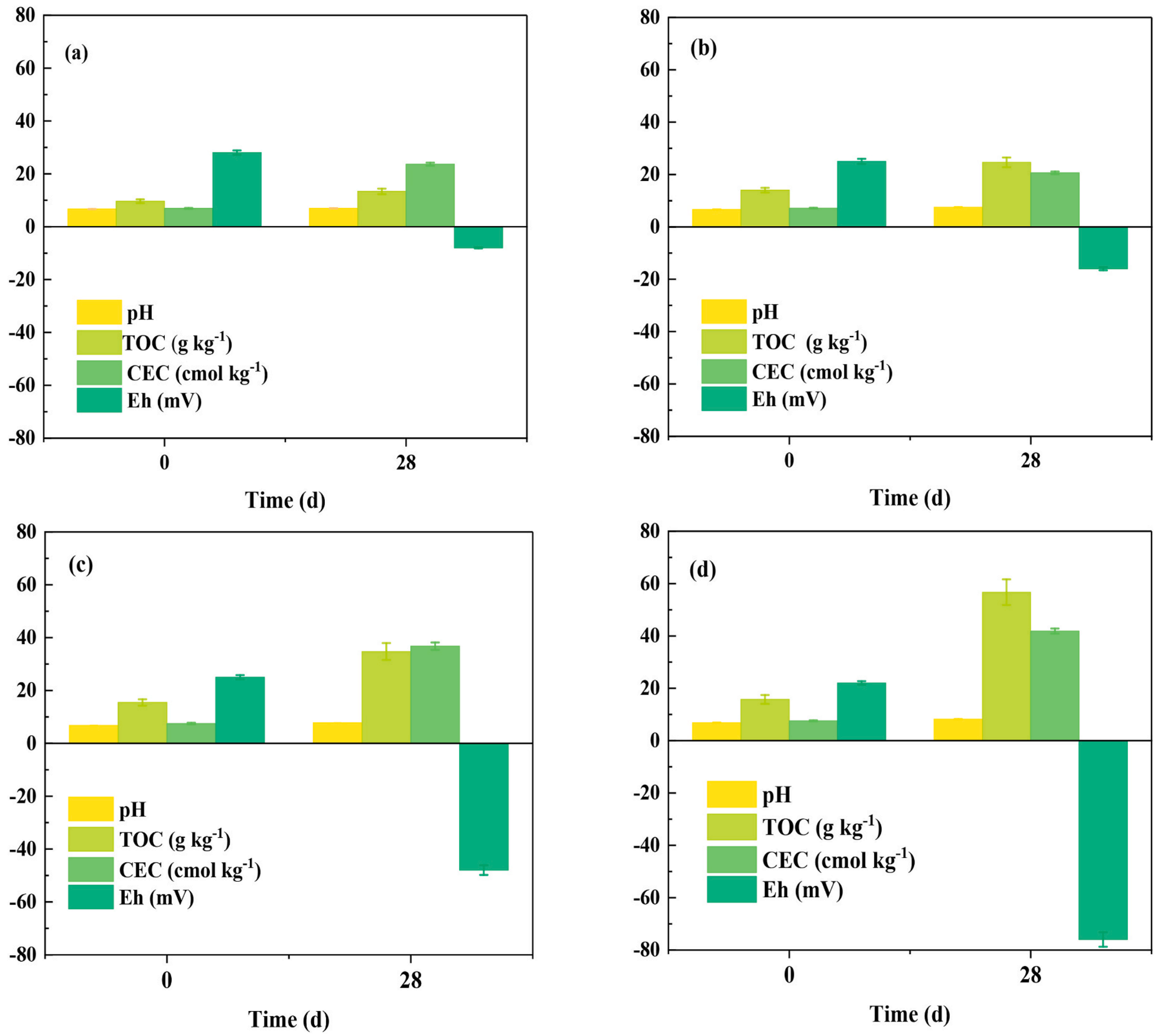

Fig. 5. Change of soil properties after treating with BC and strain L1. (a) CK, (b) strain L1, (c) BC, (d) BL1.

group had more compact pores. This result is similar to (Rasa et al., 2018). Table S2 shows the changes in specific surface area and pore structure after addition of BL1. The addition of the extractant decreased the specific surface area of the soil mixture but increased the average porosity of the soil mixture due to its porous structure. Biochar was shown to increase soil porosity due to its large specific pore structure and surface area. In addition, the rich organic macromolecules and pore morphology of biochar are likely to influence the interaction between biochar and water through surface roughness and contact angle, and thus contribute to soil water storage (Liu et al., 2017). As a result, larger soil clusters were produced, thus enhancing the adsorption capacity of the soil. Biochar was found to enhance water retention and permeability of the soil, thereby significantly improving the microbial habitat. Moreover, many small crystalline particles can be observed on the BL1 group, which are presumed to be complexes generated by heavy metal ions and biochar indicating functional groups.

3.5.2. FTIR and XRD

Fig. 6a shows the FTIR spectra of the BL1 and CK groups after 28 d. The vibration of stretching from 3427 cm^{-1} to 3439 cm^{-1} indicated the involvement of O-H in the reaction (Deng et al., 2017). In addition, the symmetric stretching at 778 cm^{-1} belongs to -COOH (Liu et al., 2018). Combined with the analysis of XRD results (Fig. 6b), functional groups of BL1, such as -OH and -COOH, complexed with heavy metal ions to generate $Cu(OH)_2H_2O$ (PDF#42–0638 2θ = 42.51°) and $Zn(OH)_2$ (PDF#20–1427 2θ = 29.46°). The carbonate and phosphate peaks are represented by the telescopic vibrations at 1035 cm^{-1} and 694 cm^{-1}, respectively (Zięba-Palus et al., 2017; Zhang et al., 2020). It was shown that BL1 released carbonate and phosphate complexed with heavy metals to form $Ni_3(PO_4)_2{\cdot}8H_2O$ (PDF#33–0951 2θ = 39.46°), $NiCO_3(H_2O)_4$ (PDF#24–0523 2θ = 52.45°) and $CrPO_4{\cdot}6H_2O$ (PDF#29–0455 2θ = 20.95°). The spectrum at 797 cm^{-1} was assigned as -CH twist. At 1428 cm^{-1} the peak belongs to the amide vibration peak of the protein. This indicates that the proteins of strain L1 were involved in the process of heavy metal stabilization. Furthermore, the distinctive peak observed at a wavenumber of 468 cm-1 was identified as the Si-O-Si bond (Li et al., 2008). Based on the XRD findings, it can be shown that Zn_2SiO_4 (PDF#37–1485, 2θ = 22.07°), $CuSiO_3{\cdot}H_2O$ (PDF#34–0077, 2θ = 28.03°), and Ni_2SiO_4 (PDF#15–0388, 2θ = 23.12°) were generated. 1637 cm^{-1} might be attributed to C=O (Liu et al., 2018). Cr_2O_3 (PDF#38–1479 2θ = 50.21°) production may be the result of the loss of protons by the positively charged functional groups and the reduction of Cr(VI) to Cr(III) by electrons acceptance from the biochar (Ren et al., 2023). FTIR and XRD confirmed that -OH, -CH, C=O and Si-O-Si functional groups on the surface of BL1 contributed to the stabilization of the heavy metal. In addition, the abundance of carbonate and PO_4^{3-} on BL1 also promoted the immobilization of heavy metals.

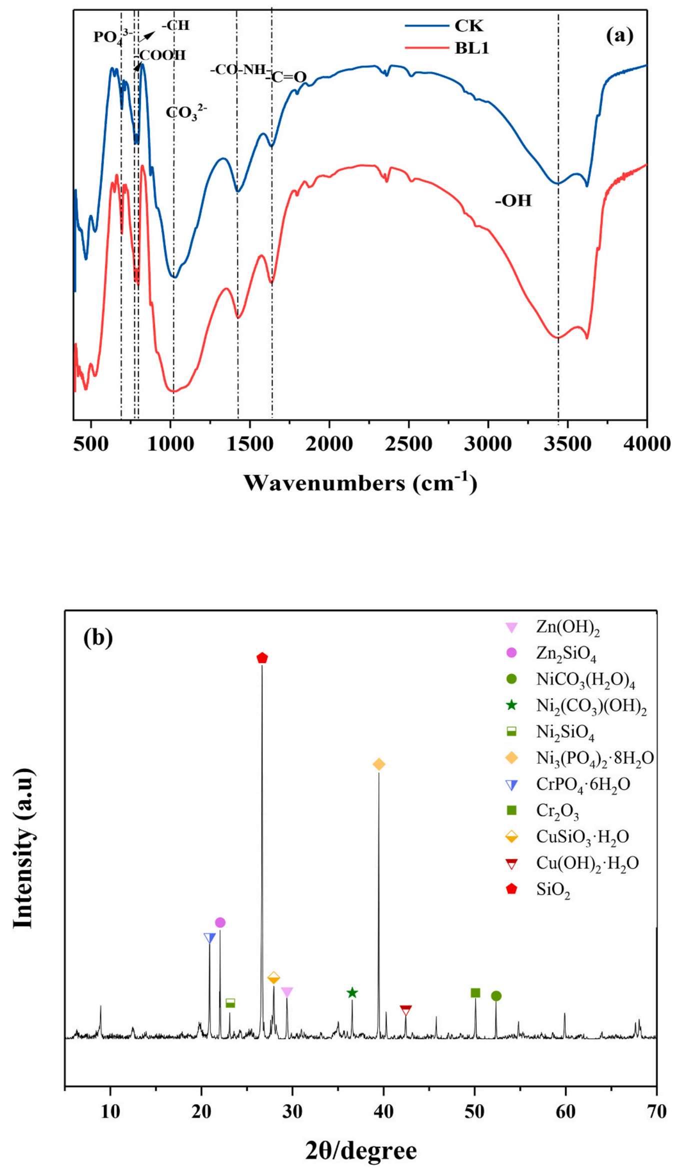

Fig. 6. (A) FITR of soil after 28 d of incubation; (b) XRD of soil after 28 d of incubation.

3.6. Microbial community diversity analysis in soil

Following the completion of the soil column experiment after 28 days, the assessment of bacterial composition in the soil subjected to BC treatment and strain L1 was conducted using high-throughput DNA sequencing. The investigation focused on the assessment of species variety in each soil sample by the utilization of alpha diversity analysis. The Ace, Chao 1, Shannon, and Simpson x indices were computed for each individual sample. Additionally, the findings indicated that the Coverage values for both groups approached 1.0, suggesting that the data possess scientific validity and provide meaningful insights (Chen and Achal, 2019). Shannon, Feature, Ace, Chao 1 and Simpson indices are commonly associated with the Alpha diversity of microbial communities, and higher values of these indicators represent higher diversity of the community (Qiu et al., 2023). These indices were higher in the added BC and L1 groups than in the CK group (Table 1). This finding implies that BL1 facilitated a rise in both the variety and population size of soil bacteria. Qi et al., (2021) similarly found an increase in microbial diversity in soils corrected with *Bacillus* biochar material. Furthermore, the implementation of biochar immobilization microbial technology has the potential to mitigate the detrimental impacts of heavy metal-contaminated soils. This is achieved by the stimulation of microbiota proliferation, hence facilitating the bioremediation process of heavy metals (Lebrun et al., 2021; Qi et al., 2022).

Fig. 7a shows the microbial composition at the phylum level, with the three dominant groups being Proteobacteria, Firmicutes, and Actinobacteriota. Most of the *Proteobact*eria phylum belonged to denitrifying bacteria (Si et al., 2018). In the CK group and BL1 group, the phylum *Proteobacteria* accounted for: 69.99% and 72.98%, respectively. The relative abundance of Actinobacteriota exhibited a significant rise from

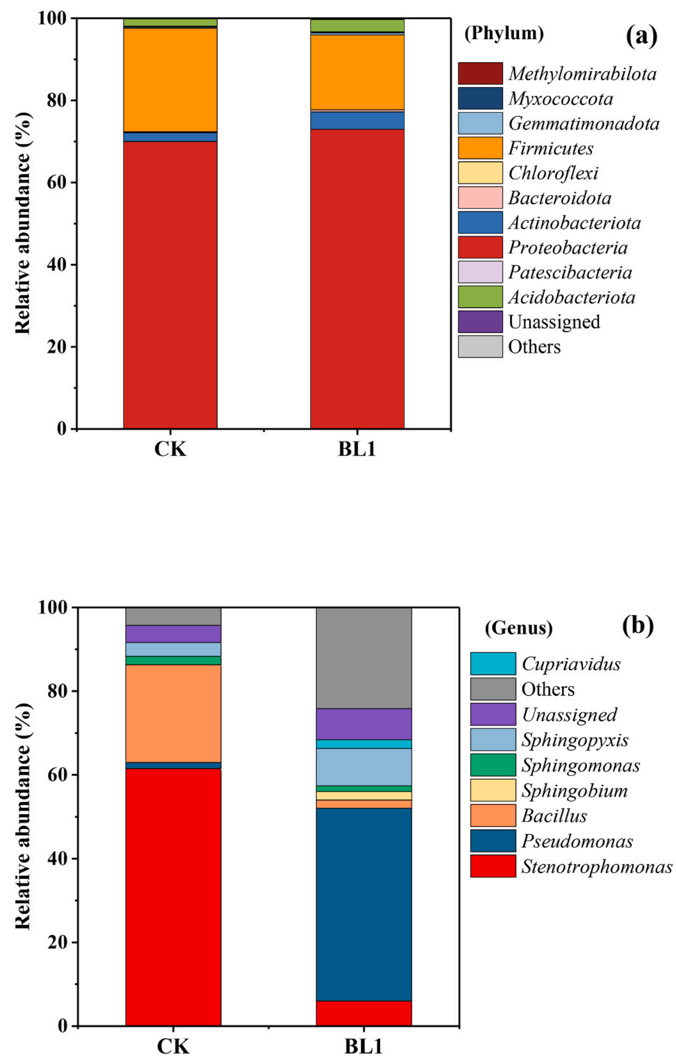

Fig. 7. Relative abundance of bacterial communities in soils (a) phylum, (b) genus (Species with less than 1% abundance were combined to others).

Table 1
Alpha diversity index statistics.

Sample	Feature	ACE	Chao	Simpson	Shannon	PD-whole-tree	Coverage
CK	291	301.3321	302.6667	0.8306	3.5835	21.1868	0.9996
BL1	404	423.0145	419.5294	0.8271	4.203	25.2816	0.9994

2.16% in the CK to 4.22% in the BL1 group, indicating a notable difference between the two groups. The majority of Actinobacteriota exhibited the capacity for nitrification. The findings of this study indicate that the application of BL1 resulted in an augmentation of both nitrifying and denitrifying bacterial populations, thus promoting the removal of ammonia and nitrate. Fig. 7b shows that *Pseudomonas* was obviously higher in the BL1 group than in the CK group, while strain L1 belonged to *Pseudomonas*, indicating that strain L1 successfully colonized the soil and performed a vital role in soil heavy metal immobilization and NH_4^+-N transformation. In conclusion, the BL1 addition increased the diversity of the soil microbial community and enriched functional bacteria, thus promoting the stabilization of heavy metals and the removal of ammonia and nitrate.

4. Conclusion

The synergistic effect of biochar and strain L1 (BL1) effectively reduced the risk of groundwater contamination by electroplating wastewater. The contribution of biochar to the stabilization of four heavy metals was higher than that of strain L1, while the removal of ammonia nitrogen and nitrate nitrogen by strain L1 was higher than that of biochar. The removal mechanism was divided into four parts: (1) Increase the pH, CEC, TOC and enzyme activity of the soil, and reduce the Eh value, which makes the soil function self-recovery ability faster. (2) Increasing the total pore volume and average pore size of the soil, which enhances the water retention and permeability of the soil. (3) Increase the diversity and abundance of soil bacteria. Special functional flora with nitrification, denitrification function and heavy metal removal ability. (4) The surface functional groups of BL1 such as SiO_2, -OH, C=O, C-H, Si-O, etc. have an effect on the heavy metal.

5. Perspectives and shortcomings

In this study, It was found that biochar synergized with strain L1 can effectively reduce the risk of electroplating wastewater contamination of soil and groundwater, but the specific mechanism of biochar to promote the reaction and the gene expression mechanism to promote nitrification and metabolic accumulation of strain L1 are not clear. Meanwhile, it was found that the addition of biochar and strain L1 could effectively promote the immobilization of heavy metals, but the co-transport between multiple heavy metals was not clear, which needs to be further investigated in the future.

CRediT authorship contribution statement

Binbin Ran: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Qiang An:** Project administration, Methodology, Funding acquisition, Conceptualization. **Shuman Deng:** Writing – review & editing, Visualization, Validation, Supervision, Investigation. **Jiali Song:** Visualization, Validation, Investigation. **Zhiruo Huang:** Visualization, Validation, Formal analysis. **Bin Zhao:** Resources, Project administration, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

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.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2024.105926.

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