

# Biotechnology of phosphate solubilizing microorganisms for metabolites regulation: Present and future

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# Biotechnology of phosphate solubilizing microorganisms for metabolites regulation: Present and future

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# Editorial: Biotechnology of phosphate solubilizing microorganisms for metabolites regulation: present and future

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## KEYWORDS

biotechnology, heavy metal remediation, phosphate solubilizing microorganisms, phosphate dissolution, metabolism

## Editorial on the Research Topic

Biotechnology of phosphate solubilizing microorganisms for metabolites regulation: present and future

Phosphate solubilizing microorganisms (PSM) can transform insoluble phosphate into plant-absorbable forms in soil by producing metabolites, including phosphate solubilizing fungi (PSF), phosphate solubilizing bacteria (PSB), and phosphate solubilizing actinomycetes. However, promoting the capacity of metabolism *via* biotechnology approaches is a current challenge for PSM. This paper overview the primary functional metabolites of PSM and the related biotechnology approaches to improve their application in agriculture and environmental remediation.

Sun *et al.* found manure is a potential substitute for mineral phosphorus (P) fertilizers, which can enhance the bacterial organic P mineralization and increase soil available P content. Jiang *et al.* overviewed the electrochemical technology for enhanced P solubilization by PSM. The application of this technology not only provides a suitable environment for the metabolism of PSM but also promotes the growth of PSM. Tian *et al.* overviewed lead (Pb) remediation by phosphate solubilizing fungi (PSF) and apatite. The primary pathway for the stable Pb minerals formation needs to enhance the production of oxalic acid by PSF. Pan *et al.* studied the physiological response of PSF under Pb and cadmium (Cd) stress *via* the technology of scanning electron microscopy and transmission electron microscopy. Compared with Cd, PSF has a higher Pb accumulation in both the extracellular and intracellular. Zhang *et al.* isolated a phosphate solubilizing bacteria (PSB) strain from saline-alkali soil. This PSB has high phosphate solubilizing capacity and can remove 90.8% Pb cations under a 500 mg/L Pb level. Chen *et al.* summarized that biochar can enhance PSM survival in a high heavy metal concentration. The biotechnology of biochar and PSM combination is a promising approach in heavy metal remediation. Wang *et al.* overviewed the technology of biofertilizers, soil conditioners, remediation agents, etc., which could be potential pathways to solve the PSM commercialization and application. Wu and Zhao

summarized a new technology of machine learning in assessing the efficiency of PSM in phosphate dissolution and heavy metal remediation. In addition, [Chen et al.](#) found that the PSM can also apply to mine phytoremediation as a driving force. PSM can function in the establishment and development of plants and ecosystems, which has great potential to reduce heavy metal toxicity and promote the growth of plants.

This Research Topic generally reviews the metabolic characteristics of PSM and the biotechnology approaches to enhance metabolic production. These biotechnologies have promoted the application of PSM in agriculture and heavy metal remediation. Significantly, these potential biotechnologies could also extend the application of PSM in food fermentation and biocontrol of plant pathogens.

## Author contributions

DT: Formal analysis, writing–review and editing. HC: Formal analysis, writing–review and editing. GM: Formal analysis,

writing–review and editing. YF: Formal analysis, writing–review and editing.

## Conflict of interest

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# Substitution of manure for mineral P fertilizers increases P availability by enhancing microbial potential for organic P mineralization in greenhouse soil

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The shortage of phosphorus (P) as a resource represents a major challenge for the sustainable development of agriculture. Manure has a high P content and is a potential substitute for mineral P fertilizers. However, little is known about the effects on soil P availability and soil microbial P transformation of substituting manure for mineral P fertilizers. In this study, variations in soil P availability and bacterial P mobilization were evaluated under treatment with manure as compared to mineral P fertilizers. In the greenhouse fruit and vegetable production system that provided the setting for the study, substitution of manure for mineral P (PoR treatment) resulted in a similar level of soil total P and a similar fruit and vegetable yield as compared to traditional fertilization, but a significantly increased level of soil available P. In addition, PoR treatment enhanced bacterial organic P mineralization potential and decreased inorganic P dissolution potential. These results demonstrate that manure application increases the availability of soil P primarily by enhancing soil microbial P mineralization, indicating the potential feasibility of applying manure instead of mineral P fertilizers in greenhouse farming.

## KEYWORDS

P fertilizer, manure, bacterial community, organic P mineralization, greenhouse soil, phosphate solubilizing microbes

## Introduction

Phosphorus (P) is a fundamentally important element in agricultural production. Around 80% of phosphate rock use occurs in agricultural systems in fertilizers, and total global P consumption is expected to continue increasing (Van Vuuren et al., 2010). Thus, a shortage of P fertilizers may greatly restrict the development of agriculture in the near future, as phosphate rock is a non-renewable resource. Effective management of phosphorus resources and reduction of the dependence on mineral P fertilizers is of great importance for the sustainable development of agriculture, as well as for society as a whole (Withers et al., 2014; Fu et al., 2021; Rong et al., 2021; Xu et al., 2021; Zheng et al., 2022). Many approaches are considered to have potential to reduce mineral P input, one of which is recycling of the P content of animal excreta (Van Vuuren et al., 2010; Withers et al., 2014).

Global animal stocks are extremely large. Livestock production greatly impacts global nutrient cycles, and improved integration of animal manure into crop production can effectively reduce nutrient flows (Bouwman et al., 2013). Manure has a high P content and contains a variety of forms of P, including both organic P (Po) and inorganic P (Pi), and nearly 70% of the total P in manure is labile (Shen et al., 2011); thus, manure represents a good substitute for mineral P fertilizers. In addition, manure also contains various organic substances that could reduce P adsorption to soil particles, thereby increasing soil P availability. Finally, manure can also alter soil P availability by modifying the biochemical conditions of the soil, such as its pH (Shen et al., 2011).

Soil microbes play a vital role in regulating soil P dynamics and bioavailability (Chen H. M. et al., 2019; Lai et al., 2022), especially in organic P mineralization (Weihrauch and Opp, 2018), as well as in influencing P uptake by plants (Zhang et al., 2021). Not only is it possible that manure application may alter soil P pools, but it may also shape the constitution of the soil microbial community (Sun et al., 2020). Our previous study has revealed that the high levels of easily degradable carbon found in manure significantly enhance microbial Po mineralization through enrichment of Po-mineralizing microbial taxa (Chen et al., 2020), suggesting that support of soil microbial function in phosphorus cycling is a key mechanism in enabling manure application to modify soil P availability.

Greenhouse farming is an important aspect of agricultural production. Through recent advancements in greenhouse technology, greenhouse farming has become increasingly productive and is considered to be a promising approach to ensuring that demand for food is satisfied in future (Aznar-Sánchez et al., 2020). However, as a resource-intensive production system, greenhouse farming requires heavy fertilizer input; this makes it highly challenging in terms of mineral resources, especially non-renewable ones, such as phosphate rocks. As mentioned above, manure has a high P content, and it

has great potential to function as an alternative to mineral P fertilizers. However, the composition of P in manure in terms of form differs greatly from that of P in mineral P fertilizers. Therefore, there is a need to evaluate the impact of manure application on soil P availability and soil microbial P transformation; doing so can provide valuable information on the possibility of replacing mineral P fertilizers with manure in greenhouse production.

## Methods

### Experimental design

A field experiment was conducted to investigate the impact of replacement of mineral P with manure on soil P availability and microbial phosphorus transformation. Two treatments were implemented as part of the experiment: one (the control) consisting of conventional fertilization with mineral nitrogen (N, 90 kg N·ha<sup>-1</sup>·year<sup>-1</sup>), P (90 kg P<sub>2</sub>O<sub>5</sub>·ha<sup>-1</sup>·year<sup>-1</sup>), and potassium (K, 90 kg K<sub>2</sub>O·ha<sup>-1</sup>·year<sup>-1</sup>) fertilizers and cattle manure (84 t ha<sup>-1</sup>·year<sup>-1</sup>), and the other (PoR treatment) consisting of Po fertilization with manure (134.4 t ha<sup>-1</sup>·year<sup>-1</sup>) in place of the mineral P fertilizer. The total input of P under the PoR treatment was same as under the control. Implementation of the experiment began in 2017 in a solar greenhouse located in Raoyang County, Hebei Province, China (38°15'N, 115°44'E). The soil was silt loam. Each treatment was replicated across three plots, and the plant system was a tomato and muskmelon rotation.

### Soil sampling and measurement of soil properties

On 10 June 2020, surface soil (0–20 cm) was collected from each plot in accordance with procedures described in a previous study (Sun et al., 2015). The soil samples were sieved through a 2 mm sifter to mix thoroughly and remove impurities, such as plant roots and stones.

Soil pH, total carbon (TC), total nitrogen (TN), soil organic matter (SOM), ammonia (NH<sub>4</sub><sup>+</sup>-N), nitrate (NO<sub>3</sub><sup>-</sup>-N), available P (AP), and total P (TP) were measured as described in a previous study (Sun et al., 2022).

### DNA extraction and high-throughput sequencing

Soil total DNA was extracted from 0.5 g fresh soil using a FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, United States).

The soil bacterial community was characterized using high-throughput sequencing as described in a previous study (Sun et al., 2018). In brief, primer sets 515F/806 R targeting the

V4 region of the bacterial 16S rRNA gene were used for polymerase chain reaction (PCR) analysis. PCRs were performed in a 50- $\mu$ L mixture containing 25  $\mu$ L PCR premix (TaKaRa Ex TaqR), 1  $\mu$ L forward primer (10  $\mu$ M), 1  $\mu$ L reverse primer (10  $\mu$ M), 1  $\mu$ L DNA template (20 ng), and 22  $\mu$ L PCR-grade water under the following conditions: initial denaturation at 94°C for 10 min; 30 cycles in a series of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. After quality checks and purification, the PCR products were sequenced using an Illumina HiSeq 2,500 system.

## Determination of microbial P transformation profiles

Soil bacterial functional profiles with respect to P cycling were predicted using PICRUST2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas et al., 2020). Genes involved in P transformation were extracted to reveal the potential capacity for microbial P transformation under each of the treatments applied.

Soil alkaline phosphatase activity was measured using a Soil Alkaline Phosphatase (S-AKP/ALP) Activity Assay Kit (Beijing Solarbio Science & Technology Co., Ltd. China), and expressed in the form of nM *p*-PNP (*p*-nitrophenyl phosphate)  $\text{h}^{-1}\text{g}^{-1}$  dry soil. The abundance of the *phoD* gene, which encodes for alkaline phosphatase, was determined using real-time fluorescent quantitative PCR (qPCR), which was conducted using primer sets *phoD*-F733/*phoD*-R1083 (5'-TGGGAYGATCAYGARGT-3'/5'-CTGSGCSAKSACRTTCCA-3') under the following amplification conditions: 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 55°C for 1 min. After amplification, melting curve analysis and gel electrophoresis were performed to measure the specificity of the reaction (Chen X. et al., 2019).

The microbial dissolving potential for calcium phosphate was determined for each treatment using an incubation method, in accordance with procedures described in our previous study (Sun et al., 2022). Briefly, 1 g soil was added to 90 ml sterilized water and mixed thoroughly. Next, 1 ml of the mixture was added to 50 ml sterile PVK liquid medium and incubated at 28°C (180 rpm) for 5 days. A blank control with 1 ml sterilized water was included. Dissolved P in the culture was measured at 0, 12, 24, 48, 72, 96, and 120 h.

## Bioinformatic analysis of the high-throughput sequencing data

VSEARCH (version 2.21.1) (Rognes et al., 2016) was used for bioinformatic analysis of the high-throughput sequencing data

TABLE 1 Soil properties and crop yield following different treatments.

Treatment	pH	TC (%)	TN (%)	SOM (%)	$\text{NH}_4^+-\text{N}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	$\text{NO}_3^--\text{N}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	AP ( $\text{mg}\cdot\text{kg}^{-1}$ )	TP ( $\text{g}\cdot\text{kg}^{-1}$ )	Tomato yield ( $\text{t}\cdot\text{ha}^{-1}$ )	Muskmelon yield ( $\text{t}\cdot\text{ha}^{-1}$ )
Control	7.82 (0.15)a	2.50 (0.06)b	0.18 (0.02)a	1.54 (0.08)a	1.41 (0.56)a	17.39 (0.19)a	179.79 (16.57)b	2.17 (0.08)a	132.80 (1.20)a	85.70 (6.90)a
PoR	7.62 (0.04)b	2.51 (0.08)a	0.20 (0.01)a	1.57 (0.04)a	1.30 (0.45)a	17.74 (1.15)a	243.63 (13.02)a	2.22 (0.10)a	126.50 (8.00)a	88.30 (1.40)a

Data was averages with standard deviations in (brackets),  $n = 3$ .

TC, total carbon; TN, total nitrogen; SOM, soil organic matter;  $\text{NH}_4^+-\text{N}$ , ammonia nitrogen;  $\text{NO}_3^--\text{N}$ , nitrate nitrogen; AP, available P; TP, total P.

Different lowercase letters beside data points in the same column indicate a significant difference between treatments according to the Kruskal–Wallis rank sum test ( $p < 0.05$ ).

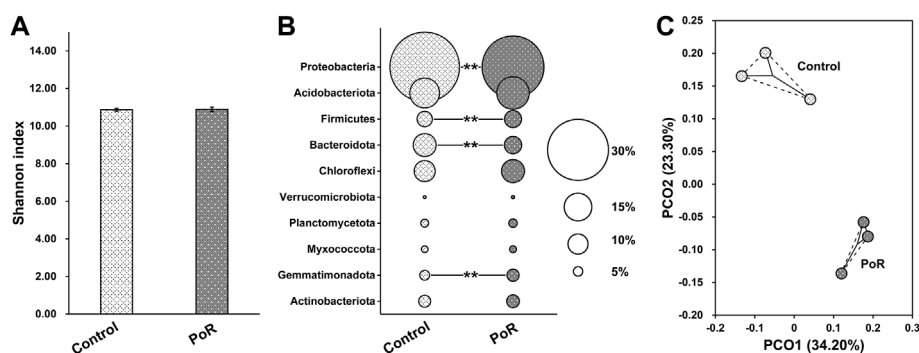


FIGURE 1

Shannon diversity (A) and taxonomic composition (B) of the bacterial community under different treatments; PCoA plot (C) showing variation in the bacterial community under different treatments. \*\* indicates a significant difference between PoR and control (Kruskal–Wallis rank sum test,  $p < 0.01$ ).

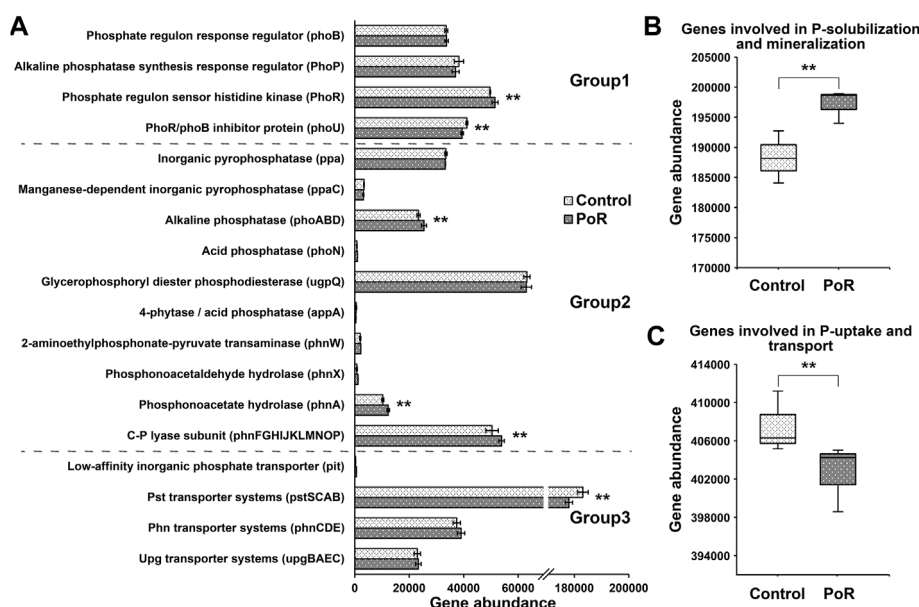


FIGURE 2

The abundance of genes involved in P transformation in soils having undergone different treatments (A). Group 1: genes coding for regulation of the P-starvation response; Group 2: genes coding for inorganic P-solubilization and organic P-mineralization; Group 3: genes coding for P-uptake and transport. Total abundance of genes involved in P-solubilization and mineralization (B) and P-uptake and transport (C). \*\* indicates a significant difference between PoR and control (Kruskal–Wallis rank sum test,  $p < 0.01$ ).

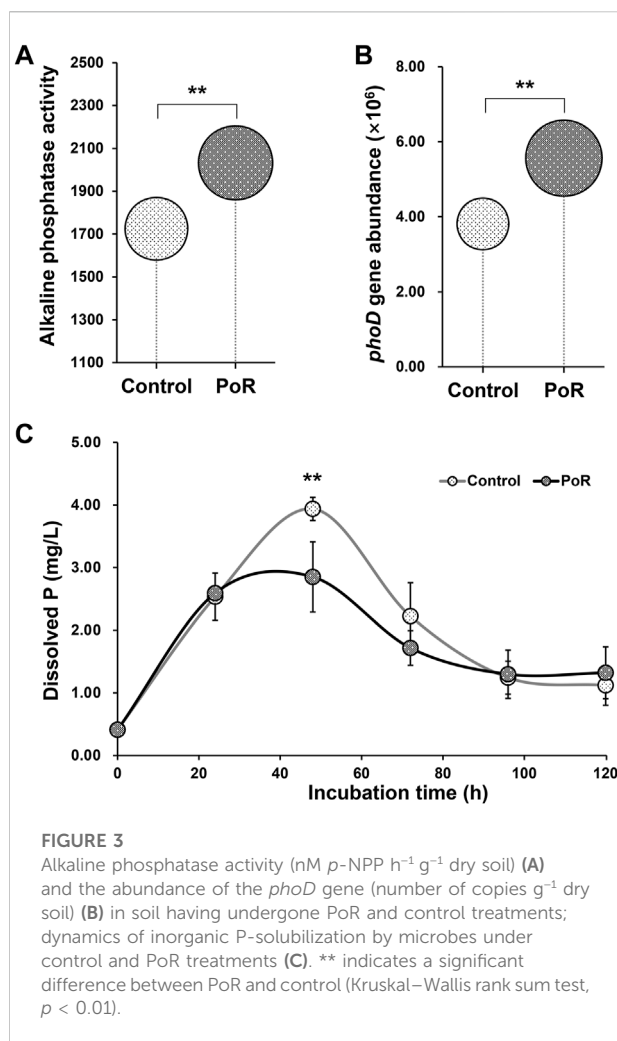
following the protocols described in previous work (Liang et al., 2020; Sun et al., 2022). The paired-end reads were first merged, and low-quality and chimeric reads were then removed. Subsequently, the clean reads were further denoised and zOTUs (zero-radius operational taxonomic units) were generated using the UNOISE algorithm (version 3). The taxonomic details of each zOTU were determined using the RDP Classifier tool based on the SILVA rRNA database (version 138). After removal of zOTUs not annotated as

bacteria, the zOTU tables were subsampled to extract 48,000 reads per sample for statistical analysis.

## Statistical analysis

Statistical analysis was conducted and figures generated using R (version 4.0.2) as described in our previous study (Sun et al., 2022). The Kruskal–Wallis rank sum test was used





**FIGURE 3**  
Alkaline phosphatase activity (nM p-NPP h<sup>-1</sup> g<sup>-1</sup> dry soil) (A) and the abundance of the *phoD* gene (number of copies g<sup>-1</sup> dry soil) (B) in soil having undergone PoR and control treatments; dynamics of inorganic P-solubilization by microbes under control and PoR treatments (C). \*\* indicates a significant difference between PoR and control (Kruskal–Wallis rank sum test,  $p < 0.01$ ).

to test for significant differences between treatments for each variable. Principal coordinate analysis (PCoA) was performed based on Bray–Curtis distance using the “vegan” library.

## Results

### Effects of PoR treatment on soil properties and crop yield

PoR treatment significantly lowered soil pH, but had no significant impact on soil contents in terms of total carbon (TC), total nitrogen (TN), soil organic matter (SOM), ammonia nitrogen (NH<sub>4</sub><sup>+</sup>–N), or nitrate nitrogen (NO<sub>3</sub><sup>-</sup>–N) (Table 1). Levels of soil total P (TP) were similar under the two treatments (Table 1), while PoR treatment significantly increased the level of soil available P (AP), which was 35.51% higher in samples having undergone PoR treatment compared to the control (Table 1).

Tomato and muskmelon yields did not differ significantly between the control and PoR plots (Table 1), indicating that PoR treatment has a similar productivity to that of conventional fertilization.

### Variation in microbial communities under different treatments

The Shannon index was calculated as a measure of variation in bacterial diversity. The results showed that there was no significant difference in bacterial α-diversity between samples having undergone control and PoR treatment (Figure 1A), indicating that PoR treatment had little impact on bacterial α-diversity. However, soil bacterial community composition was impacted by PoR treatment. Proteobacteria was the most dominant phylum in the control soil, but it featured in a significantly lower proportion in the PoR soil. Bacteroidota was also diluted under PoR treatment, while Firmicutes and Gemmatimonadota were enriched (Figure 1B). This variation in bacterial community structure is further illustrated by the corresponding 2D PCoA plot (Figure 1C), which shows the clear separation in bacterial community between control and PoR samples. ANOSIM (Analysis of Similarities) also confirmed that there was a significant difference between control and PoR samples in terms of bacterial community ( $p < 0.05$ ).

### Effects of PoR treatment on microbial P-mobilization profiles

Through functional prediction (using PICRUSt2), 38 genes involved in P transformation were detected (Park et al., 2022). PoR treatment significantly decreased the abundance of the *phoU* and *pst* genes, while it significantly increased the abundance of the *phoR* and *phnA* genes and genes coding for alkaline phosphatase and C-P lyase (Figure 2A). Considering genes in terms of functional groups, those involved in Pi solubilization and Po mineralization were significantly increased under PoR treatment (Figure 2B). In contrast, the abundance of genes involved in P uptake and transport was significantly decreased under PoR treatment (Figure 2C).

Soil alkaline phosphatase activity was significantly higher under PoR treatment than under control treatment (Figure 3A). A similar pattern was observed in the abundance of the *phoD* gene, which was significantly increased by PoR treatment (Figure 3B).

Taking calcium phosphate as the sole P resource, the incubation experiment indicated that, over the entire incubation period, the levels of dissolved P in the culture liquid followed an approximate bell curve, peaking at 48 h



(Figure 3C). At the 48 h time point, the dissolved P content of the culture with PoR-sourced microbes was significantly lower than that of the culture with control-sourced microbes, showing that the potential P solubilization of the microbial community was significantly lower under PoR treatment than under the control treatment.

## Discussion

As a typical form of intensive production system, greenhouse agriculture depends on heavy resource input; thus, a shortage of resources would represent a major challenge to its productivity. In the present study, we have demonstrated that PoR treatment significantly increases soil P availability without exerting an adverse effect on other nutrient levels or on crop yield, indicating that the substitution of manure for mineral P fertilizers is feasible in greenhouse production.

The increase in P availability in soil that has undergone PoR treatment may be partly associated with the significant decrease in soil pH (Table 1). Manure contains a large amount of organic matter. However, in the present study, soil TC and SOM did not significantly differ between the PoR and control treatments, indicating strong degradation of the organic matter contained in the manure. Various organic acids would be released during the degradation of this organic matter, thereby decreasing soil pH and consequently enhancing the dilution of inorganic phosphorus compound. An existing study has also found that lower pH enhances the dilution of Ca phosphates in alkaline soil (Devau et al., 2009). In addition to impacting soil pH, organic acids also act as “chelates” for phosphates (Kalayu, 2019), as they contain very large amounts of negative charges, carboxyl, and hydroxyl groups, which decrease the precipitation of Al, Fe, and Ca phosphates by competing with Pi for adsorption sites (Shen et al., 2011).

As the primary driver of P transformation in soil, the soil bacterial community underwent a major shift as a result of PoR treatment. The results of high-throughput sequencing showed that PoR treatment reshaped the soil bacterial community into one with a high potential for P mobilization. This was further confirmed by the greater abundance of *phoD* genes in samples that had undergone PoR treatment; this is the most common alkaline phosphatase gene and is widely used as a molecular marker for the detection of alkaline phosphatase-producing bacteria in soil (Chen H. M. et al., 2019). This finding was coincident with the results of alkaline phosphatase activity measurement, which showed that alkaline phosphatase activity was 17.83% higher under PoR treatment than under the control treatment (Figure 3B). Alkaline phosphatase plays a crucial role in releasing Pi from Po in

alkaline soil. Studies have found that bacteria are the primary producers of alkaline phosphatase in soil (Chen X. et al., 2019). The results of this study showed that application of manure enhances the mineralization of Po. However, PoR treatment also decreases the bacterial Pi solubilization potential (Figure 3C), indicating that the high levels of AP in PoR soil are largely the result of Po mineralization, while bacterial Pi solubilization may make little contribution to the increase in P bioavailability.

The impact of manure application on bacterial community structure and P mobilization may be largely associated with the resultant changes in the soil components functioning as resources for the microbial community. The type, quantity, and availability of resources are key regulators of the soil microbial community, as microbial taxa have different preferences in terms of resources (Estrela et al., 2021). A large amount of Po input may enhance the competitiveness of the bacterial taxa involved in Po mineralization, resulting in a bacterial community that harbors a high proportion of Po-mineralizing taxa. As a result, the secretion of alkaline phosphatase is enhanced. In addition, application of manure also leads to variation in the soil bacterial community by introducing a large amount of organic carbon, which is also an important driver of microbial community interactions (Bergk Pinto et al., 2019).

In summary, this work has revealed that substitution of manure for mineral P fertilizer significantly increases P availability in greenhouse soil, which can largely be attributed to the enrichment of Po-mineralizing microbes. The results also indicate that manure is a feasible substitute for mineral P fertilizers in greenhouse farming. However, the high AP content resulting from manure application may increase the risk of P leaching; reducing the amount of manure applied may be a possible way to mitigate this problem, but identification of the appropriate amount of manure to use in substitution is an important issue requiring further study.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

RS, JN, FW, and XY designed the experiment. BL, XW, WL, and WZ performed laboratory measurements. RS and CZ performed the data analysis. RS, JN, FW, CZ, and XY wrote the article. All authors read and approved the final version of the manuscript.

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# REDOX physical-chemical method boosted phospholytic bacteria technology for enhanced phosphorus solubilization

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## KEYWORDS

redox process, phospholytic bacteria, phosphorus solubilization, electrochemical technology, resource recycle

## Introduction

Phosphate-solubilizing bacteria are the bacteria that secrete organic acids and phosphatases to convert the insoluble phosphorus fixed in soil into soluble phosphorus (such as monovalent  $\text{H}_2\text{PO}_4^-$  and divalent  $\text{HPO}_4^{2-}$ ) (Rodriguez et al., 2006). Among the species, the main bacteria with phosphorus solubilizing ability were *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Erwinia*, *Aerobacter* and *Flavobacterium* (Ma et al., 2022). Representative phosphate-solubilizing bacteria are *Pseudomonas*, *Rhizobium* and *Bacillus*, which have the best phosphate-solubilizing capability (Maheshwari et al., 2013).

The mechanism of these bacteria can be explained based on the form of phosphorus, namely, mineral phosphorus and organic phosphorus. From the perspective of mineral phosphorus dissolution, the most accepted theory is that phosphate-solubilizing bacteria synthesize organic acids that acidify the cells of microorganisms and the surrounding environment, releasing phosphorus from mineral phosphorus through proton ( $\text{H}^+$ ) exchange of  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ , etc., (Goldstein, 1994; Kim et al., 2005). Research has found gluconic acid to be the most effective organic acid for dissolving mineral phosphorus. It is generated from the action of phosphate-solubilizing bacteria such as *Pseudomonas sp.*, *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia*. Another representative organic acid is 2-ketogluconic acid, which is produced from the action of phosphate-solubilizing bacteria such as *Rhizobium leguminosarum*, *Rhizobium meliloti* and *Bacillus firmus*. In addition, there are also reports on other organic acids including glycolic acid, oxalic acid, malonic acid and succinic acid.

From the perspective of organic phosphorus dissolution, it is in its nature the mineralization of organic phosphorus. Saprophytes mineralize organophosphates when decomposing organic matter in soil, which releases orthophosphate and thus dissolves phosphorus (Illmer and Schinner, 1992). The mineralization of organic phosphorus is completed by the action of phosphatase, the main process of which is the hydrolysis of phosphate or phosphoric anhydride bonds. The function of phosphorus solubilizing bacteria is related to the type of phosphorus

source itself. It has been confirmed that tricalcium phosphate and hydroxyapatite are more easily degraded than phosphates in inorganic phosphorus, while phospholipids and sugar phosphates are relatively easily decomposed and polyphosphates are more slowly decomposed (Adnane et al., 2021). In addition, the mineralization of organic phosphorus by bacteria is significantly influenced by environmental factors; for organic phosphorus, medium alkalinity is more favorable for its mineralization, unlike for inorganic phosphorus.

Although mechanisms for phospholytic bacteria technology has been discovered in a certain degree, several challenges are still remained in its practical employment. First, it usually causes a considerable delay for the phosphorous conversion at the early stages of phosphorus solubilization, when phosphorus-solubilizing bacteria have not yet formed large colonies or become dominant, such as in the initial stage of fertilization. Second, the accumulation of aromatic hydrocarbons and heterocyclic compounds (e.g., pesticide or herbicide) in the soil, which could not be utilized as the microbial carbon source, greatly inhibited the functioning of phospholytic bacteria. Moreover, the low temperature or the turbulence of environmental pH conditions also decreases the activity of bacteria, thus slowing down the phosphorus conversion rate. All these issues have great impacts on the practical applications of the phospholytic bacteria technology. Herein, seeking a facile but efficient technology to make a synergistic interaction with bio-tech for boosting of phosphorus solubilization efficiency is challenging but of great significance.

## Electrochemical enhancement of biological phosphorus solubilization

Electrochemical technology is a kind of multi-functional physical-chemical process, containing electro-adsorption, electro-migration, anodic oxidation and cathodic reduction reactions (known as REDOX process), which plays an important role in the water and soil remediation. The high content of organic matter and a variety of electrolytes (such as the mineral salt and inorganic fertilizer) in soil and its wetness enabled electrochemical process to be realized in diverse ways. Electrochemical degradation of organic matter can be achieved directly by electron transfer between the electrode and the organic matter, or by the following reactions: at the anode with water molecules as the medium:  $\text{H}_2\text{O} \rightarrow \cdot\text{OH} + \text{H}^+ + \text{e}^-$ , generating hydroxyl radical to oxidize the organic matter while lower the pH, and at the cathode:  $2\text{H}^+ + \text{e}^- = \text{H}_2$ , consuming  $\text{H}^+$  to raise the pH (Wang et al., 2022). In general, electrochemistry can not only achieve the transformation of organic matter (such as conversing into low molecular acid or directly degrading the organophosphorus), but also alter ambient pH through the adjusting the above reactions on the anode and cathode, so as to adjust the soil pH according to the demand (Teklit et al., 2023). On the other hand, electrochemistry can stimulate the phosphate-solubilizing bacteria and enhance the activity of the bacteria, thus enhancing the metabolism of phospholytic bacteria (Huang et al., 2021; Liu et al., 2022). Combined with the phosphate-solubilizing mechanism of phosphate-solubilizing bacteria, both oxidation and reduction will to a certain extent enhance biological phosphorus solubilization. The illustration of the electrochemical reduction-oxidation process on the phosphorus solubilization performance conducted by phospholytic bacteria technology is referred to Figure 1.

## Phosphate solubilization of phosphate-solubilizing bacteria enhanced by anodic oxidation

Anodic oxidation is closely related to the properties of the electrode. Different oxygen evolution potential (OEP) of the anode will lead to different processes and endpoints of organic degradation. When OEP is less than 1.8V, the organic matter is degraded by the high-valence oxides generated from interaction between hydroxyl radical and anode rather than by hydroxyl radical, for which the degradation endpoint will be low molecule organic acids (such as formic acid and acetic acid) and other intermediates. Noble metal electrodes like ruthenium or ruthenium-iridium exhibit this property (Eleftheria et al., 2019). Therefore, introducing such electrodes to the phosphate-solubilizing bacteria system will enhance the content of organic acids and  $\text{H}^+$  in the system and thus improving inorganic phosphorus dissolution. In particular, a significant effect of anodic oxidation is that when soil contains some refractory organic matter, such as residual organic pollutants from pesticide spraying or fertilization, anodic oxidation can degrade such organic matter into low molecular acids, which can not only reduce soil toxicity and its harm to the flora (such as phosphorous solubilizing bacteria), but also improve the concentration of organic acid in the system (Brillas, 2021). On the other hand, extensive previous studies have confirmed that anodic oxidation could directly degrade organic phosphorus and convert it into soluble phosphate, thus enhancing the dissolution of organic phosphorus (Wang et al., 2022).

## Phosphate solubilization of phosphate-solubilizing bacteria enhanced by cathodic reduction

The mechanism of phosphate solubilization of phosphate-solubilizing bacteria enhanced by cathodic reduction is relatively simple—it is mainly the cathodic reduction that consumes  $\text{H}^+$  and transfer the pH of the system to neutral or alkaline (Cheng et al., 2021). As mentioned above, phosphate-solubilizing bacteria dissolve organic phosphorus through the hydrolysis of phosphate or phosphoric anhydride bonds by phosphatase, for which medium alkalinity is more favorable—fits right in with the cathode. Another important effect is that in an atmosphere with enough air, the cathode will react as follows:  $\text{O}_2 + 2\text{H}^+ + 2\text{e}^- = \text{H}_2\text{O}_2$ . The generated strong oxidizing substance, hydrogen peroxide (Brillas, 2022), promotes the conversion of organophosphorus to phosphoric acid roots, thus enhancing the dissolution of organophosphorus. In addition, it has been reported that cathode can not only reduce organic matter itself, but also enhance the reducing ability of bacteria. The electron giving ability of cathode can strengthen the electron transfer between cathode, microbiota and organic matter, and improve the reducing ability of the system (Yang et al., 2020). When there are nitro compounds in the system, cathodic reduction can achieve the enhanced removal of the substance and ensure the biological activity of phosphorus solubilizing bacteria (Hu et al., 2022).

## Future perspective

First of all, the high content of organic matter and a variety of electrolytes in soil and its wetness facilitate the introduction of



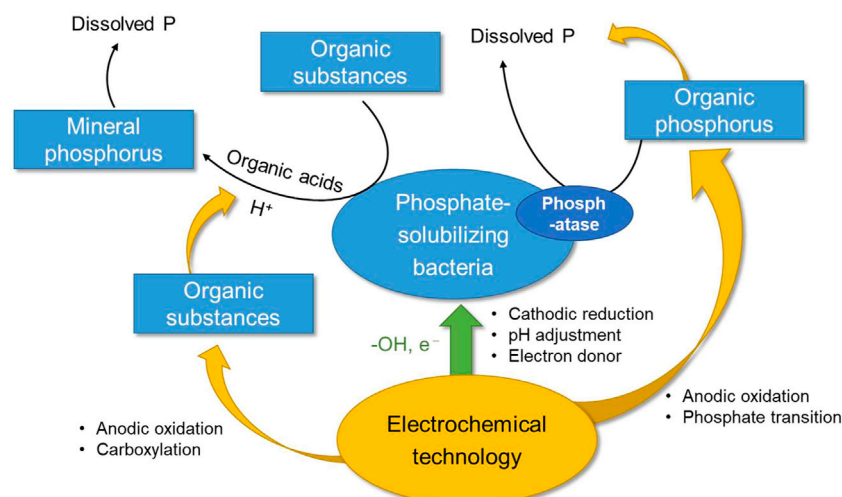


FIGURE 1

Schematic illustration of the electrochemical reduction-oxidation process on the phosphorus solubilization performance conducted by phospholytic bacteria technology.

electrochemical technology. Such characteristics of electrochemical technology as the oxidized organic matter staying in an organic acid state, producing  $H^+$  and  $H^+$  being consumed in reduction agree well with the phosphorus solubilization mechanism of phosphorus-solubilizing bacteria (Nancharai et al., 2016). Therefore, the introduction of electrochemical technology will more significantly improve the efficiency of phosphorus solubilization than a system with phosphorus-solubilizing bacteria alone. As stated above, anodic oxidation can enhance the dissolution of both inorganic phosphorus and organophosphorus, while cathodic reduction mainly enhances the dissolution of organophosphorus. In particular, the strengthening mechanism of anodic oxidation on phosphorus dissolution may play a positive role in promoting phosphorus circulation and even plant growth (Yang et al., 2021). Because the largest reserves of phosphorus in nature exist in the form of inorganic phosphorus, which is chelated into insoluble complexes such as  $Fe^{3+}$ ,  $Ca^{2+}$  and  $Al^{3+}$  in the soil. The content of soluble phosphorus in nature is very low, which greatly hinders the absorption and acquisition of plants (Wei et al., 2018). At the same time, electrochemical technology can not only create an environment suitable for the metabolism of phosphorus solubilizing bacteria, but also remove toxic organic matter in the surrounding environment which is not conducive to the growth of phosphorus solubilizing bacteria. On the other hand, the functioning of phosphorus solubilizing bacteria could be promoted under assistance of the REDOX process of the electrodes (Ren et al., 2022). Electrochemical enhanced phosphorus solubilization can be used in the early stages of phosphorus solubilization to ensure phosphorus conversion when phosphorus-solubilizing bacteria have not yet formed large colonies or become dominant; or after fertilization to enhance phosphorus solubilization when organic/inorganic phosphorus is not converted in time. The cathodic reduction could also maintain the medium alkalinity of the soil and provide electron to activate the metabolisms of the bacteria. As for the scale application, the challenges may be located at the distribution of the anode and

cathode pair in the practical soil, the anti-corrosion of the electrode materials by the salt and bacteria and the cost control of the integrated electro-bacteria system. Overall, it is very promising to have the introduction of electrochemical technology into the phosphorus-solubilizing bacteria system, which is of great benefits for the transformation of phosphorus elements and plant growth (Rodríguez and Fraga, 1999; Acevedo et al., 2014; Wang and He, 2022).

## Author contributions

YJ and TC wrote the manuscript. LC, JH, YT, YC, AX, JZ and MN assisted the data collection. YZ and KW conceived the idea, revised the manuscript and led the project.

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# Electron microscopic imaging and NanoSIMS investigation on physiological responses of *Aspergillus niger* under Pb(II) and Cd(II) stress

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In the bioremediation process, coexistence of lead (Pb) and cadmium causes complex toxicity, resulting in the difficulty of bioremediation. This study investigated the physiological responses and bioaccumulation mechanisms of the typical filamentous fungus *Aspergillus niger* under the coexistence of Pb and Cd. Four treatments were set up, i.e., control, sole Pb, sole Cd, and coexistence of Pb and Cd. The morphology of *A. niger* were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. Then, nano-scale secondary ion mass spectrometry (NanoSIMS) was applied to accurately investigate the distribution of heavy metals in the fungal cells under the coexistence of Pb and Cd. Finally, the metallogenic process and mineral types were simulated by Geochemist's Workbench (GWB). The electron microscopic and NanoSIMS imaging showed that Pb and Cd were accumulated in both the extracellular and intracellular regions of the *A. niger* cells. In particular, the accumulated Pb content was ten times higher than that of Cd. However, Cd showed stronger toxicity than Pb to *A. niger*. Compared with the control treatment, Cd stress resulted in a two-fold increase of cell diameter and more extracellular substances, whereas the cell diameter increased nearly four times in the coexistence treatment. Moreover, the bioaccumulation of Pb was more intense than that of Cd during competitive sorption. The GWB simulation confirmed that Pb<sup>2+</sup> can form multiple minerals (e.g., PbC<sub>2</sub>O<sub>4</sub>, PbHPO<sub>4</sub>, and Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, etc.), which significantly weakened its toxicity on the cell surface. This study elucidated the morphological characteristics of *A. niger* and competitive bioaccumulation under the coexistence of Pb and Cd, which would facilitate the application of microorganisms to the bioremediation of coexisted metals.

## KEYWORDS

lead, cadmium, *Aspergillus niger*, electron microscopy, NanoSIMS, GWB simulation

## 1 Introduction

Heavy metal pollution caused by anthropogenic activities is increasing (Jarup, 2003; Hou, 2021). The coexistence of heavy metals in sewage and solid wastes derived from mining, smelting, and electroplating industries usually causes compound pollution (Song et al., 2022). Lead (Pb) and cadmium (Cd) are the two most common heavy metals (Yang et al., 2018).



According to the national commune of soil pollution survey by the Ministry of Environmental Protection of China, the over-limit rates of Pb and Cd were 1.5% and 7.0%, respectively (MEP of China, 2014). Thus, the coexistence of Pb and Cd is one of the most common combined pollutions (Gao et al., 2010; Chen et al., 2015).

*Aspergillus niger* is a representative phosphate-solubilizing fungus in soil (Khan et al., 2014). It could produce abundant organic acids and extracellular degradative enzymes to accelerate the release of phosphate (Tian et al., 2021). Thus, *A. niger* has been widely applied to bioremediation (Tian et al., 2020; Yang et al., 2020). *Aspergillus niger* had more stable heritability and a strictly stronger acid-producing capacity than bacteria and many other fungi (Sharma et al., 2013; Yu et al., 2021). The oxalic acid ( $H_2C_2O_4$ ) secreted by *A. niger* could efficiently precipitate heavy metal cations (Yakout, 2014). In addition, heavy metals could be accumulated in both intracellular and extracellular regions of fungal cells (Kapoor and Viraraghavan, 1997; Qiu et al., 2021; Geng et al., 2022). Thus, the filamentous fungus *A. niger* has been considered as an ideal strain for heavy metal bioremediation (Ahluwalia and Goyal, 2007; Ren et al., 2009; Zegzouti et al., 2020). However, previous studies mostly focused on remediation of a single heavy metal (Gola et al., 2016; Tian et al., 2019; Okolie et al., 2020). Therefore, lacking knowledge of bioaccumulation under the coexistence of Pb and Cd impeded the application of microorganisms in the remediation.

The sorption capacity of *A. niger* to Pb was usually higher than that of Cd due to their different affinity to negative charges on cell surface (Amini & Younesi, 2009; Okolie et al., 2020). *Aspergillus niger* had higher tolerance concentrations of Pb than Cd, i.e., >1,500 mg/L for Pb and only 100 mg/L for Cd (Tian et al., 2019; Okolie et al., 2020). Moreover, Pb-oxalate was easier to be precipitated than Cd-oxalate, as the solubility product constant ( $K_{sp}$ ) of Pb-oxalate is nearly three orders of magnitude less than Cd-oxalate (Pb oxalate:  $K_{sp} = 2.74 \times 10^{-11}$ ; Cd oxalate:  $K_{sp} = 1.42 \times 10^{-8}$ ) (Benitez and Dubois, 1999; Johansson et al., 2008). Therefore, the responses of *A. niger* to Pb and Cd should be correlated to a series of factors under the coexistence system.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were suitable for observing the surface morphology and internal structure of microorganisms, respectively (Jiang et al., 2021; Su et al., 2021). In the interaction between microorganisms and heavy metals, the metallogenesis and mineral crystal structure were observed by SEM (Bhattacharya et al., 2018; Chen et al., 2019; Tian et al., 2019; Xu et al., 2021). Significant changes in the sizes of microbial cells have been observed under metal stimulation based on SEM imaging (Gola et al., 2016; Sharma et al., 2017; Jiang et al., 2020). In addition, TEM could identify intracellular and extracellular adsorption of heavy metals based on its resolution up to nanometre scale (Zhu et al., 2017). Furthermore, the fine observation by TEM elucidated a new cell wall formation under Pb stress (Tian et al., 2019).

Nano-secondary ion mass spectrometry (NanoSIMS) owns high sensitivity when investigating microchemistry (Guerquin-Kern et al., 2005). Recently, the potential of NanoSIMS as a new tool in the study of bio-interface has been demonstrated (Yu et al., 2020). The high sensitivity, high lateral resolution (50 nm for  $Cs^+$  primary ion beam source), and high mass resolution ( $\sim 4,000X$ ) for secondary ions qualify the NanoSIMS as a powerful tool for investigating elemental composition (e.g., C, N, P, and halogen elements) on microbial samples (Popa et al., 2007). However, NanoSIMS technology is rarely applied to the studies of microbial responses to heavy metals.

In this study, we investigated the morphological responses and metallogenetic mechanisms of *A. niger* to the coexistence of Pb and Cd. SEM and TEM were used to elucidate morphology characteristics and internal structures of *A. niger* cells. Then, NanoSIMS was applied to identify the distribution of cell composition elements and heavy metals. Finally, based on Geochemist's Workbench (GWB), the mineralization of Pb and Cd cations was simulated, which would provide a theoretical understanding of bioremediation.

## 2 Materials and methods

### 2.1 Fungal strain and incubation

*Aspergillus niger* strain information can be referred to our previous study (Qiu et al., 2021). The fungus accession number in China General Microbiological Cultural Collection Center (CGMCC) is No. 11544. *Aspergillus niger* was cultured in potato dextrose agar (PDA) medium at 28 °C for 5 days. After spore formation, the medium was drenched with sterile water. The spores were scraped carefully from the plate surface with a fine brush. Then, the suspension was filtered through a three-layer sterile cheesecloth to eliminate mycelial fragments. The concentration of spores was measured by haemocytometer. The initial count of spores was  $10^7$  cfu mL<sup>-1</sup>.

### 2.2 Experimental design

Four treatments were performed, i.e., CK (no metal addition), TPb (sole Pb addition), TCd (sole Cd addition), and TPbCd (addition of Pb and Cd). The concentrations of Pb and Cd addition were both .893 mmol/L. Three replicates were set for each treatment. The solid  $Pb(NO_3)_2$  powder (Xilong Scientific Ltd.) and  $Cd(NO_3)_2$  powder (98% cadmium nitrate tetrahydrate, Sigma Aldrich Inc.) were added to 100 mL potato dextrose broth (PDB) medium. After sterilizing the medium, 1 mL spore suspensions were added to the medium for incubation. The initial pH value of the inoculation system was set as 6.5. All the treatments were incubated at 28 °C for 5 days under 180 rpm shaking.

### 2.3 Experimental instruments and analytical methods

After the incubation, the precipitates and supernatant were separated by centrifugation (2,504 rcf, 10 min). The precipitates were dried at 65 °C for 24 h for subsequent analyses.

#### 2.3.1 SEM analysis

The samples were fixed by 2.5% glutaraldehyde for 4 h. After the samples were rinsed with .1 M sodium phosphate buffer (pH = 7.4), ethanol of 30%, 50%, 70%, 85%, 90%, and 100% was used for dehydration of the precipitates. Finally, isoamyl alcohol was applied to dry the precipitates in a freeze-dryer for 48 h. The samples were pasted on the platform with conductive adhesive for SEM analysis. The image acquisition was tested by Carl Zeiss SUPRATM 55 system. Gold particles by Gressington 108 Autosputter coated the samples to improve electrical conductivity and prevent thermal damage. Semi-

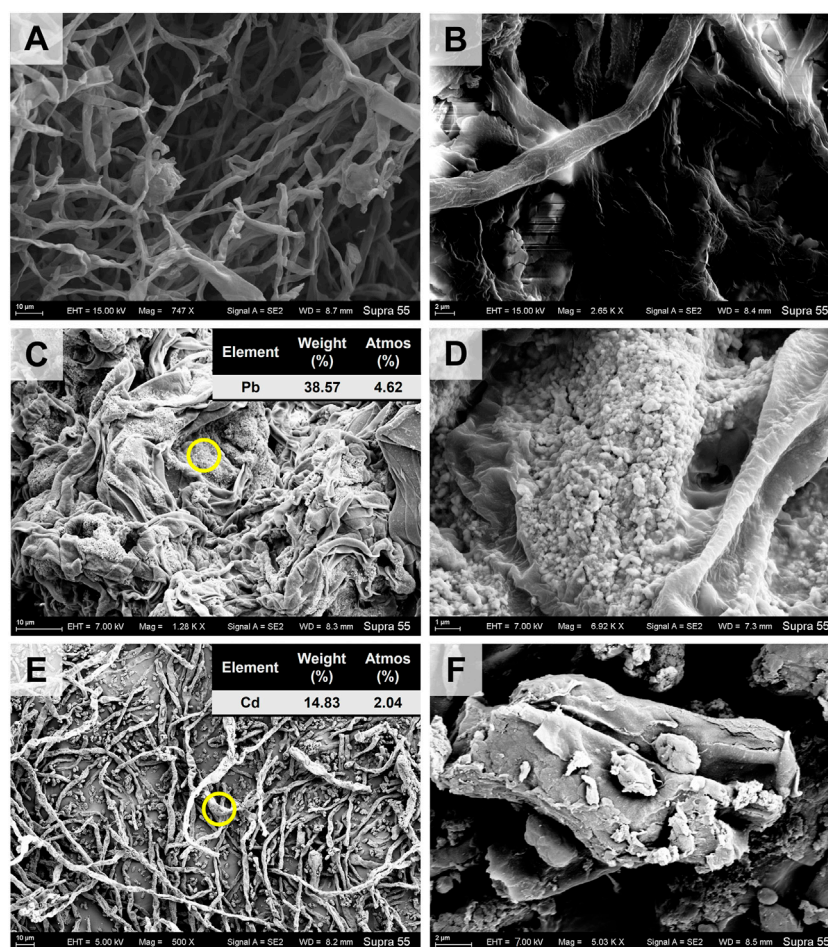


FIGURE 1

The SEM images of *Aspergillus niger* after 5 days incubation in the CK (A, B), TPb (C, D), and TCd (E, F) treatments. The representative particles on the mycelial surface in images C and E were selected for EDS analysis.

quantitative analysis was performed by Oxford Aztec X-Max 150 energy dispersive X-ray spectrometer (EDS).

### 2.3.2 TEM analysis

The processing of the samples for TEM analysis can refer to our previous study (Tian et al., 2019). The precipitate was pre-fixed with electron microscopy fixative (G1102, Servicebio, Wuhan, China) and fixed again by osmic acid. The samples were prepared as ultrathin sections (60–80 nm thickness). The field-emission transmission electron microscope was performed by FEI Tecnai G2 F20S-TWIN system equipped with AZtec X-Max 80T energy dispersive spectrometer (EDS).

### 2.3.3 NanoSIMS analysis

The precipitates collected from the TPbCd treatment were analyzed by NanoSIMS. The sample preparation processes were similar to the process for preparation of TEM samples. After embedding, the sample was sectioned with 400 nm thick slices. The element observations were performed with a NanoSIMS 50 (Cameca, Courbevoie, France). A Cs + primary ion beam was used to

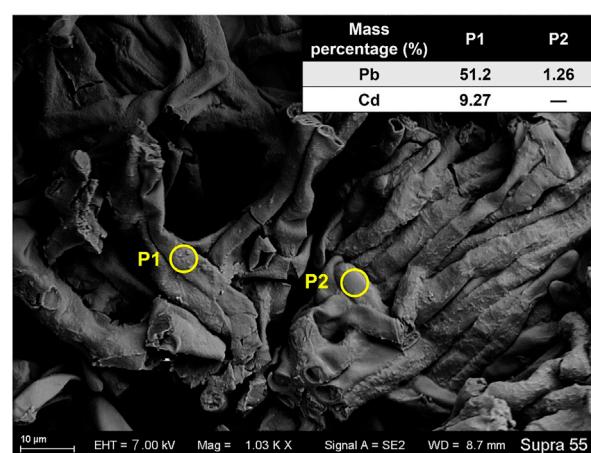
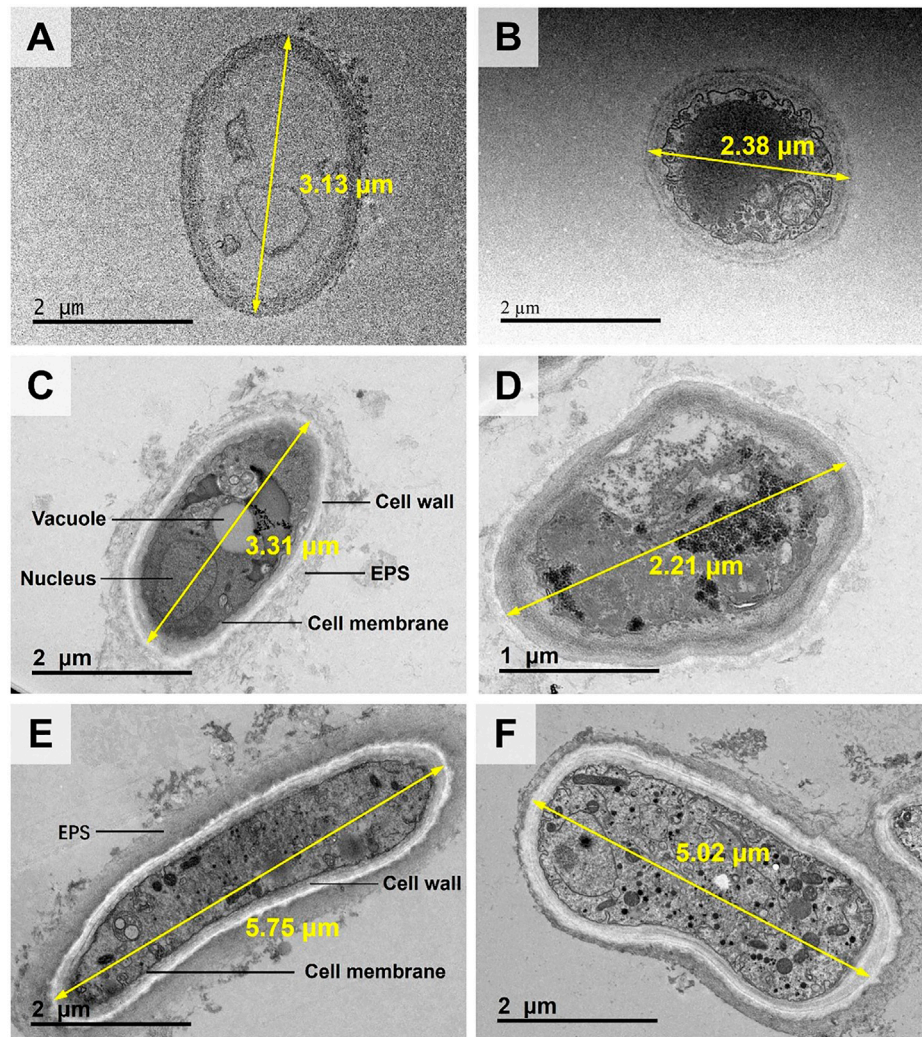


FIGURE 2

The SEM image of *Aspergillus niger* after 5 days incubation in the TPbCd treatment. The representative spots of P1 and P2 were selected for EDS analysis.





**FIGURE 3**

The TEM images of morphological changes of *Aspergillus niger* after 5 days incubation in the CK (A, B), TPb (C, D), and TCd (E, F) treatments. Cell diameters of representative cells were shown.

continuously bombard microbial cells on the sample surface. Then, the secondary ions were sputtered and liberation from the upper surface. These secondary ions were sorted based on their energy in the electrostatic sector before being dispersed in a mass spectrometer according to their mass/charge ratios. By acquiring a series of spatially referenced spectra, maps of  $^{16}\text{O}^-$ ,  $^{12}\text{C}^{14}\text{N}^-$  (characterize nitrogen (N)),  $^{208}\text{Pb}^{16}\text{O}^-$  and  $^{114}\text{Cd}^{16}\text{O}^-$  were produced for the atomic mass.

#### 2.3.4 GWB modeling

Geochemist's Workbench (GWB 11, Aqueous Solutions LLC.) was applied to simulate mineralization of the metals. Under the Titration mode, using React module to simulate ion concentration changes with pH value in the system. The concentrations of  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  in the system were set based on the experimental design. The maximum concentration of  $\text{H}_2\text{PO}_4^-$  was set to 10 mmol/L (Qiu et al., 2021). The phase diagram of the dominant minerals was drawn by Act2 module. The mineralization of Pb and Cd were

subsequently simulated when reaching an equilibrium state at each site of the system.

## 3 Results

### 3.1 SEM and EDS analyses

In the CK treatment, the typical diameter of *A. niger* hypha was  $\sim 2 \mu\text{m}$  (Figures 1A,B). In the TPb treatment, the hypha has a typical diameter of  $\sim 3 \mu\text{m}$  (Figures 1C,D). The value increased to 5–6  $\mu\text{m}$  in the TCd treatment (Figures 1E,F). Moreover, the hyphae were tightly interwoven under Pb stress, but loosely arranged under Cd stress.

The mycelia showed rough surface with the enrichment of particles. In the TPb treatment, the particle diameter varied between .1–.5  $\mu\text{m}$  (Figure 1D). In contrast, the particle diameter in the TCd treatment was larger, i.e., 1–2  $\mu\text{m}$  (Figure 1F). The representative particles on the mycelial surface were selected for

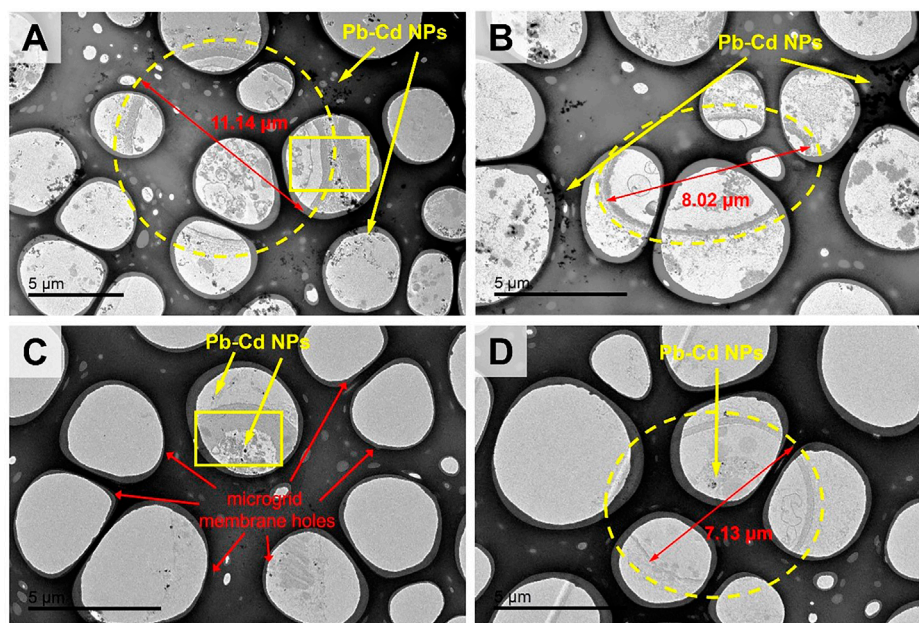


FIGURE 4

The TEM images of morphological changes of *Aspergillus niger* after 5 days incubation in the TPbCd treatment. Cell diameters of several representative cells were shown. Representative Pb and Cd nano-particles (NPs) were shown.

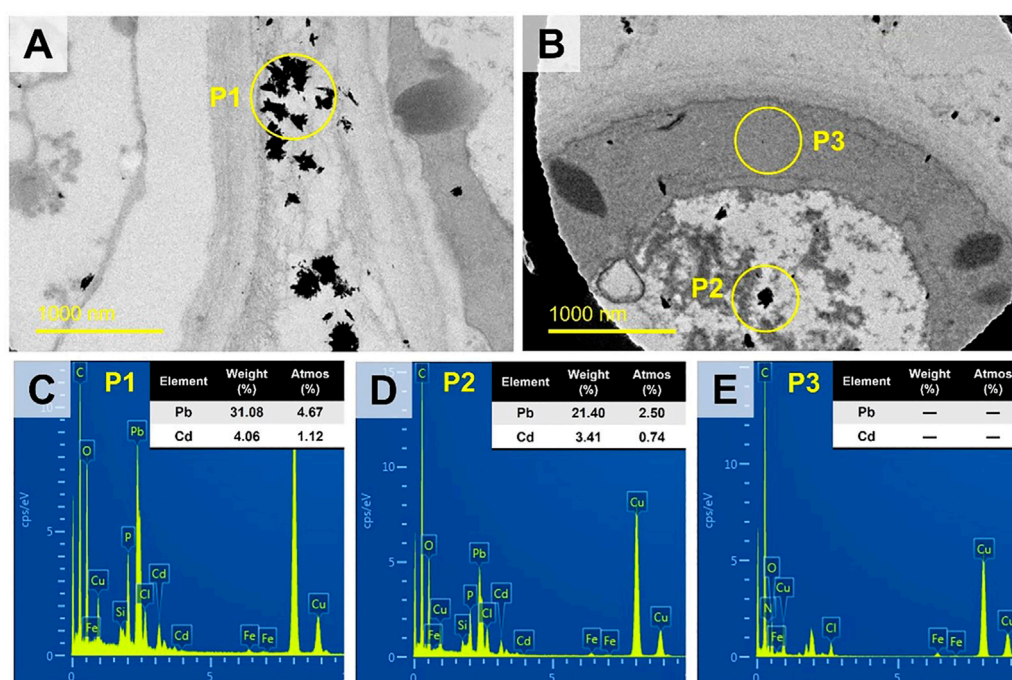


FIGURE 5

The TEM images of the rectangular regions in Figures 4A image (A) and 4C image (B) at high-resolution (slight offset might occur). Three representative spots (P1, P2, P3) were selected for EDS analysis as shown in images (C–E) respectively.

EDS analysis. In the TPb and TCd treatments, the weight percentage of Pb and Cd accounted for 38.57% and 14.83%, respectively (Figures 1C,E). In the TPbCd treatment, the mycelia were arranged tightly and

orderly. The diameter of hypha was ~5–6 μm, which was much larger than those in the other treatments (Figure 2). Meanwhile, the Pb and Cd weight percentage at P1 was 51.2% and 9.27%, respectively. The



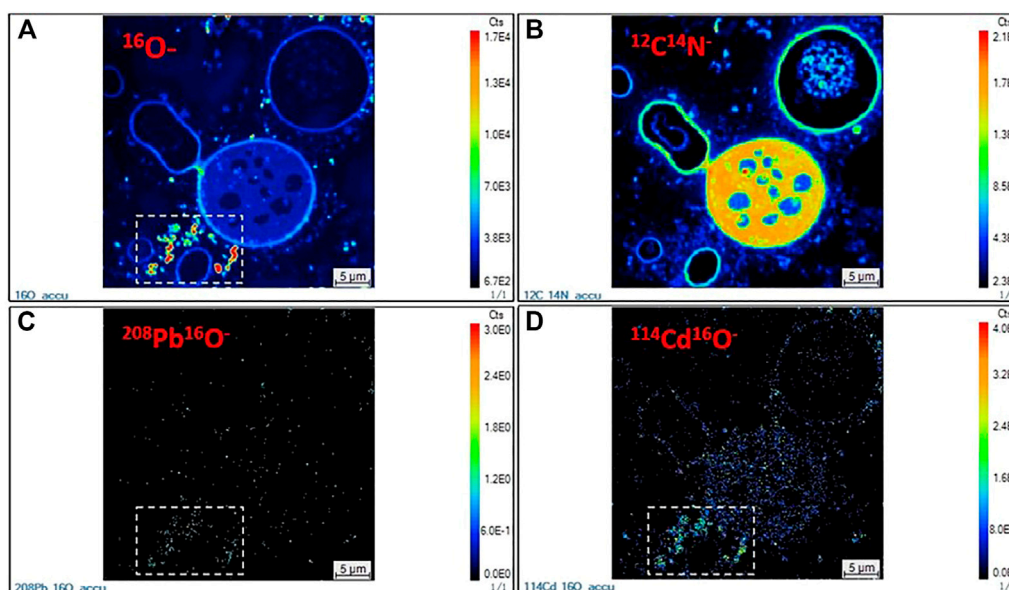


FIGURE 6

NanoSIMS images of *Aspergillus niger* cells in the TPbCd treatment. (A)  $^{16}\text{O}^-$  secondary ion image; (B)  $^{12}\text{C}^{14}\text{N}^-$  secondary ion image; (C)  $^{208}\text{Pb}^{16}\text{O}^-$  secondary ion image; (D)  $^{114}\text{Cd}^{16}\text{O}^-$  secondary ion image.

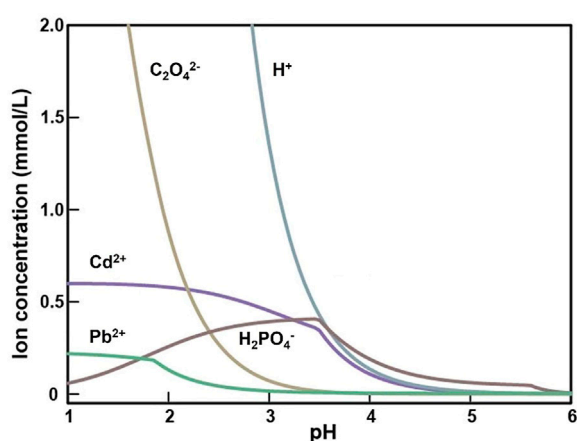


FIGURE 7

The concentration curve of the main ion species varying with pH value after 5 days incubation in the TPbCd treatment.

content of Pb was nearly five times higher than Cd. The Pb content at P2 was 1.26 wt%, yet Cd was under the detection line (Figure 2).

### 3.2 TEM and EDS analyses

In the CK treatment, the cell diameter was about 2–3  $\mu\text{m}$  (Figures 3A,B). There was almost no extracellular substances. The cell wall thickness was around 0.1  $\mu\text{m}$ . Moreover, no evident black particles were observed (see Figures 3A,B). In the TPb treatment, the cell size or the cell wall thickness showed no significant change (Figures 3C,D). However, the abundance of extracellular substances was increased,

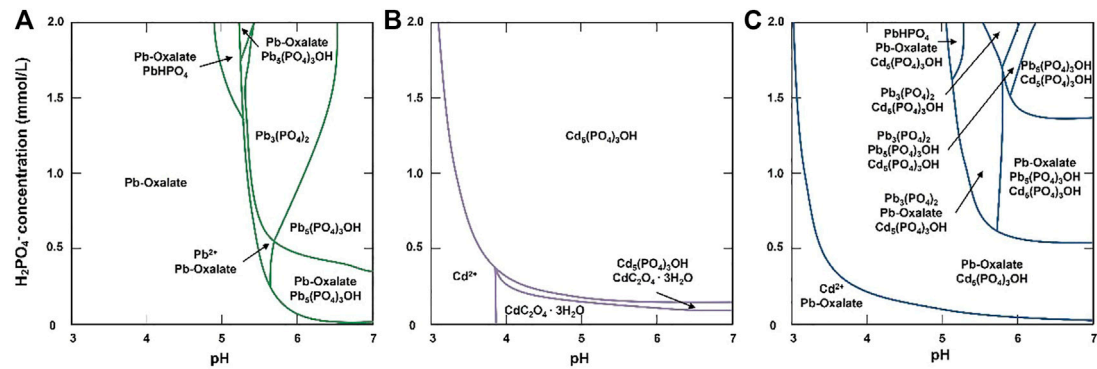
which were attached loosely to the cell walls. The particles were enriched near the vacuoles (Figures 3C,D). In the TCd treatment, the cell diameters were increased to 5–6  $\mu\text{m}$ , and the cell wall thickness was increased to 0.3  $\mu\text{m}$ . Meanwhile, the extracellular substances were secreted to form a dense layer outside the cells. Moreover, the particles were not only distributed in the intracellular region, but also adsorbed on the extracellular substance surfaces (Figures 3E,F).

In the TPbCd treatment, the cell diameter was enlarged to 7–11  $\mu\text{m}$  under TEM, which was about twice of that in the TCd treatment and four times of that in the CK treatment (Figure 4). Moreover, the particles were enriched in both the extracellular and intracellular regions of the cells (Figure 4). It should be noted that the circles with a diameter of  $\sim 5 \mu\text{m}$  were the microgrid membrane holes. The cells of *A. niger* were marked by dotted circles (Figure 4).

The representative micro-regions (marked as rectangular in Figure 4C) were selected for high-resolution observation. It showed that the particles aggregated in the extracellular region, while dispersedly distributed in the intracellular region (Figures 5A,B). The weight percentage of Pb in P1 and P2 was 31.08% and 21.40%, while that of Cd was as low as 4.06% and 3.41%, respectively. No signal of Pb or Cd was detected in P3 (Figures 5C–E).

### 3.3 NanoSIMS analysis

The spatial distribution of the secondary ions  $^{16}\text{O}^-$ ,  $^{12}\text{C}^{14}\text{N}^-$ ,  $^{208}\text{Pb}^{16}\text{O}^-$ , and  $^{114}\text{Cd}^{16}\text{O}^-$  under the TPbCd treatment were displayed in Figure 6. The intense  $^{16}\text{O}^-$  signals were indicated in the dashed rectangular area (see Figure 6A). In contrast, the strong  $^{12}\text{C}^{14}\text{N}^-$  signals appeared in the intracellular region (Figure 6B). The  $^{12}\text{C}^{14}\text{N}^-$  was used to characterize the contour and position of cells as N has been considered as an indicator of biogenic matters (Romer et al., 2006). Moreover, several weak  $^{12}\text{C}^{14}\text{N}^-$  signal circles were observed,



**FIGURE 8**  
Diagrams of Pb and Cd phase with the changes of pH and  $\text{H}_2\text{PO}_4^-$  concentrations in the TPb treatment (A), TCd treatment (B), and TPbCd treatment (C).

**TABLE 1** Solubility product constants ( $K_{sp}$ ) of typical Pb and Cd compounds.

Functional groups	Chemical formula	$K_{sp}$	References
<b>Pb</b>			
Carboxyl groups	-COOH	$2.74 \times 10^{-11}$	Lee et al. (1999)
Phosphate groups	- $\text{H}_2\text{PO}_4$	$8.0 \times 10^{-43}$	Martinez et al. (2004)
Hydroxyl	-OH	$1.2 \times 10^{-15}$	Frost and Williams (2004)
Carbonate	- $\text{CO}_3$	$7.4 \times 10^{-14}$	Yao et al. (2013)
Chromate	- $\text{CrO}_4$	$2.8 \times 10^{-13}$	Zheng et al. (2017)
Sulfate radical	- $\text{SO}_4$	$1.6 \times 10^{-8}$	DeSantis et al. (2018)
<b>Cd</b>			
Carboxyl groups	-COOH	$1.42 \times 10^{-8}$	Wang et al. (2017)
Phosphate groups	- $\text{H}_2\text{PO}_4$	$2.53 \times 10^{-33}$	Dmitrevskii et al. (2008)
Hydroxyl	-OH	$5.27 \times 10^{-15}$	Canepari et al. (1998)
Carbonate	- $\text{CO}_3$	$1.0 \times 10^{-12}$	Remacle et al. (1992)

which might be attributed to the dead cells undergoing/after cytoplasm decomposition. In addition, the enrichment of both  $^{208}\text{Pb}^{16}\text{O}^-$  and  $^{114}\text{Cd}^{16}\text{O}^-$  were higher in the extracellular region than that in the intracellular region (Figures 6C,D).

3.4 GWB simulation

The geochemical modeling under the TPbCd treatment showed that the concentrations of  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  were decreasing along with the decline of  $\text{H}^+$  and  $\text{C}_2\text{O}_4^{2-}$  concentrations (Figure 7). In addition, the  $\text{Pb}^{2+}$  concentration was always lower than that of  $\text{Cd}^{2+}$ , which indicated that  $\text{Pb}^{2+}$  was easier to form mineralized precipitation (with occurrence of  $\text{C}_2\text{O}_4^{2-}$ ) than  $\text{Cd}^{2+}$  (Figure 7).

The phase diagrams revealed the mineralization processes of Pb and Cd (Figure 8). In the TPb treatment, the mineral was Pb-oxalate when  $\text{pH} < 5.2$ , while the mineral types increased to  $\text{Pb}_3(\text{PO}_4)_2$ ,  $\text{PbHPO}_4$ , and  $\text{Pb}_5(\text{PO}_4)_3\text{OH}$  when  $\text{pH} > 5.2$  (Figure 8A). In the TCd treatment, most Cd existed as free cations when  $\text{pH} < 3$ . When  $\text{pH} < 3.8$  and  $\text{H}_2\text{PO}_4^- > 0.4 \text{ mmol/L}$ , the system was dominated by  $\text{Cd}_5(\text{PO}_4)_3\text{OH}$ . When  $\text{pH} > 3.8$  and  $\text{H}_2\text{PO}_4^- < 0.4 \text{ mmol/L}$ , oxalate minerals dominate the mineralization (Figure 8B).

In the TPbCd treatment, Pb presented as Pb-oxalate when pH value and  $\text{H}_2\text{PO}_4^-$  concentrations were relatively low (Figure 8C). Compared with the TCd treatment, Cd-oxalate was not formed when  $\text{pH} > 3.8$  and  $\text{H}_2\text{PO}_4^- < 0.4 \text{ mmol/L}$  (Figures 8B,C). Only when pH and  $\text{H}_2\text{PO}_4^-$  concentrations continue to increased,  $\text{Cd}^{2+}$  cations were mineralized to  $\text{Cd}_5(\text{PO}_4)_3\text{OH}$ . In addition, Pb induced a variety of minerals, such as  $\text{Pb}_3(\text{PO}_4)_2$ ,  $\text{PbHPO}_4$ , and  $\text{Pb}_5(\text{PO}_4)_3\text{OH}$  (Figure 8C).

4 Discussion

In this study, *A. niger* showed distinct responses to Pb and Cd stresses. This was consistent with the conclusion that Pb concentrations  $< 1,000 \text{ mg/L}$  could promote biological activity (Sayer et al., 1999). Therefore, the secretion of extracellular substances would subsequently be promoted (Figure 3). However, the tolerance of *A. niger* to Cd was much weaker due to its high toxicity and migration (Wu et al., 2016). The biomass of *A. niger* was significantly lower under Cd stress than the CK and Pb treatments (Wang, et al., 2017; Qiu, et al., 2021). The dead cell lysis would also release intracellular organic matters (Li, et al., 2021), which were

adsorbed around the living cells to isolate the contact between the cells and heavy metals. In addition, the extracellular substances and cell debris had similar functional groups (Li, et al., 2012), which were mainly composed of proteins, polysaccharides, lipids, and humic acids (Lin et al., 2014). Their negatively charged functional groups could adsorb heavy metal cations (Comte et al., 2008; Dang et al., 2018). Therefore, the abundance of extracellular organic substances are able to form a protective layer of *A. niger* to immobilize metals.

Microbes can adapt to environmental changes by regulating their morphology (Guan et al., 2020). This study revealed the phenomenon of the enlargement of the cross-section of hyphae under the coexistence of Pb and Cd (Figure 3). Moreover, appropriate  $Pb^{2+}$  in the coexistence system could significantly enhance microbial activities by promoting the tricarboxylic acid cycle of *A. niger* (Qiu et al., 2021). This response to heavy metals was also observed in *A. niger* sporangia which were increased by 50% (Xu et al., 2021). When *A. niger* was exposed to heavy metals, it preferred to promote the surface area by expanding the cell volume. The larger cell surface provided more active sites for adsorbing more heavy metal ions (Smyth, 1989). This mechanism was also consistent with the study regarding the resistance of bacteria to Cd toxicity (Keene et al., 2008).

The NanoSIMS mapping showed that the bioaccumulation of Pb and Cd was more intense in the extracellular than intracellular region. *Aspergillus niger* could secrete a variety of low-molecular-weight organic acids (LMWOAs) (Strobel, 2001; Li et al., 2016). Oxalic acid was the most abundant LMWOAs (Yakout, 2014). Compared with other LMWOAs, oxalic acid had a higher acidity constant ( $pK_{a1} = 1.25$ ;  $pK_{a2} = 4.27$ ), which facilitated the formation of oxalate precipitation to reduce metal toxicity (Green and Clausen, 2003). In addition, Pb had higher competitive accumulation than Cd in the coexistence system. The competitive accumulation of Pb and Cd also existed in the bioremediation by bacteria. For example, a study of *Pseudomonas putida* showed that  $Pb^{2+}$  had almost the same sorption sites as  $Cd^{2+}$  on the cell surface (Du et al., 2016). Moreover, the bioaccumulation efficiency of *Exiguobacterium* sp. to Pb was also higher than that of Cd (Park and Chon, 2016).

The GWB simulation showed that  $Pb^{2+}$  and  $Cd^{2+}$  competed for oxalate species ( $C_2O_4^{2-}$ ) in the coexistence system (Figure 7).  $Pb^{2+}$  was preferred to generate oxalate minerals due to that Pb-oxalate usually has a lower  $K_{sp}$  value than Cd-oxalate (Benitez and Dubois, 1999). Furthermore,  $Pb^{2+}$  could form a variety of mineral species (e.g., Pb oxalate ( $PbC_2O_4$ ),  $PbHPO_4$ , and  $Pb_3(PO_4)_2$ ). However,  $Cd^{2+}$  cations were commonly mineralized as  $Cd_5(PO_4)_3OH$ . Additionally, it was attributed to the stronger affinity between Pb and amino acid residues, which induces the  $K_{sp}$  of Pb-containing compounds lower than that of Cd (Table 1). Therefore, in the coexistence of Pb and Cd,  $Pb^{2+}$  was more easily mineralized. The mineralization finally immobilized and detoxified the cations.

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## 5 Conclusion

This study identified the physiological responses and metallogenetic mechanisms of *A. niger* to Pb and Cd stress. Our findings confirmed that the filamentous fungus *A. niger* had multiple pathways to effectively adsorb heavy metal ions, e.g., producing LMWOAs, secreting extracellular substances, and enlarging the cell surface area. Therefore, *A. niger* shows evident advantages in the bioremediation of heavy metals. In the coexistence system, Pb had preferential bioaccumulation than Cd, which allowed that most Pb cations could be mineralized and detoxified. This study sheds a light on the remediation of the coexistence of metals by functional fungi.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

ZL and SP conceived the research. SP, LM, and MS designed and conducted the experiments. ZYL, JW, XL, and YC performed the data analyses. SP and ZL wrote the manuscript with significant inputs from all other authors.

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## Conflict of interest

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# Isolation and screening of phosphorus solubilizing bacteria from saline alkali soil and their potential for Pb pollution remediation

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The high pH and salinity of saline alkali soil not only seriously restrict the growth of crops, but also aggravate the pollution of heavy metals. The fixation of heavy metals and the regulation of pH by phosphorus solubilizing microorganisms may become a new way to repair heavy metal and improve saline alkali soil. In this study, a saline-alkali resistant bacteria (CZ-B1, CGMCC No: 1.19458) was screened from saline-alkali soil, and its tolerance to salt/alkali/lead stress was investigated by shaking flask experiment. The strain was identified as *Bacillus amyloliquefaciens* by morphology and 16S rRNA gene sequence analysis. The optimum growth temperature of CZ-B1 is about 35°C–40°C. The maximum salt stress and pH that it can tolerance are 100 g/L and 9 respectively, and its tolerance to Pb<sup>2+</sup> can reach 2000 mg/L. The phosphorus release amount of CZ-B1 to Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> within 72 h is 91.00–102.73 mg/L. The phosphate solubilizing index in PVK agar medium and NBRIP agar medium are more than 2, which can be defined as phosphate solubilizing bacteria. Moreover, the dissolution of CZ-B1 to phosphorus is mainly attributed to tartaric acid, citric acid and succinic acid in inorganic medium. In addition, the removal rate of Pb<sup>2+</sup> by CZ-B1 can reach 90.38% for 500 mg/L. This study found that CZ-B1 can immobilize Pb through three biological mechanisms (organic acid, extracellular polymers and mineralization reaction). The release of succinic acid (10.97 g/L) and citric acid (5.26 g/L) may be the main mechanism to promote the mineralization reaction of CZ-B1 (phosphate and oxalate) and resistance to Pb stress. In addition, the high enrichment of Pb<sup>2+</sup> by EPS can increase the rate of extracellular electron transfer and accelerate the mineralization of CZ-B1. The screening and domestication of saline-tolerant phosphorus-solubilizing bacteria not only help to remediate Pb contamination in saline soils, but also can provide P element for plant growth in saline soil.

## KEYWORDS

phosphorus solubilizing bacteria, salt and alkaline tolerant, Pb stress, organic acid, extracellular polymers, mineralization reaction

## Highlights

- A strain of phosphorus solubilizing bacteria (CZ-B1) tolerant to salt and alkaline stress was successfully screened.
- The CZ-B1 tolerated Pb<sup>2+</sup>, salinity and alkalinity up to 2000 mg/L, 10% and 9.

- The resistance of CZ-B1 to Pb is mainly achieved by cellular and secretions adsorption under low Pb stress.
- CZ-B1 can form pyromorphite and Pb oxalate minerals through secreted citric, succinic and oxalic acids.
- CZ-B1 increases extracellular electron transfer rate and mineral precipitation efficiency by EPS adsorption and enrichment of  $\text{Pb}^{2+}$ .

## 1 Introduction

Soil salinization has always been a major threat to the sustainable development of agriculture and the improvement of land use efficiency (Meena et al., 2020; Liang et al., 2021; Chen Q. et al., 2022). It is reported that more than 100 million hectares of land in the world are being destroyed by soil salinization (Wicke et al., 2011), and the area of saline alkali soil continues to grow at a rate of 10% every year (Cui et al., 2021). There are over 20,000 km<sup>2</sup> of saline soils in China's coastal mudflats that need to be improved (Cui et al., 2021). The high salinity and high pH of saline alkali soil will lead to severe degradation of soil structure, nutrient deficiencies and disruption of microbial activity, among other hazards (He et al., 2020; Zhao et al., 2020; Zhang et al., 2022), which disrupts the normal physiological functions of plant cells (Shao et al., 2018; Jia et al., 2019). Not only that, but high salinity of saline alkali soil will affect the diffusion of heavy metals (HMs) ions, thus increasing the bioavailability of HMs in soil and their toxicity to plants (Liu et al., 2019). Therefore, in the context of the global food crisis, as an important reserve of land resources for food production and ecological environment construction, the improvement and utilization of salinized soil is imminent (Zhao et al., 2020; Zhang et al., 2022).

As a common HMs pollution, lead (Pb) has high toxicity, bioaccumulation and persistence (Jing et al., 2004; Gladysz-Plaska et al., 2017). Pb can be accumulated and amplified in the organism through the food chain. Excessive Pb intake will seriously affect the human nerve center, hematopoietic function, and cause cancer and other diseases (Kolodynska et al., 2018; Chen H. M. et al., 2022; Lai et al., 2022). Under neutral or alkaline conditions,  $\text{Pb}^{2+}$  is not active in the soil due to the formation of  $\text{Pb}(\text{OH})_2$  precipitation (Wang et al., 2015). This lead to a large accumulation of  $\text{Pb}^{2+}$  in saline alkali soil (tilth). However,  $\text{Pb}(\text{OH})_2$  has poor stability and can be dissolved by acid substances secreted by plant roots or soil microorganisms, which has great potential risks (Reboreda and Cacador, 2007). It has been proved that the activity of  $\text{Pb}^{2+}$  in saline alkali soil will increased after reclamation, which leads to Pb in rice approaching the food safety threshold (Wang et al., 2015). Common remediation technologies for HMs pollution include physical and chemical methods, such as soil cleaning, heat treatment (Phieler et al., 2014), electric remediation (Gupta and Kumar, 2017), stable solidification method (Li et al., 2021) and chemical elution (Leyval et al., 1997). These methods have the disadvantages of high cost, low remediation capacity and secondary contamination (Glick, 2010). Also, these methods are difficult to apply to the remediation of HMs in saline soils (Lefevre et al., 2009). Therefore, a low-cost, sustainable and ecologically friendly measure is urgently needed to alleviate soil salinization stress and improve plant productivity and safety of saline soil.

In recent years, the use of microorganisms for soil remediation and improvement has proved to be a safe and economic potential measure (Sheng et al., 2008). Phosphorus solubilizing microorganisms (PSM),

as a type of environmental functional microorganism, can enhance phosphorus utilization by plants via releasing organic acids, enzymes and other biological secretions to dissolve insoluble phosphorus sources in the environment (Rodriguez et al., 2006; Shao et al., 2021; Zhu et al., 2022). In addition, with the further study of PSM, it has been proved that they can be used in the remediation of HMs in soil (Park et al., 2011; Teng et al., 2019; Feng et al., 2022; Lai et al., 2022). The greatest advantage of PSM in HMs remediation is their ability to release phosphate ions that combine with HMs ions to form more stable metal-phosphate compounds (Chen et al., 2020; Lai et al., 2022). For example, under  $\text{Pb}^{2+}$  stress of 1000 mg/L, phosphorus solubilizing bacteria (*Enterobacter* sp.) can repair Pb pollution of 114.2–231.4 mg/L by forming Pb phosphate precipitation (Chen et al., 2019). The percentage amount of Pb immobilized in solution after 14 days was 98.18% for the PSM (*Citrobacter farmer* CFI-01) (Li et al., 2022). However, the saline alkali environment has a significant inhibitory effect on the growth of microorganisms. High salt content will cause the microbial cells to shrink and lose their activity due to dehydration (Michel et al., 2022). Meanwhile, high concentrations of  $\text{Pb}^{2+}$  significantly inhibit the growth of phosphorus-dissolving bacteria. For example, PSM (*Enterobacter* sp.) can only survive at  $\text{Pb}^{2+}$  concentrations of <500 mg/L (Jiang et al., 2020). Therefore, if PSM can be screened from saline alkali soil and their Pb tolerance can be domesticated, it may become a new way to effectively passivate Pb in saline alkali environment.

This study aims to screen a phosphorus solubilizing bacteria that can repair HMs Pb in saline alkali environment, and verify the tolerance of the strain to salt/alkali/Pb stress. At the same time, X-ray diffraction (XRD), infrared spectroscopy (ATR-IR), three-dimensional fluorescence (3D-EEM) and scanning electron microscopy (SEM/EDX) were used to explore the biological mechanism of Pb passivation and repair. It is expected to provide a new idea for remediation of Pb pollution in saline alkali soil.

## 2 Materials and methods

### 2.1 Isolation of salt alkali and Pb resistance bacteria

Soil samples were collected from coastal saline alkali soil in Dongying City, Shandong Province (118.78112°E, 37.83379°N). 10 g soil was added to 100 mL sterilized sterile water containing  $\text{Pb}^{2+}$  stress for shaking ( $\text{Pb}^{2+}$  concentration was 50 mg/L, 180 r/min, 30°C, 2 h). Then, the supernatant was continuously diluted and coated on Luria Bertani (LB) solid medium, and incubated in a 36°C constant temperature incubator for 72 h. The strains with Pb tolerance and salt alkali tolerance can be obtained by picking single colonies of different forms for purification.

### 2.2 Identification of salt alkali and Pb resistance bacteria

A salt alkali resistant strain screened was labeled CZ-B1, and was incubated in LB medium for 72 h (180 rpm, 30°C) by shaking. Then the strain was identified by 16 S rRNA gene sequence. Use FastDNA SPIN Kit (Bio Teke, Co., China) to extract genomic DNA (Zhou et al., 2018). Primers 27F (5'-AGA GTTTGATCTMTGCTCAG-3') and



TABLE 1 The culture conditions of stress experiment.

Condition	Value
temperature (°C)	25, 30, 35, 40, 45
pH	5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0
NaCl concentration (w/v %)	1, 5, 10, 15, 20, 30
Pb <sup>2+</sup> concentration (mg/L)	500, 1000, 2000, 3000, 4000
Sampling time (h)	1, 3, 6, 12, 24, 48, 72

1492R (5'-CRGYTACCTGTTACGA-3') were used for PCR amplification of 16S rRNA gene (Biswas et al., 2018).

## 2.3 Phosphorus dissolving capacity experiments

In this study, two different methods were used to verify the phosphorus dissolving function of CZ-B1, namely, the plate phosphorus dissolving method and the liquid medium phosphorus dissolving method. The plate phosphorus dissolving method is to inoculate CZ-B1 onto PVK (Pikovskaya) solid medium and NBRIP (National Botanical Research Institute's Phosphate) solid medium for 4–7 days, and record the size of its halo (Liu et al., 2015). The method of dissolving phosphorus in liquid medium is to culture CZ-B1 in modified PVK, PVK and NBRIP liquid medium by shaking for 72 h (180 rpm, 30°C). The mixed solution was centrifuged and filtered, and the total soluble phosphorus content in the supernatant was determined (after deducting the control). The PVK medium contains glucose 10 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g, NaCl 0.3 g, KCl 0.3 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.3 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.3 g, MnSO<sub>4</sub>·4H<sub>2</sub>O 0.03 g, yeast powder 0.5 g, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 5 g, H<sub>2</sub>O 1 L, solid medium contains 2% agar (Li et al., 2018). Meanwhile, modified PVK medium (HAP) was obtained by replacing Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> in PVK medium with hydroxyapatite (insoluble). NBRIP medium consisted of glucose 10 g, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 5 g, MgCl<sub>2</sub>·6H<sub>2</sub>O 5 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.25 g, KCl 0.2 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g, H<sub>2</sub>O 1 L, solid medium contains 2% agar (Park et al., 2011).

## 2.4 Stress experiments

The stress culture of CZ-B1 was carried out at different temperatures, pH, salt concentration and Pb<sup>2+</sup> concentration (Pb(NO<sub>3</sub>)<sub>2</sub>) (Table 1). The absorbance (wavelength 600 nm) of CZ-B1 at different times (1–72 h) was measured separately to characterize the growth of the strain, no stress treatment was used as control. The samples of Pb stress experiment with different concentrations (72 h) were divided into solid and liquid fractions. One part of the liquid samples are used to determine the content of organic acids (formic acid, oxalic acid, malic acid, succinic acid, tartaric acid, and citric acid), 3D-EEM, electrochemical characteristics and EPS. The other part of the liquid samples were centrifuged (5000 rpm, 5 min) and solid-liquid separated, the Pb content in the filtrate was determined by ICP-OES. One part of the solid sample is used to determine ATR-IR, XRD, and the other part is fixed with 2.5% glutaraldehyde for 24 h, then dehydrated with ethanol gradient and used for scanning electron microscopy analysis (Su et al., 2019).

## 2.5 Instrumentation

The OD<sub>600</sub> value of the bacterial liquid was determined at 600 nm using a UV visible spectrophotometer (752 NPlus, Shanghai Yidian, China). The Pb content in the solution was determined by ICP-OES (icap7000, ThermoFisher Scientific Inc iCAP PRO, United State). The 3D-EEM excitation wavelength is 200–600 nm, the emission spectrum wavelength was 200–600 nm, and the diffraction slit is 5 nm (F-7100, Hitachi, Japan). Organic acids are tested by high performance liquid chromatography (U3000, ThermoFisher Scientific Inc, United State) (Su et al., 2019). Refer to our previous research for the extraction and testing methods of EPS (Chen et al., 2021). Used the electrochemical workstation (CHI760D, CHInstrument Company, China) to conduct the electrochemical test of the Pb stress bacterial liquid by cyclic voltammetry (CV) (the working electrode is bacteria modified glassy carbon electrode, auxiliary electrode is platinum wire electrode, and the reference electrode is saturated calomel electrode). XRD (D8 Advance, Bruker AXS GMBH, Germany, Cu Kα, λ = 1.540 60 Å; 40 kV; 40 mA; and 2θ rotation range of 10°–80°) and ATR-IR (Nicolet iS5 Fourier-transform infrared spectrometer, ThermoFisher Scientific Inc.) were used to determine the mineral composition and functional group characteristics in solid samples. The morphology and elements of solid samples were determined by SEM (Hitachi Regulus 8100, Japan) and EDX (INCA 300 Oxford, UK).

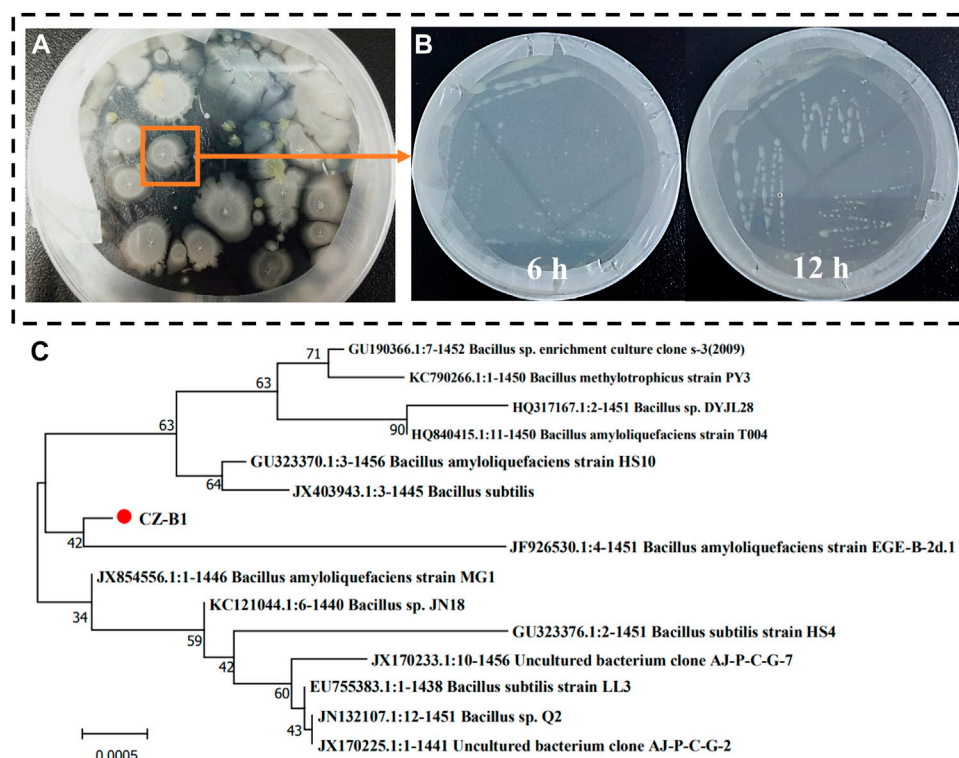
## 2.6 Statistical analysis

Repeat three times for each experiments. The means and standard deviations in each treatment were calculated and presented. The 16S rRNA sequences were compared by BLAST program, and then the phylogenetic tree was constructed by MEGA 7.0 software (Zhu et al., 2011; Biswas et al., 2018). Charting with Origin 2022 and Office 365.

# 3 Results and discussion

## 3.1 Screening and identification of CZ-B1

CZ-B1 obtained by screening and purification from saline alkali soil and its state after 6 and 12 h culture are shown in Figures 1A, B. The colony of CZ-B1 is milky white, opaque, rough and diffuse. In addition, CZ-B1 reached a good growth state within 12 h, which indicates that CZ-B1 grows rapidly and has strong environmental adaptability. The 16S rRNA amplification of strain CZ-B1 showed that its molecular weight was 1457 bp. The results showed that the 16S rRNA of strain CZ-B1 had the highest homology with *Bacillus velezensis* (GenBank registration number NR-116240.1) *Bacillus amyloliquefaciens* (GenBank registration number NR-117946.1), and the sequence matching degree were both 99.10% (Supplementary Figure S1). Meanwhile, phylogenetic tree results showed that CZ-B1 and *B. amyloliquefaciens* EGE-B-2d. 1 (registration No. JF926530.1) were on the same branch, and the unit length of difference between sequences was 0.0005 (Figure 1C). Therefore, we confirmed CZ-B1 as a strain of *B. amyloliquefaciens*. The strain CZ-B1 was sent to China General Microbiology Preservation Center for preservation, with the preservation number of CGMCC11.19458.



**FIGURE 1**  
Preliminary screening (A), growth status (B) and phylogenetic tree (C) based on 16S rRNA sequence of CZ-B1.

## 3.2 Phosphorus solubilizing capacity of CZ-B1

Figures 2A, B are divided into photos of CZ-B1 cultured on NBRIP solid medium and PVK solid medium for 7 days. The phosphoric acid solubility index (PSI) of CZ-B1 on NBRIP medium and PVK medium are 2.5 and 2.3 respectively, which can be used to determine that CZ-B1 was a phosphate solubilizing bacteria (Sarkar et al., 2012). In addition, the phosphorus content of liquid medium showed that the amount of dissolved phosphorus in CZ-B1 to HAP medium, PVK medium and NBRIP medium increased with time (Figure 2C). The phosphorus content in all three media increased slowly during the first 6 h, increased rapidly from 6 to 24 h, and then tended to be stable. Moreover, the trend of dissolved phosphorus of CZ-B1 in the three media at 72 h was PVK > NBRIP > HAP, and the dissolved phosphorus in PVK medium was 102.73 mg/L, 1.13 times of that in NBRIP medium.

In PVK medium, the concentration of formic acid, tartaric acid, citric acid and succinic acid increased with the increase of culture time, while the concentration of malic acid increased first and then decreased (Figure 2D). In NBRIP medium, the content of formic acid, tartaric acid, citric acid and malic acid fluctuated continuously, and the content of succinic acid increased with the increase of culture time. After 12 h, the tartaric acid, citric acid and succinic acid in PVK were gradually higher than those in NBRIP, and it was at this time that the phosphorus content in PVK medium was also gradually higher than that in NBRIP medium. Therefore, tartaric acid, citric acid and succinic acid may be the key for CZ-B1 to dissolve  $\text{Ca}_3(\text{PO}_4)_2$  in PVK medium. In addition, the amount of succinic acid secreted in the two media increased with the increase of culture time, which was in perfect agreement with the change trend of

phosphorus content. Therefore, succinic acid may make a great contribution to the dissolution of  $\text{Ca}_3(\text{PO}_4)_2$  by CZ-B1.

## 3.3 Stress tolerance experiments with CZ-B1

The growth curves of CZ-B1 at different temperatures are shown in Figure 3A. The most suitable growth temperature of CZ-B1 was 40°C, and it can keep good survival in the temperature range of 25°C–45°C. At 25°C–40°C, the growth of CZ-B1 tends to be stable after 24 h. However, at 45°C, the growth of CZ-B1 gradually decreased after 24 h and the final  $\text{OD}_{600}$  value was only 51.8% of the  $\text{OD}_{600}$  value at 40°C. Nevertheless, CZ-B1 was able to maintain a high activity between 25°C–40°C, which indicates that CZ-B1 has a good adaptability to temperature.

In the salt stress experiment, the activity of the strain decreased with the increase of NaCl concentration (Figure 3B). When NaCl concentration reaches 15%, CZ-B1 cannot grow. Therefore, the maximum NaCl tolerance concentration of CZ-B1 is 100–150 g/L, which proves that CZ-B1 can grow in a high salinity environment. In addition, the growth of CZ-B1 showed rapid growth within 24 h under the stress of 1%–10% NaCl concentration. After 24 h, CZ-B1 growth remained stable under 1% NaCl stress, grew slowly under 5% NaCl stress, and appeared to decrease under 10% NaCl stress. Although the final  $\text{OD}_{600}$  of CZ-B1 under 10% NaCl stress was only 42.33% of that of 1% NaCl, CZ-B1 was still stable enough to survive. Therefore, CZ-B1 has good salt tolerance.

The  $\text{OD}_{600}$  value (72 h) of CZ-B1 was maximum at pH 7–9 (Figure 3C), and there was no significant difference among the

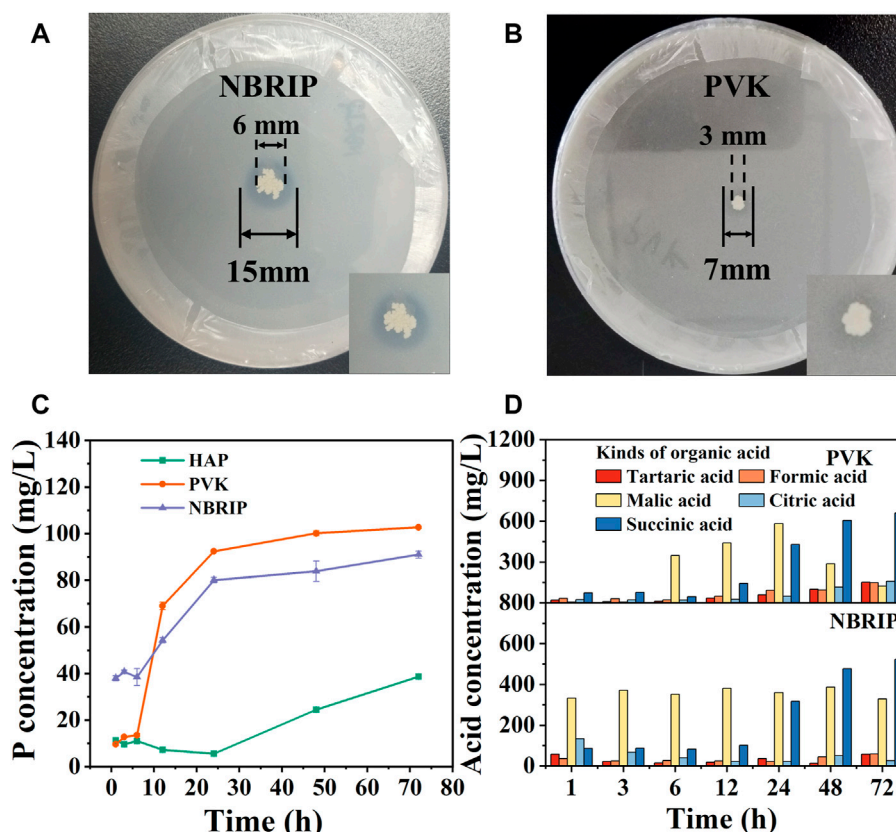


FIGURE 2

The ability of CZ-B1 to dissolve phosphorus and secrete organic acid. Transparent zone of phosphate solubilization on PVK (A) and NBRIP (B) for 7 days of CZ-B1. The soluble phosphorus (C) and organic acid (D) content of CZ-B1 in different media.

three, which indicates that CZ-B1 is more suitable for survival under weak alkaline conditions. With the increase of pH ( $\text{pH} > 10$ ), the growth of CZ-B1 was obviously restricted. Furthermore, although CZ-B1 can survive and maintain a high biomass at pH 9, it needs a longer lag period to adapt to pH stress (1–24 h). The growth rate of CZ-B1 was greatest at pH = 6 and was able to reach the stabilization phase faster. In addition, since CZ-B1 can also survive and maintain high activity in weakly acidic conditions ( $\text{pH} = 5\text{--}6$ ), it may also be adapted to a less acidic environment. Therefore, the phosphorus solubilizing bacteria CZ-B1 can survive in a very wide pH range ( $\text{pH} 5\text{--}9$ ) and the most suitable pH environment for CZ-B1 growth is 7–8.

The growth of CZ-B1 under different  $\text{Pb}^{2+}$  stresses was shown in Figure 3D. When the concentration of  $\text{Pb}^{2+} < 1000 \text{ mg/L}$ , CZ-B1 shows a certain tolerance to Pb. The  $\text{OD}_{600}$  values (72 h) under 500 mg/L and 1000 mg/L  $\text{Pb}^{2+}$  stress were 87.2% and 69.2% of those without stress, respectively. However, when  $\text{Pb}^{2+}$  reached 2000 mg/L, the  $\text{OD}_{600}$  of CZ-B1 at 72 h decreased significantly and was only 16.0% of that without stress, which indicates that the growth of CZ-B1 was significantly inhibited. Although the initial stress effect of 2000 mg/L  $\text{Pb}^{2+}$  concentration on CZ-B1 was obvious, the  $\text{OD}_{600}$  of CZ-B1 gradually increased after 48 h. Therefore, CZ-B1 may need a longer time to adapt to the  $\text{Pb}^{2+}$  stress of 2000 mg/L. Although CZ-B1 cannot survive well at  $\text{Pb}^{2+}$  concentration higher than 3000 mg/L, its excellent tolerance made it have the potential for further domestication.

## 3.4 The mechanism of Pb remediation by CZ-B1

### 3.4.1 Pb removal and organic acid

The strain CZ-B1 has a significant removal effect on Pb while resisting Pb stress (Figure 4A). The removal rate of  $\text{Pb}^{2+}$  in the treatment of 500 mg/L  $\text{Pb}^{2+}$  and 1000 mg/L  $\text{Pb}^{2+}$  by CZ-B1 at 72 h was 90.38% and 39.3% respectively. With the inverse ratio of Pb stress concentration to  $\text{Pb}^{2+}$  removal, this may be due to the fact that increased  $\text{Pb}^{2+}$  toxicity severely inhibits bacterial reproduction and growth functions, thus interfering with their  $\text{Pb}^{2+}$  adsorption. In addition, the removal of  $\text{Pb}^{2+}$  by CZ-B1 under 500 mg/L  $\text{Pb}^{2+}$  stress showed three stages of increase (0–12 h), decrease (12–24 h) and increase (24–72 h). Under the  $\text{Pb}^{2+}$  stress of 1000 mg/L, CZ-B1 was able to adapt to the  $\text{Pb}^{2+}$  stress within 24 h at the earliest and continued to perform its ecological restoration function.

Organic acid secretion is a key process for PSM to dissolve phosphorus sources in the environment (Su et al., 2019). Phosphate dissolved/released by PSM can form stable phosphate minerals with HMs ions (Xiao et al., 2021). In addition, the chelation of organic acids with HMs can reduce the toxicity of HMs, which is also a common way for microorganisms to resist HMs stress on their own (Que et al., 2020). Malic acid, citric acid, oxalic acid, etc. are small molecule organic acids usually secreted by PSM (Rawat et al., 2020; Sarmah and Sarma, 2022). In order to explore the mechanism of  $\text{Pb}^{2+}$  removal by CZ-B1, we determined six organic acids in the solution (Figure 2D). In LB medium without  $\text{Pb}^{2+}$  stress, oxalic acid, citric acid, malic acid and succinic acid, formic acid and



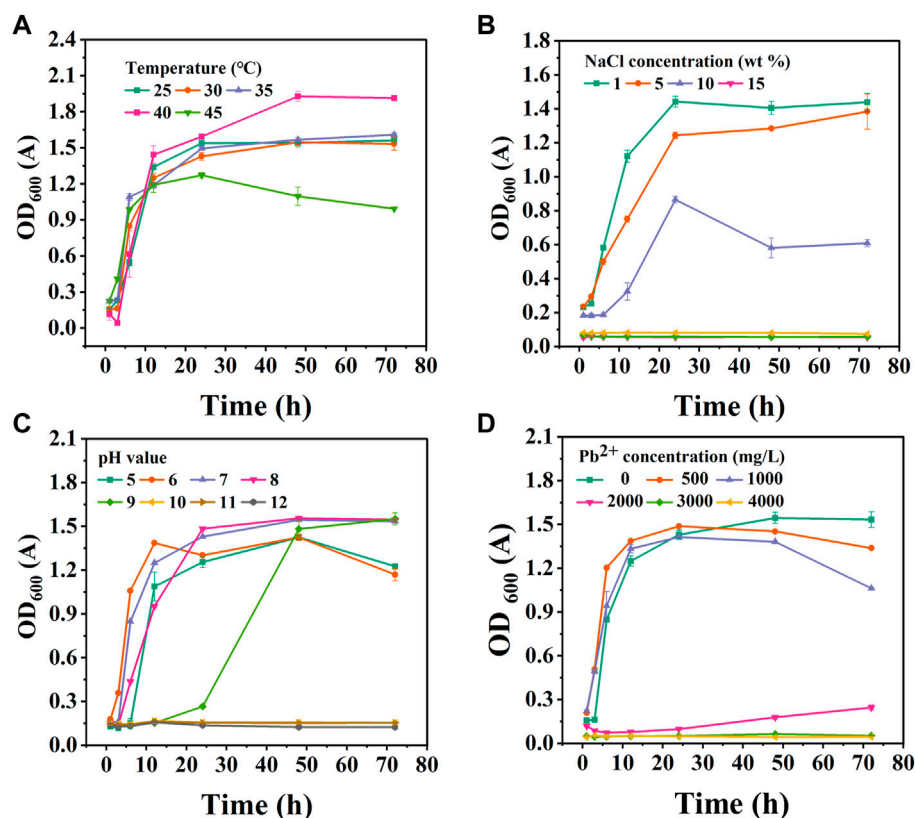


FIGURE 3

The growth status of CZ-B1 under different stress conditions. Temperature effect (A), NaCl stress (B), pH stress (C), Pb<sup>2+</sup> stress (D).

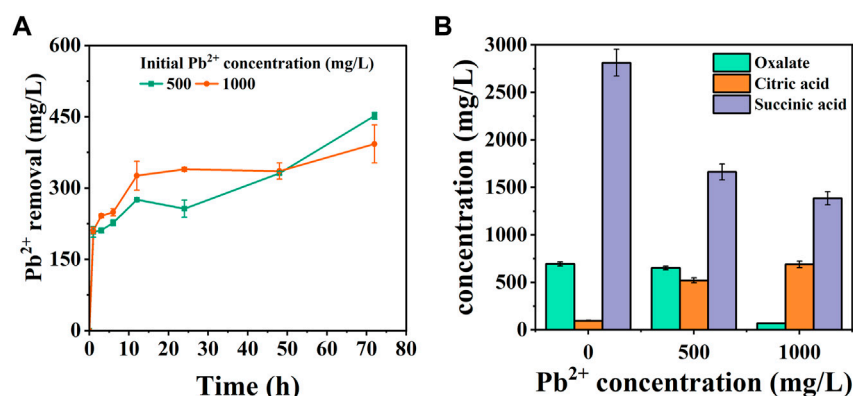
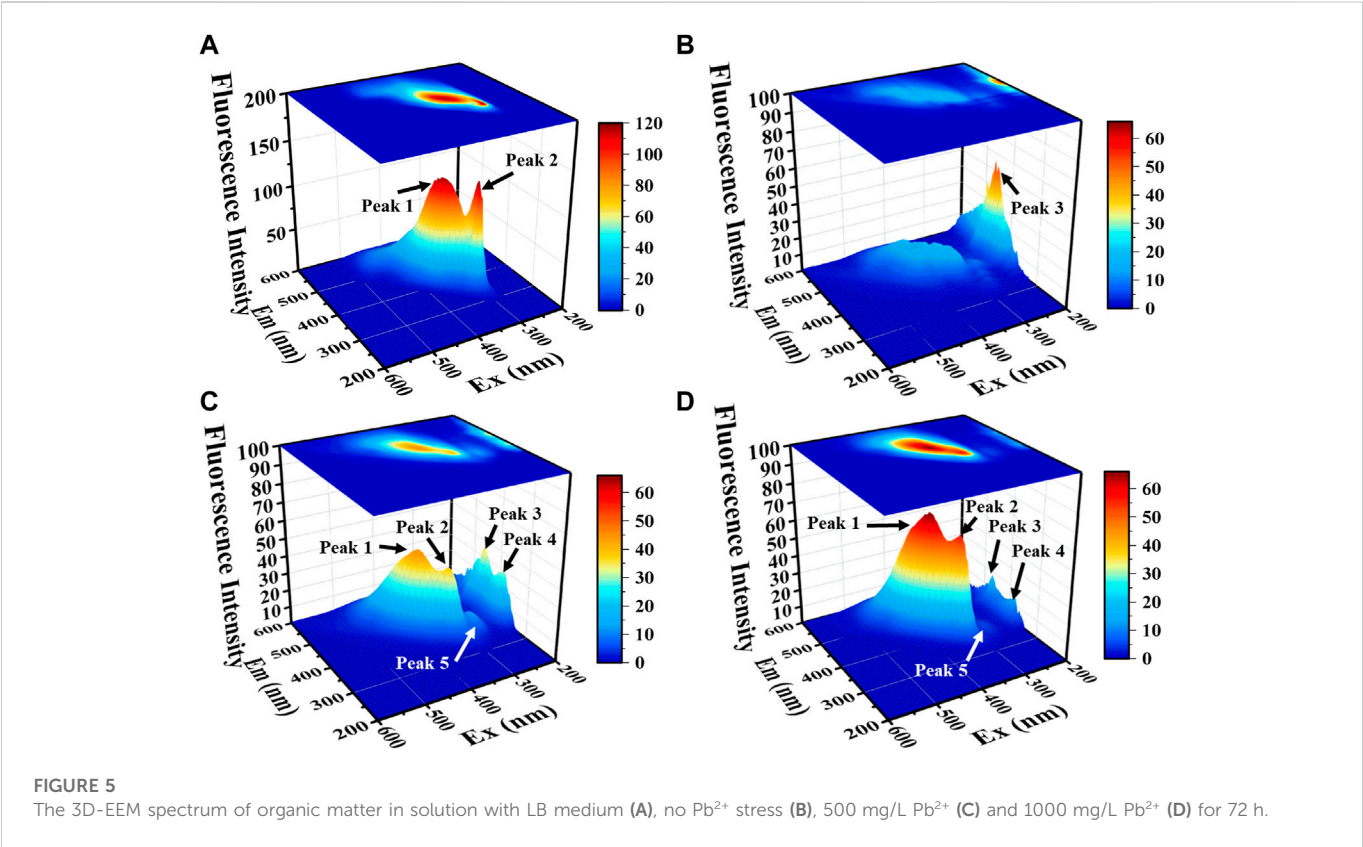


FIGURE 4

Pb<sup>2+</sup> removal amount (A) and organic acid content (B) in solution under 500 mg/L and 1000 mg/L Pb<sup>2+</sup> stress.

tartaric acid were detected in the solution. This result was different from the oxalic acid content in the two inorganic media (Figure 2D), which could be attributed to the different stress of CZ-B1 itself to inorganic and organic phosphorus sources. Among them, the content of malic acid (6902.0 mg/L) > succinic acid (2812.7 mg/L) > citric acid (96.4 mg/L) > oxalic acid (68.7 mg/L) at 72 h (Supplementary Table S1). The maximum level of organic acid release from CZ-B1 in organic medium was significantly higher than that in inorganic medium.

Formic acid, malic acid and tartaric acid were not detected in solution under 500 mg/L–1000 mg/L Pb<sup>2+</sup> stress, which suggesting that Pb stress may directly inhibit the secretion of CZ-B1 for the three acids. Meanwhile, oxalic and succinic acids in solution kept decreasing with increasing concentration of Pb stress, while citric acid gradually increased. The content of oxalic acid and succinic acid in the solution under 1000 mg/L Pb<sup>2+</sup> stress was 10.5% and 83.3% of that under 500 mg/L Pb<sup>2+</sup> stress, respectively, which indicated that the effect



**FIGURE 5**  
The 3D-EEM spectrum of organic matter in solution with LB medium (A), no Pb<sup>2+</sup> stress (B), 500 mg/L Pb<sup>2+</sup> (C) and 1000 mg/L Pb<sup>2+</sup> (D) for 72 h.

**TABLE 2** Unit concentration of different organic acids under 500 mg/L and 1000 mg/L Pb<sup>2+</sup> stress.

Organic acid content per unit OD	Pb <sup>2+</sup> concentration (mg/L)		
	0	500	1000
Oxalic acid (mg/L)	452.08	486.58	64.68
Citric acid (mg/L)	62.87	389.63	649.16
Succinic acid (mg/L)	1834.76	1242.77	1304.42

of Pb stress on oxalic acid content was greater than that on succinic acid content. Also, the increase of citric acid content in the solution (by 32.2%) with increasing Pb<sup>2+</sup> concentration indicates that Pb<sup>2+</sup> coercion stimulates the secretion of citric acid by CZ-B1. In addition, the decrease in oxalic and succinic acid content in solution could both be caused by slowed cellular metabolism and reduced activity or inactivation due to HMs stress (Babu et al., 2015). Studies have shown that environmental stress significantly inhibits the rate of tricarboxylic acid cycle in microorganisms, thereby reducing the secretion of succinate (an intermediate of the tricarboxylic acid cycle) (Guarnieri et al., 2017; Teng et al., 2019). In addition, some studies have shown that oxalic acid secreted by *Aspergillus niger* can form PbC<sub>2</sub>O<sub>4</sub> precipitation with Pb<sup>2+</sup> (Ceci et al., 2015; Feng et al., 2022). Therefore, the reduction of oxalic acid in solution may also be transferred to the precipitate in the form of Pb oxalate. On the other hand, the results of organic acid content per unit cell of CZ-B1 showed that the oxalic acid concentration of unit cell under 1000 mg/L Pb<sup>2+</sup> stress was higher than that under 500 mg/L Pb<sup>2+</sup> stress, which further confirmed that CZ-B1 could still release oxalic acid under Pb stress (Table 2).

3.4.1 3D-EEM result

Figure 5 there are five characteristic peaks were mainly present in the 3D-EEM of CZ-B1, among which peak 1 (Ex/Em 370/450) and peak 2 (Ex/Em 330/400) belonged to humic acid (Baker, 2001; Guo et al., 2014) and fulvic acid (Wang et al., 2009; Lin and Guo, 2020), which were mainly derived from the nutrients in LB. Peak 3 (Ex/Em 200/450) and peak 4 (Ex/Em 200/400) may originate from the secretion of CZ-B1. In addition, there is a small peak 5 at Ex/Em 280/380. Studies have shown that Ex/Em 280/380 may be a by-products of microorganisms (Chen et al., 2003) or tryptophan (Baker, 2001). When the Pb<sup>2+</sup> concentration increased from 500 mg/L to 1000 mg/L, the fluorescence intensity of peak 1 and peak 2 increased by 44.8% and 47.1%, respectively. Meanwhile, peak 3, peak 4, and peak 5 from microbial secretions were decreased by 50.1%, 52.6% and 60.0%, respectively. It can be concluded that the metabolic activity of the microorganisms decreased and the consumption of nutrients diminished with increasing Pb stress concentration and that microbial by-products such as tryptophan might be produced simultaneously under stress conditions.

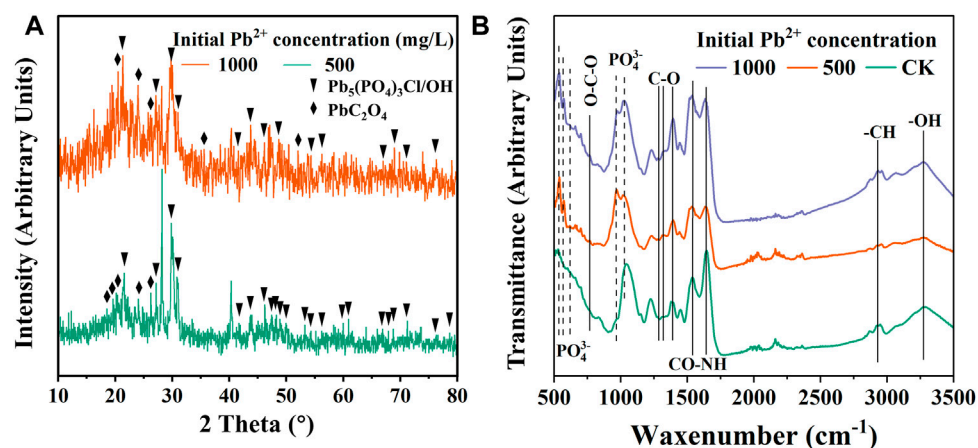


FIGURE 6

XRD (A) and ATR-IR (B) of precipitate under 500 mg/L Pb<sup>2+</sup> and 1000 mg/L Pb<sup>2+</sup> stress for 72 h.

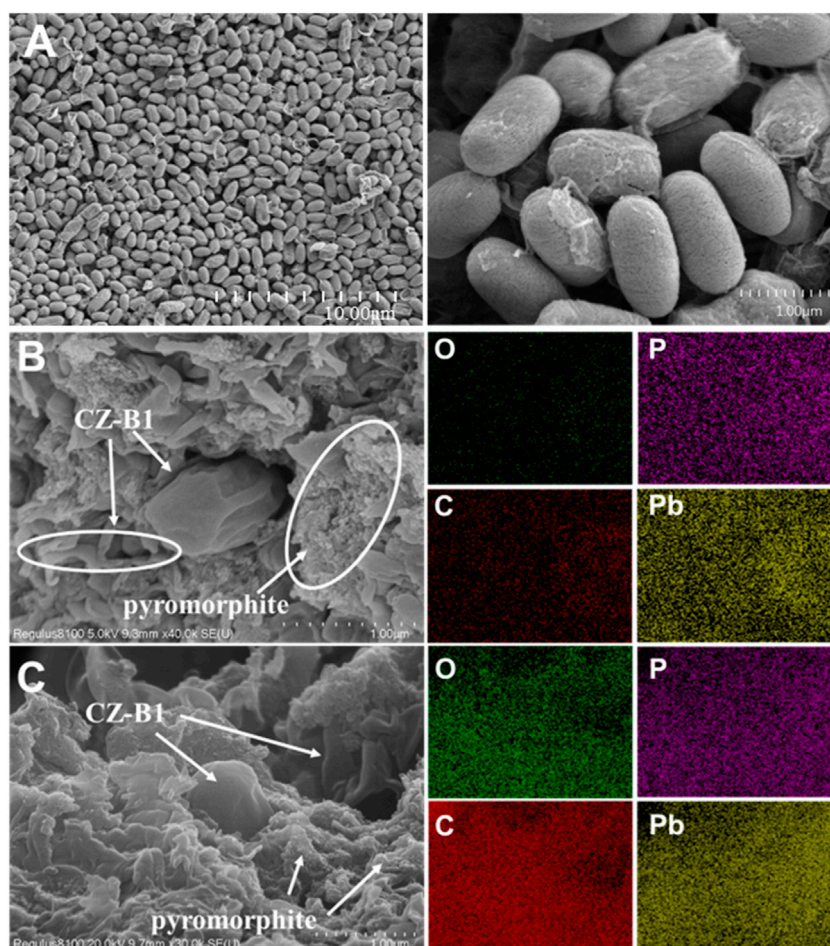


FIGURE 7

The SEM and EDX of precipitate under no Pb<sup>2+</sup> stress (A), 500 mg/L Pb<sup>2+</sup> (B) and 1000 mg/L Pb<sup>2+</sup> (C) for 72 h.

Tryptophan as a precursor of indoleacetic acid (Shahid et al., 2012), both of which possess the property of promoting phosphorus solubilization (Ji et al., 2012; Alemneh et al., 2021), which

undoubtedly provides a gaining effect for Pb<sup>2+</sup> passivation. Therefore, the increase of tryptophan can indirectly prove the enhanced bioimmobilization of Pb<sup>2+</sup> by CZ-B1. This



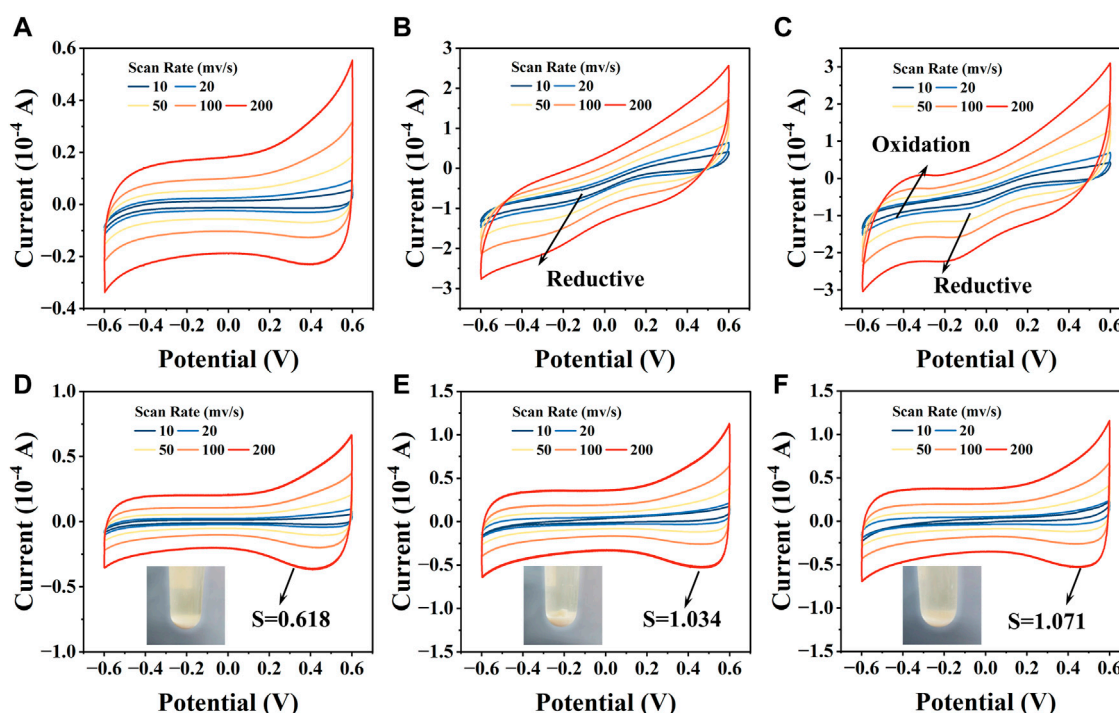


FIGURE 8

The CV curves of cell precipitates and EPS under no  $\text{Pb}^{2+}$  stress (A, D), 500  $\text{Pb}^{2+}$  (B, E), and 1000  $\text{Pb}^{2+}$  (C, F) stress.

phenomenon also proves that under low-concentration stress, CZ-B1 are more inclined to use their own cells for  $\text{Pb}^{2+}$  fixation.

### 3.4.2 XRD, ATR-IR and SEM result

XRD results showed that Pb-phosphate and Pb-oxalate were present in the sediment (Figure 6A). Many strong diffraction peaks were present at  $29.8^{\circ}$ – $30.9^{\circ}$ ,  $21.6^{\circ}$ ,  $26.3^{\circ}$ – $27.2^{\circ}$  and  $43.9^{\circ}$  in both concentrations of  $\text{Pb}^{2+}$  stress, which may be attributed to pyromorphite  $[\text{Pb}_5(\text{PO}_4)_3\text{Cl}/\text{OH}]$  (Meng et al., 2020). Meanwhile, the diffraction peaks at  $20.78^{\circ}$ ,  $23.6^{\circ}$ ,  $26.4^{\circ}$  and  $35.5^{\circ}$  indicate the formation of  $\text{PbC}_2\text{O}_4$  (Choi et al., 1994; Ma et al., 2016). Compared with 1000 mg/L  $\text{Pb}^{2+}$  stress, the pyromorphite peak signal of CZ-B1 under 500 mg/L  $\text{Pb}^{2+}$  stress was more significant. The formation of pyromorphite indicates that CZ-B1 can fix  $\text{Pb}^{2+}$  by releasing phosphorus. However, the Pb oxalate signal under 1000 mg/L  $\text{Pb}^{2+}$  stress is lower than that under 500 mg/L  $\text{Pb}^{2+}$  stress. Therefore, according to the oxalic acid content in solution under different Pb stress (Figure 4A), the decrease of oxalic acid under high concentration Pb stress may be more attributed to the decrease of cell number of CZ-B1 than to Pb oxalate precipitation. However, the formation of Pb phosphate and Pb oxalate can reduce the Pb concentration in the environment, which is also one of the main mechanisms of CZ-B1's resistance to Pb stress.

ATR-IR results showed (Figure 4B) that the cell sediment under Pb stress increased the peak position related to  $\text{PO}_4^{3-}$  at  $565\text{ cm}^{-1}$ ,  $603\text{ cm}^{-1}$ ,  $965\text{ cm}^{-1}$  (Botto et al., 1997; Wei et al., 2021). In addition, the O-C-O stretching vibration peak of  $782\text{ cm}^{-1}$  (Mancilla et al., 2009) and the C-O bond peak of  $1289\text{ cm}^{-1}$ ,  $1312\text{ cm}^{-1}$ ,  $1386\text{ cm}^{-1}$  were enhanced (Mancilla et al., 2009; Salavati-Niasari et al., 2009). O-C-O and C-O may be related to Pb oxalate (Mancilla et al., 2009).

This result further proves that there are Pb phosphate compounds and Pb oxalate compounds in the cell sediments under Pb stress. In addition, the peaks representing amides and vibration peaks of saturated aliphatic ( $-\text{CH}$ ) substances were observed at  $1533\text{ cm}^{-1}$ ,  $1640\text{ cm}^{-1}$  and  $2930\text{ cm}^{-1}$  (Doshi et al., 2007; Wang et al., 2020), indicating that the hydrocarbon functional groups and cellular proteins produced by CZ-B1 stress, which may be attributed to pyromorphite also participated in  $\text{Pb}^{2+}$  adsorption.

SEM images showed not only the presence of intact cells of CZ-B1 in the precipitate, but also the presence of obvious mineral grains (Figure 7). CZ-B1 cells are rod-shaped, about  $2\text{ }\mu\text{m}$ , the surface is smooth (Figure 7A). Under  $\text{Pb}^{2+}$  stress, CZ-B1 cells appear deformation and shrinkage to varying degrees, and the cell morphology was significantly smaller, which indicates that Pb stress hinders the growth and development of CZ-B1 (Figures 7B, C). In addition, the individual CZ-B1 cell becomes smaller under the stress of 500 mg/L  $\text{Pb}^{2+}$ , but the cell surface was smooth. The deformation of CZ-B1 under 1000 mg/L  $\text{Pb}^{2+}$  stress was more serious than that under 500 mg/L  $\text{Pb}^{2+}$  stress, and even broken cell remains were present in the precipitate (Figure 7C). In addition, EDX spectroscopy found that there were sharp particles with highly overlapped P and Pb signals around CZ-B1 cells, which may form pyromorphite. However, the pyromorphite signal was stronger under 1000 mg/L  $\text{Pb}^{2+}$ , so more Pb phosphate mineral precipitation may be produced under the stress of high concentration of Pb. Combined with the OD values it can be confirmed that under high  $\text{Pb}^{2+}$  stress, the solubilization and utilization of P by CZ-B1 may be preferentially supplied to mineral precipitation rather than used by itself. The resistance mechanism of microorganisms under high stress may be dominant over the reproduction mechanism.

### 3.4.3 Electrochemical analysis results

The CV curves of CZ-B1 cell precipitates showed no significant redox peak positions in the absence of stress (Figure 8A). The CV curves under 500 mg/L  $Pb^{2+}$  stress showed significant reduction peaks between  $-0.4$  V and  $-0.2$  V, but not significant oxidation peaks (Fig. B). When  $Pb^{2+}$  stress was increased to 1000 mg/L, the reduction peak position of the CV curve shifted to between  $-0.4$  V and  $0.0$  V, and a clear oxidation peak appeared around  $-0.4$  V (Figure 8C). The generation and loading of minerals can change the electron transfer capacity of cell (Zhang et al., 2021), so the shift in the redox peak position of the CV curve may originate from the Pb minerals generated by CZ-B1. In addition, there were no significant oxidation or reduction peaks on the CV curves of the three different treatments of EPS (Figure 8D, E, F). However, the EPS peak area of CZ-B1 increased significantly by 67.15%–73.16% under Pb stress, which suggests that EPS may assist CZ-B1 in the removal of  $Pb^{2+}$  through adsorption. In addition, the area of CV closure increased continuously with increasing Pb stress concentration. The enrichment of  $Pb^{2+}$  by EPS was beneficial to increase the extracellular electron transfer rate and accelerate the mineralization response of cells to  $Pb^{2+}$ , which is consistent with Figure 8C and XRD results. Compared to the cells, the main change in the CV curve of EPS was the peak area rather than the redox peak position. Therefore, we speculate that EPS mainly acts as an enriched adsorption for  $Pb^{2+}$  rather than generating Pb minerals on it. It is worth mentioning that the mineralization and precipitation of cells to  $Pb^{2+}$  were improved precisely because  $Pb^{2+}$  was adsorbed around CZ-B1 cells by EPS.

In summary, the resistance and repair mechanism of CZ-B1 to Pb stress may be mainly from three aspects: 1) CZ-B1 can combine with  $Pb^{2+}$  to form phosphate, succinic acid and oxalate minerals *via* secreted citric, succinic and oxalic acids. 2) CZ-B1 can secrete tryptophan and other microbial products for complexation to enhance the absorption of  $Pb^{2+}$ , thus weakening the stress of  $Pb^{2+}$  on itself. 3) CZ-B1 adsorbs and enriches  $Pb^{2+}$  through pericellular and surface EPS to increase the extracellular electron transfer rate, which can increase the reaction rate of CZ-B1 with  $Pb^{2+}$  mineralization and promote mineral precipitation.

## 4 Conclusion

In this study, a phosphorus solubilizing bacteria strain CZ-B1 with Pb and salt tolerance was successfully screened. CZ-B1 is highly adaptable to temperature and can survive from  $25^{\circ}\text{C}$  to  $45^{\circ}\text{C}$  (the optimum temperature is about  $40^{\circ}\text{C}$ ). CZ-B1 can survive in alkaline environment with  $\text{pH} < 10$  and high salt environment with NaCl concentration  $< 15\%$ . In addition, the solubility of CZ-B1 for insoluble phosphorus source  $\text{Ca}_3(\text{PO}_4)_2$  could reach 102.8 mg/L within 72 h. Its efficient solubility for insoluble phosphorus source was attributed to succinic acid. The removal rate of 500 mg/L  $Pb^{2+}$  by CZ-B1 in liquid LB medium can reach 90.38%. With the increase of Pb concentration, the number of CZ-B1 cells decreased significantly, which greatly affected its cell adsorption function. However, the release of organic acids by CZ-B1 under Pb stress was very different from that under no Pb stress. Therefore, in addition to cell adsorption, the resistance/repair mechanism of CZ-B1 to Pb stress may be related to organic acids. The cells mainly secrete oxalic acid, citric acid and succinic acid under 500–1000 mg/L  $Pb^{2+}$  stress. Among them, citric acid increased with the increase of  $Pb^{2+}$

stress concentration, which may be the main mechanism of CZ-B1 resistance to  $Pb^{2+}$  stress. In addition, XRD and SEM results indicate that CZ-B1 reduces  $Pb^{2+}$  toxicity mainly through the formation of a large amount of pyromorphite minerals and a small amount of Pb oxalate precipitation. The formation of phosphate and oxalate benefited from the release of succinic acid and oxalic acid from CZ-B1, respectively. More than that, CV results showed that Pb minerals were mainly concentrated on CZ-B1 cells and Pb stress could affect the electron transfer process of CZ-B1. Therefore,  $Pb^{2+}$  may undergo mineralization reactions on or inside the cell after EPS enrichment.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

## Author contributions

HC and ZH contributed to conception and design of the study. CZ, YD, YC, and XT performed the statistical analysis. CZ and HC wrote sections of the manuscript. HC and ZH directed, supervised, coordinated, and corrected the review. All authors contributed to manuscript revision, and read and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fbioe.2023.1134310/full#supplementary-material>



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# Biochar: An effective measure to strengthen phosphorus solubilizing microorganisms for remediation of heavy metal pollution in soil

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## 1 Introduction: Phosphorus solubilizing microbial remediation is a sustainable technology for soil heavy metal remediation

### 1.1 Microbial remediation is an effective measure to achieve sustainable remediation of heavy metals in soil

Heavy metals (HMs) pollution of soil caused by human activities is a serious threat to human health and sustainable social development. The insidious, lagging, long-term, inhomogeneous and irreversible nature of heavy metal pollution has led to serious degradation of soil ecological structure and function. Most importantly, heavy metals in soil can be enriched into animals or humans through the food chain (food crops), threatening human health and life (Jan et al., 2015). Therefore, remediation of heavy metals for soils has been one of the key issues in the field of environmental remediation. Compared with physical or chemical remediation technologies, microbial remediation technology is gradually recognized for its green, low cost, easy operation and long sustainability (Maity et al., 2019). Although most HMs are difficult to degrade and remove by microorganisms in soil, microorganisms (microbial uptake, transformation, mineralization or immobilization) can convert HMs to less toxic forms or reduce their mobility (Kotrba and Ruml, 2000; Lin et al., 2023). Among them, biomineralization is a common and effective method for remediation of HMs pollution in soil, which is mainly through the interaction between microorganisms and HMs to form mineral crystals (such as, phosphate, carbonate, sulfate, arsenate, fluoride, oxide, hydroxide, and manganese oxide, etc.) outside, between or within the cells of microorganisms (Tayang and Songachan, 2021; Lin et al., 2023). Not only that, among up to 60 kinds of biomineralization products, metal phosphates have been of concern due to their high stability.

## 1.2 Mineralization allows phosphorus solubilizing microorganisms (PSM) to be successfully used for the solidification/stabilization of HMs in soil

PSM are known for their ability to convert the insoluble state of phosphorus (e.g., iron phosphorus, calcium phosphorus, highly stable organic phosphorus, etc.) in the environment into a form that can be used directly by organism (Qian et al., 2019; Deb et al., 2016). The recognized mechanisms of phosphorus dissolution by PSM can be classified into two categories according to the type of phosphorus source. Firstly, dissolution of inorganic phosphorus sources by means of low molecular weight organic acid release, proton secretion to generate  $H^+$ , and direct oxidation (Gadd, 2010; Wang YY. et al., 2020). Secondly, the organophosphorus source is solubilized by the production of extracellular enzymes, such as phytase, phosphatase/carbon phosphorus lyase (Rawat et al., 2020). It is noteworthy that PSM is widely used for solidification and stabilization of HMs in soil because the  $PO_4^{3-}$  released by PSM can combine with HMs to form stable phosphate precipitation. In addition, PSM can also repair HMs pollution through adsorption and transformation. For instance, PSM is capable of biosorption, accumulation and complexation of HMs ions by cells themselves (extracellular adsorption, surface adsorption and intracellular absorption), surface functional groups and secreted organic substances (extracellular polymers, glutathione, etc.) (Ahemad, 2015). Alternatively, the valence of HMs is changed and their toxicity is reduced by the metabolic action of the PSM themselves (e.g., redox, methylation, etc.) (Gadd, 2004; Vassileva et al., 2010). However, unstable HMs forms (such as biosorption, complexation, etc.) that remain in the soil for a long time will inevitably be subject to environmental changes and transform again into bioavailable forms. Therefore, mineralization precipitation is the key to guarantee that PSM reduce the mobility and bioavailability of HMs in the soil, which gives PSM a place in the field of microbial solidification and stabilization remediation.

## 2 The solubilizing effect of PSM makes it difficult to remediate high concentrations of soil HMs pollution alone

Although PSM have a good remediation effect on soil HMs, the environment with high concentrations of HMs still seriously threatens the normal microbial metabolism process (Chen X. et al., 2020). For example, HMs ions can damage protein folding or the combination of cofactors and enzymes by binding with sulfhydryl groups on proteins, thus damaging the normal biological activity of proteins (Sharma and Melkania, 2018). Also, the hydroxyl radicals and reactive oxygen species (ROS) formed by the transition metals participating in Fenton reaction can also destroy biological macromolecules (He et al., 2017). Most notably, the solubilization of PSM is a “double-edged sword”. Organic acids secreted by PSM, such as formic acid, acetic acid, citric acid, oxalic acid, etc., can cause changes in soil pH and redox potential, and promote the weathering and dissolution of HMs minerals in soil (Ahemad, 2015). The solubilizing effect of PSM

on HMs undoubtedly greatly increased the difficulty of PSM to repair HMs in soil alone. Herein, seeking a facile but efficient technology to make a synergistic interaction with PSM for boosting of HMs remediation efficiency is challenging but of great significance.

## 3 Biochar is a green and sustainable material that can assist phosphorus-dissolving microorganisms to remediate HMs in soil

### 3.1 Biochar is a promoter to enhance soil microbial remediation

Biochar is a carbon-rich material obtained by pyrolysis of biomass under anoxic conditions, which exhibits excellent adsorption and solidification effects on soil HMs. Multiple studies have demonstrated that surface complexation, electrostatic adsorption, ion exchange, mineral precipitation, etc. are the main mechanisms for the adsorption of HMs by biochar (Chen et al., 2022). As an excellent green sustainable material, biochar still has long-term stability to HMs, even when it faces the effects of aging in the environment (Wang L. et al., 2020), which is certainly a guarantee for its own application. On the other hand, adding biochar to soil will have a positive impact on soil environment, including soil aggregate, pH value, nutrient conservation, and improvement of microbial community (Shaaban et al., 2018). It is of interest that the porous structure of biochar can provide a good refuge for microorganisms inside the soil from HMs attacks. Meanwhile, the rich nutrients of biochar itself can also guarantee the reproduction of microorganisms (Cooper et al., 2020). Blanco-Vargas et al. (2022) also confirmed that biochar is an excellent biological inoculant to support the growth of phosphate-solubilizing bacteria. Therefore, the use of biochar to improve the efficiency of soil microbial remediation is a more effective strategy.

### 3.2 Toxicity attenuation and mineral precipitation mechanisms of biochar are key to the protection of phosphorus-dissolving microorganisms

As mentioned earlier, it is because of its ability to reduce the transport, leaching and biotic stress of potentially toxic elements in soils that biochar has been successfully applied to assist microbial remediation of HMs contamination in soil. For example, pine cone biochar enhanced inorganic ion transport and metabolic rate by increasing the number of bacilli and immobile bacteria, thus in promoting the passivation and stabilization of HMs (Lan et al., 2021). Sludge and rice husk biochar improved phosphorus solubilizing bacteria (*Enterobacter* sp.) remediation efficiency and resistance to  $Pb^{2+}$  (217%–700%) and  $Cd^{2+}$  (85%–201%) under high concentration (1,000 mg/L) stress (Chen et al., 2019; Chen et al., 2020 H.). The combination of waste fungus chaff-based biochar and *Herbaspirillum huttiense* was able to solidify 85.5%  $Cu^{2+}$  and 64.4%  $Zn^{2+}$  (total  $Cu^{2+}$  and  $Zn^{2+}$  in background soil: 218.2 mg/kg and 427.1 mg/kg) (Zhang X. et al., 2022). *Bacillus cereus* with biochar



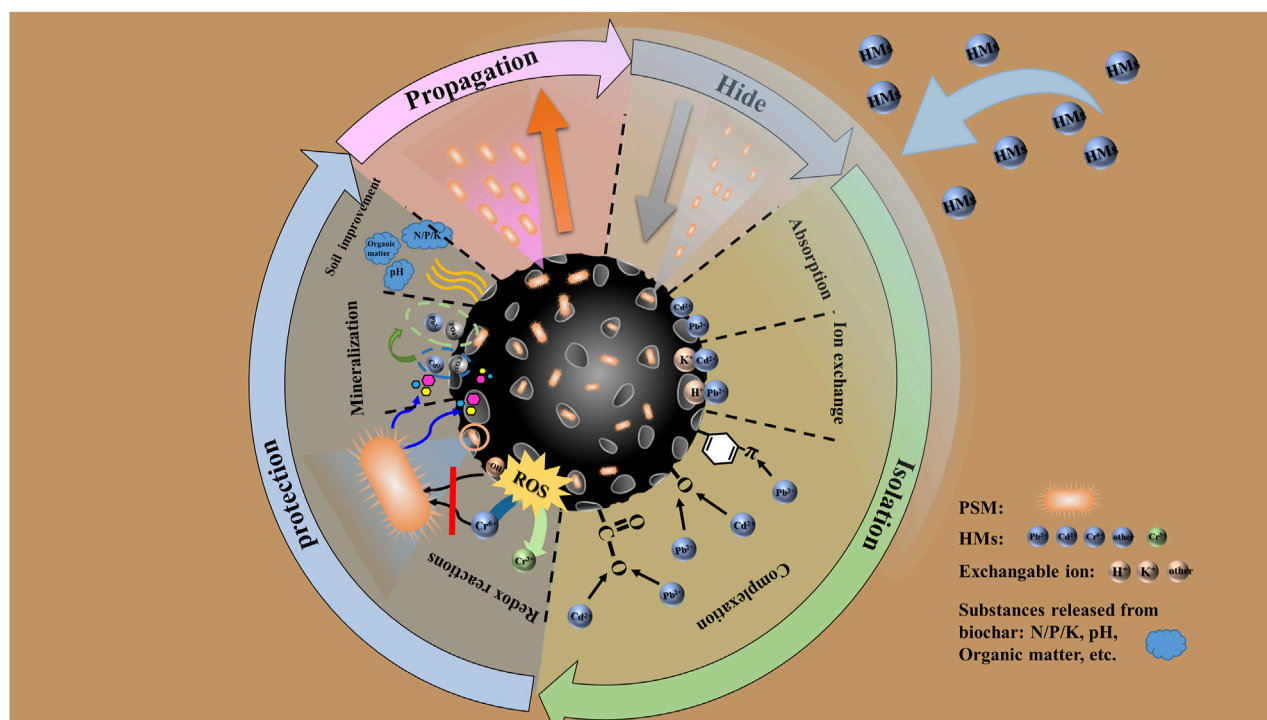


FIGURE 1

Schematic diagram of the mechanism of biochar-assisted phosphorus solubilizing microorganisms for remediation of heavy metals contamination in soil.

converted 94.22% of Cr(VI) to Cr(III) in the soil and increased the residual Cr by 60% (Chen et al., 2021). Arbuscular mycorrhizal fungi and biochar enhanced the immobilization of Cr and reduced the Cr(VI) concentration by 0.3%–64.5% (Chen et al., 2022). Some scholars have also used Fe modified biochar with PSM to mineralize  $Pb^{2+}$  to  $Pb_5(PO_4)_3Cl$  and  $Pb_5(PO_4)_3OH$  (Teng et al., 2019). In general, the protection mechanism of biochar for PSM can be divided into four aspects: hiding, isolation, protection, and proliferation Figure 1.

First, biochar has abundant grooves and large pores on the surface and inside respectively, which allow microorganisms to hide in them (Zhang R-H. et al., 2022). Secondly, biochar reduces the biological effectiveness of HMs through surface adsorption and complexation control mechanisms (e.g., cation exchange, functional groups and C- $\pi$  interactions, etc.), which sufficiently reduce the exposure of HMs to microorganisms (Chen et al., 2022; Gong et al., 2022). Thirdly, the redox effect of biochar itself can change the valence state of variable HMs ( $Cr^{6+}$ ,  $As^{3+}$ ,  $Hg^{2+}$ ) and accelerate electron transfer, which weakens the toxicity of toxic HMs (Gong et al., 2022; Lin et al., 2023). Also, biochar is able to induce the toxic groups (ROS) caused by the valence shift of HMs in advance outside the cell. Most importantly, biochar completely prevents HMs stress on PSM by providing C, P and other elements to form stable mineral precipitates with HMs (including those dissolved by PSM) (Chen et al., 2019; Lai et al., 2022). In addition, biochar can improve the physicochemical properties of the soil such as organic carbon, pH, CEC, agglomerates, etc., which may lead HMs to the formation of

organic complexes to be immobilized (Zheng et al., 2022; Fu et al., 2023). For example, biochar can improve nutrient retention and reduce leaching by increasing soil CEC (Laird et al., 2010), thereby providing nutrient reserves for microorganisms. Biochar provides lasting benefits to microorganisms by slowly releasing its own dissolved organic carbon (a nutrient for microorganisms) (Zheng et al., 2022). Finally, the protected PSM multiply in large numbers by virtue of nutrients from biochar. This allows the bacteria to dissolve the inorganic phosphate source (e.g., apatite minerals) or the organic phosphate source (e.g., phenolphthalein diphosphate, phytate, glycerophosphate) again to mineralize the heavy metals, thus sustaining the bioremediation effect (Jiang et al., 2020). On the other hand, biochar is used by PSM as a platform to form a fortress for solidifying and stabilizing HMs. Considering the dissolution/release of HMs by bacteria themselves, the mechanism of toxicity attenuation and stable mineral precipitation provided by biochar enables PSM to cope with toxic stresses of both variable (Cr, Hg, Cu, As, etc.) and non-variable (Cd, Zn, etc.) HMs. In particular, biochar weakening the toxicity mechanism of variable heavy metals can give microorganisms the time to resist the stress. For example, biochar directly reduces Cr(VI) to the less toxic Cr(III) via phenolic-OH (Xu et al., 2019). In conclusion, PSM have the potential to remediate high concentrations of HMs contamination with the support of biochar, and biochar with high P content (sludge, pig manure, etc.) can give more support.



## 4 Prospects

PSM have gradually attracted attention as an important tool for soil remediation of HMs contamination. Especially in recent years, relevant studies have confirmed that PSM can be applied in the remediation of agricultural soil and industrial sites with many different HMs. Although a number of studies have confirmed its mechanism of remediation of HMs, most of them have found that the application of PSM is often considerably less effective in scenarios with high concentrations of pollution. Biochar, as a green and friendly soil remediation agent, has been shown to enhance the survival of PSM in high concentrations of HMs. However, most biochar co-remediation with PSM has been performed in the laboratory and for a single HMs contamination. Therefore, future PSM combined biochar remediation should be tested in actual or simulated application scenarios, and used for remediation of multiple HMs combinations of pollution. In addition, the incorporation of various environmentally friendly modification techniques to assist biochar in promoting the remediation function of PSM is also a promising approach. On the other hand, the affinity of biochar with microorganisms and effectiveness of their practical applications will also be a challenge for its joint application. There is a need for us to develop large-scale applications of combined remediation technologies and to enhance the scenarios in which biochar combined with PSM remediation technologies can be applied. Finally, the riskiness of the remediated soil for HMs at long time scales also needs to be considered in order to minimize adverse consequences.

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## Conflict of interest

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# Lead remediation is promoted by phosphate-solubilizing fungi and apatite *via* the enhanced production of organic acid

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Lead (Pb) is one of the most common heavy metal pollutants in the environment, which can indirectly or directly threaten human health. Lead immobilization by apatite can reduce the effectiveness of Pb cations *via* the formation of pyromorphite (Pyro). However, the formation of Pyro is always depending on the release of phosphorus (P) from apatite. Phosphate-solubilizing fungi (PSF) can secrete large amounts of organic acid to promote the release of P from apatite. Although the combination of PSF and apatite has shown a huge potential in Pb remediation, this pathway needs to be more attention, especially for organic acid secretion by PSF. This research mainly reviews the possible pathway to strengthen Pb immobilization by PSF and apatite. Meanwhile, the limitation of this approach is also reviewed, with the aim of a better stabilizing effect of Pb in the environment and promoting the development of these remediation technologies.

## KEYWORDS

lead remediation, apatite, phosphate solubilizing fungi, organic acid, phosphorus release

## 1 Introduction

Lead (Pb) is one of the most important heavy metal pollutions in the environment, which has strong biological toxicity, wide distribution, and strong accumulation capacity (Arduini et al., 2010). The completely remove of Pb cations from soil is relatively long and complex due to the hidden and lagging performance (Shen et al., 2015). In-suit immobilization of Pb is an efficient pathway to reduce the toxicity of Pb in soil (Chen et al., 2006). Phosphate can effectively transfer Pb cations to highly insoluble Pb minerals *via* the phosphorus (P) release (Li et al., 2016b; Tian et al., 2018). However, the process of P release is unsustainable and easily chelates with metal cations in soil, e.g., Ca<sup>2+</sup>, Fe<sup>3+</sup>, etc (Tian et al., 2021a). The combination of phosphate solubilizing fungi (PSF) and phosphate is an effective and sustainable pathway in Pb in suit immobilization (Shao et al., 2021). As a new approach in Pb remediation, this technology needs to be more attention nowadays.

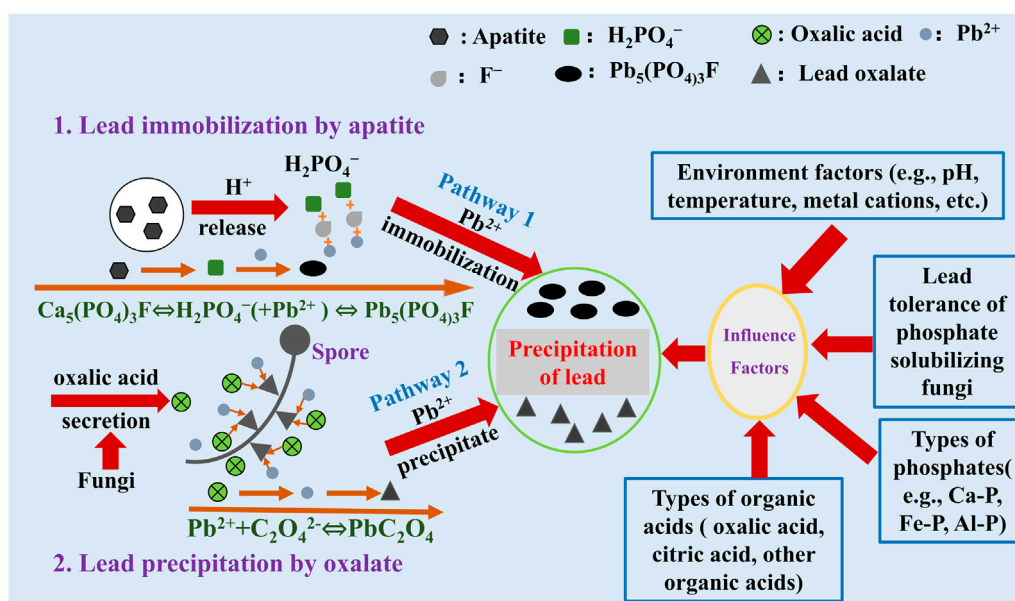
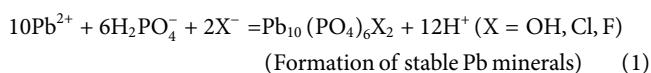


FIGURE 1

Lead remediation by phosphate solubilizing fungi and phosphate via the secretion of organic acid.

## 2 Mechanism of Pb remediation by phosphate solubilizing fungi and phosphate

In current, phosphate is generally recognized as an excellent material in Pb remediation. The P released from phosphate can react with Pb to form highly insoluble pyromorphite (Pyro) Figure 1. Pyro is highly stable and has a low  $K_{sp}$  value ( $<10^{-85}$ ), which can significantly reduce Pb toxicity and mobility in soil (Arnich et al., 2003; Chaturvedi et al., 2007; Cheyns et al., 2012). In engineering, field and indoor leaching simulation tests, phosphates can convert Pb from high-activity forms to insoluble forms (John et al., 2001; Chen et al., 20231). The application of phosphate can reduce 12%–92% available Pb content in soil, and the toxicity characteristic leaching procedure Pb (TCLP-Pb) concentration can decrease from 82 mg/L to less than 5 mg/L (Melamed et al., 2003; Cao et al., 2009; Cui et al., 2010). The reaction formula between P and Pb is as follows (Park and Bolan, 2013):



An acidic environment can significantly enhance phosphate dissolution and improve the release of P (Whitelaw, 1999; Singh and Reddy, 2011). However, the addition of chemical acid (e.g., sulfuric acid) is unsustainable and harmful to soil health. Oxalic acid is more efficient than sulfuric acid in phosphate dissolution (Mendes et al., 2020). Therefore, the utilization of oxalic acid and phosphate is a better choice in Pb remediation. Phosphate solubilizing fungi (PSF) can secrete large amounts of oxalic acid and promote the release of P from insoluble phosphate (Li et al., 2016a). Compare with bacteria, PSF not only maintains the ability of oxalic acid secretion but also can extend in soil via

the mycelium. For example, the PSF of *Aspergillus niger* can promote the dissolution of FAp and carbonate in soil via the hypha extension and oxalic acid secretion (Tian et al., 2021b). In addition, the PSF of *A. niger*, *Penicillium oxalicum*, and *Penicillium aurantiogriseum*, etc., also has a strong ability to secrete oxalic acid (Tian et al., 2021a; Hu et al., 2022; Wang et al., 2022). Therefore, the combination of PSF and phosphate is a considerable pathway in Pb remediation.

The application of PSF and phosphate have been successfully applied in Pb remediation. *A. niger* and *P. oxalicum* combined with FAp can significantly remove more than 90% Pb cations in an aqueous solution via the formation of lead oxalate and Pyro (Li et al., 2016b; Tian et al., 2018). Meanwhile, Pb remediation in soil by this combination not only promotes the formation of lead oxalate but also increased the soil available P content (Tian et al., 2022b; Meng et al., 2022). In addition, the released P can be also isolated by PSF and not easily absorbed by plants, promoting the Pb remediation process (Menezes-Blackburn et al., 2016). However, the secretion of oxalic acid by PSF is usually influenced by different factors, such as pH, nutrients, phosphate types, and Pb concentration (Tian et al., 2019; Feng et al., 2022). Therefore, the appropriate technology and conditions are needed in Pb remediation by PSF and phosphate.

## 3 Effect factors in Pb remediation by PSF and phosphate

### 3.1 Pb tolerance of phosphate solubilizing fungi

Pb remediation by PSF is usually affected by different Pb toxicity. The excessive concentration of Pb cations can limit the growth of fungi and reduce their bioremediation efficiency (Ye et al.,



2018). However, the tolerance of Pb toxicity in PSF is different. *A. niger* has a higher Pb tolerance than *P. oxalicum* (Tian et al., 2018; Tian et al., 2019). *A. niger* can survive under 1,500 mg/L Pb concentration and maintain the ability of oxalic acid secretion (Tian et al., 2019). However, the PSF of *P. oxalicum* only resists 1,000 mg/L Pb concentration, and the secretion of oxalic acid is almost lost under 1,500 mg/L Pb concentration (Tian et al., 2019). Therefore, *A. niger* has a high Pb tolerance and is efficient in Pb remediation.

## 3.2 Suitable phosphate types in Pb remediation

The type of phosphate affects the efficiency of Pb remediation mainly due to the P release capacity (Tian et al., 2021a). Hence selecting an appropriate phosphate is important in Pb remediation. The use of phosphates in Pb remediation usually contains water-soluble phosphates (WSP) and insoluble phosphates (IPs), including potassium dihydrogen phosphate, sodium dihydrogen phosphate and hydroxyapatite, fluorapatite bioapatite, etc. WSP has a high solubility of P and is efficient in Pb remediation. However, the use of WSP is easy to cause eutrophication of water and the excessive P can be fixed by metal cations in soil. Compared with WSP, IPs are more stable and need to mix with PSF in Pb remediation (Li et al., 2016b). PSF combined with IP can promote the continuous release of P via the secretion of organic acid and is suitable for long-term Pb remediation. However, the different IPs can affect the secretion of organic acid by PSF. For example, calcium phosphate (Ca-P) can stimulate *A. niger* to secrete more oxalic acid (Tian et al., 2021a). In addition, the dissolution of Ca-P is more efficient than Fe-P by *A. niger*. Therefore, Ca-P is the best choice in Pb remediation by PSF.

## 3.3 Effects of nutrients on PSF in Pb remediation

The different nutrients can significantly influence the secretion of organic acid by PSF and hence affect Pb remediation by phosphate. In the case of oxalate, the secretion of oxalate by PSF is affected by different environmental factors, such as carbon (C) source, nitrogen (N) source, environmental pH, etc (Palmieri et al., 2019). Nitrogen is a key factor affecting the metabolism of *A. niger* and the dissolution of phosphate rock (Paulo et al., 1988; Tian et al., 2018). Compare with ammonium and urea, nitrate can significantly increase the secretion of oxalate by *A. niger* and reduce the Pb concentration in Pb remediation with Ca-P (Feng et al., 2022). For nitrogen, nitrate is the suitable resource in Pb remediation by PSF and phosphate.

# 4 Ways to improve lead remediation

## 4.1 Application of fertilizers in Pb remediation

PSF and phosphate complex have been used to produce phosphate-based biofertilizers, which not only increase the P

content in the soil but also function in Pb remediation (da Silva et al., 2017). The application of PSM biofertilizer can significantly increase crop yield and soil available P content, reducing the 50% phosphate fertilizer input (Fitriatin et al., 2017). Phosphate rock combined with PSF (*P. oxalicum*) can replace chemical fertilizers, and increase crop yield. In addition, the application of PSM biofertilizer and phosphate can also reduce the Pb concentrations in soil. For example, the combination of phosphogypsum (PG) and biofertilizer (containing *A. niger*) can reduce soil Pb concentration from 365 mg/kg to 302 mg/kg (Meng et al., 2022). PG not only provide a sufficient P source for the growth of *A. niger* in highly contaminated soils but also strengthens the formation of insoluble Pb minerals. Therefore, adding phosphate and PSF as fertilizer is an effective attempt at long-term Pb remediation.

## 4.2 Application the suitable nutrients

Nitrogen sources can significantly affect the secretion of organic acids of *A. niger*, which could affect phosphate dissolution and Pb remediation (Gadd et al., 2014). The decomposition of inputted urea can produce carbon dioxide and form carbonates, which inhibits the growth of *A. niger* and the secretion of organic acids (Cintha et al., 2006; Su et al., 2021). Ammonium and nitrates are more efficient in Pb remediation by *A. niger* and phosphate (Feng et al., 2022). In addition, calcium can stimulate *A. niger* to secrete more organic acids, hence the calcium-based nitrogen fertilizer is more suitable for Pb remediation by PSF and phosphate (Tian et al., 2021a). In addition, other microorganisms such as *Rhodotorula mucilaginosa* (Rho) can secrete large amounts of extracellular polymers (EPS) to form EPS-Pb in Pb toxicity resistance (Li et al., 2019). The addition of phosphate can significantly promote the secretion of EPS by Rho (Tian et al., 2022a). The Pb remove ratio in Rho and phosphate reached 99.9% (Tian et al., 2022a). In addition, the polysaccharides and other nutrients contained in EPS can support the growth of PSF. Therefore, EPS can be applied as a synergist in Pb remediation by PSF and phosphate.

# 5 Discussion

In summary, the combination of PSF and phosphate in Pb remediation is an effective way in current research. On the one hand, PSF can secrete oxalic acid to promote the release of P from phosphate, and the released P can react with Pb cations to form highly insoluble pyromorphite. On the other hand, the secreted oxalic acid by PSF can also react with Pb to form insoluble lead oxalate Figure 1. However, this pathway is also limited due to the long-time dissolution of phosphate and the formation of insoluble Pb minerals. Increasing the secretion of oxalic acid by PSF is the key factor in Pb remediation by the combination of phosphate. Hence, the ability of oxalic acid secretion by PSF should be considered in a different environment. In the future, the enhancement of the micro-interface process in Pb remediation by PSF and phosphate should be explored, especially in strengthening the participation of oxalic acid. Improving the production of oxalic acid via the different pathways can promote Pb remediation faster and completely to reduce Pb toxicity. In addition, to obtain the best Pb remediation purpose in

the environment, choosing the suitable PSF and phosphate are needed in practical application.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

DT and XZ wrote the manuscript. LW, MH, CZ and XY assisted in the data collection. DT, CZ, and XY conceived the idea, revised the manuscript and led the project. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Machine learning: An effective technical method for future use in assessing the effectiveness of phosphorus-dissolving microbial agroremediation

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The issue of agricultural pollution has become one of the most important environmental concerns worldwide because of its relevance to human survival and health. Microbial remediation is an effective method for treating heavy metal pollution in agriculture, but the evaluation of its effectiveness has been a difficult issue. Machine learning (ML), a widely used data processing technique, can improve the accuracy of assessments and predictions by analyzing and processing large amounts of data. In microbial remediation, ML can help identify the types of microbes, mechanisms of action and adapted environments, predict the effectiveness of microbial remediation and potential problems, and assess the ecological benefits and crop growth after remediation. In addition, ML can help optimize monitoring programs, improve the accuracy and effectiveness of heavy metal pollution monitoring, and provide a scientific basis for the development of treatment measures. Therefore, ML has important application prospects in assessing the effectiveness of microbial remediation of heavy metal pollution in agriculture and is expected to be an effective pollution management technology.

## KEYWORDS

machine learning, microbial remediation, agricultural pollution, assessment and prediction, crop yield

## 1 Introduction

The safety of agricultural land as the basis for human survival and development is of increasing concern (Roy et al., 2022). In recent years, the levels of heavy metals in agricultural land have been increasing due to industrial activities, fertilizer application, coal burning, and other human activities (Xu et al., 2014). Heavy metals are characterized by non-biodegradability, high toxicity, and ease of accumulation, and are the most common type of pollutants in the agricultural environment, seriously affecting ecosystem function, food security, and human health (Sharma et al., 2022). In addition, higher levels of heavy metals may lead to changes in soil structure and nutrient loss, which in turn may affect crop quality and yield (Fei et al., 2022). Therefore, it is necessary to analyze the effects of heavy metal pollution on agricultural land and to assess the level of heavy metal pollution in agricultural land.



## 2 Phosphate solubilizing microorganisms as a green technology for improving crop growth and remediating heavy metal contamination

Microorganisms play an important role in the growth of crops in agricultural fields, and can enhance crop growth by improving the soil environment, promoting nutrient cycling, and improving plant immunity in a variety of ways. Phosphorus solubilizing microorganisms (PSM) are a group of microorganisms that can convert soil organic phosphorus compounds or insoluble inorganic phosphorus compounds into plant-available phosphorus elements. As one of the first beneficial functional microorganisms discovered in agricultural fields, the main functions of PSM include 1) increasing the effective soil phosphorus content through the release of organic acids, biological enzymes, and a series of other secretions to promote plant nutrient uptake and growth and development (Alori et al., 2017). 2) Forming a symbiotic relationship with plant roots, using inter-root substances secreted by plants for subsistence, as well as secreting hormones and enzymes (indoleacetic acid, 1-Aminocyclopropane-1-carboxylate deaminase, etc.) to help plants absorb nutrients (Rawat et al., 2020). 3) Produce plant antibodies (e.g., cell wall lysis enzymes, antibiotics, etc.) to suppress plant diseases, activate the plant's immune system, and enhance plant resistance to diseases (Zaidi et al., 2016). 4) Decomposing organic matter and promoting soil aeration and water retention capacity, thus improving soil structure and nutrient retention (Tian et al., 2021). 5) Phosphate solubilizing microorganisms are able to adsorb, accumulate and complex heavy metal ions as well as toxicity reduction through cellular self and secretions (extracellular polymers, glutathione, etc.) (Chen et al., 2023b). 6) The use of phosphate solubilizing microorganisms can also reduce the use of chemical fertilizers and pesticides, reducing the negative impact on the environment (Ahmad, 2015). In addition, the good affinity of PSM allows it to be used together with other materials to improve soil properties and remediate environmental pollution (Chen et al., 2019; Feng et al., 2022; Lai et al., 2022; Chen et al., 2023a).

## 3 Artificial intelligence—machine learning has been initially applied in agricultural environments at this stage

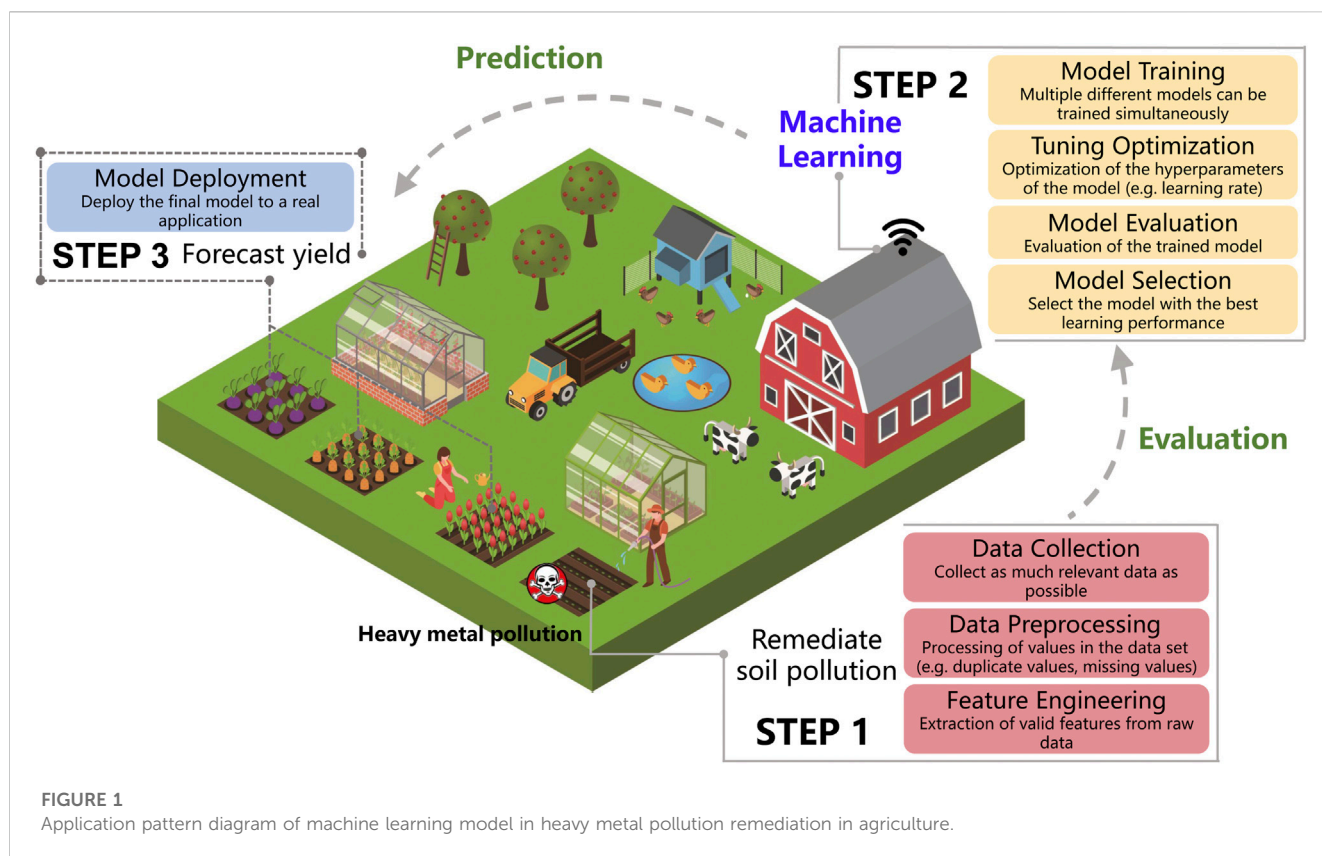
Soil contamination assessment in agriculture is a prerequisite to ensure that crops can be grown properly and safely consumed, and the classical research paradigms include three types: experiment, theory, and computational science. Traditional methods for land contamination assessment include the single factor index, single factor pollution index, potential ecological risk index, and contamination load index (El Azhari et al., 2017; Lu et al., 2021). These methods show some limitations in practical applications due to the lack of adaptability and accuracy of the built mathematical models. The era of big data has given rise to the data-driven paradigm based on big data, which refers to data sets with complex structures, including those that do not have or have not yet mastered their causal relationships. The core of the data-driven

approach lies in acquiring knowledge by analyzing large amounts of data. Common data analysis methods include classification, clustering, association (correlation, regression, etc.), discrimination, principal component analysis, statistical inference, etc. (Wu et al., 2020).

In recent years, data mining methods using artificial intelligence algorithms such as machine learning have become a hot research topic. Machine learning method is an advanced data analysis method, which is often applied to analyze the hidden information between input data and output results (Dobbelaere et al., 2021). An integrated model composed of multiple learning algorithms has the advantages of good predictive performance and high interpretability (Zhang et al., 2022). For example, random forest can directly process high-dimensional data without feature selection (Ali et al., 2021). Gradient boosting decision tree use a loss function with robustness to outliers (Yang et al., 2023). These models have been successfully applied to problems such as forecasting, engineering design, and material optimization, saving significant labor and time costs (Tian et al., 2022; Veloso et al., 2022). Currently, machine learning is widely used in agriculture. For example, Hamrani et al. successfully predicted greenhouse gas emissions in agricultural land using nine models separately, such as support vector machine, random forest, etc. Among them, long short-term memory network has the most accurate prediction for both CO<sub>2</sub> ( $R^2 = 0.87$ , RMSE = 30.3) and N<sub>2</sub>O ( $R^2 = 0.86$ , RMSE = 0.19) (Hamrani et al., 2020). Saha et al. combined a machine learning model and multi-criteria decision-making models to assess agricultural land fertility and site suitability, it was found that agricultural land with higher organic carbon content and cation exchange capacity, and low bulk weight was more advantageous (Saha and Mondal, 2022). Some studies have also shown that clay content in the soil, soluble phosphorus, and soluble organic carbon are the main factors affecting phosphorus concentration in groundwater of agricultural land using support vector machine, random forest, and neural network (Yang et al., 2023). These successful examples show that it is feasible to use machine learning methods for prediction, assessment, and analysis of pollution problems in agricultural environments.

## 4 Building machine learning models to predict crop yield and safety after remediation of heavy metal contamination is one of the hot spots for future research

In agricultural land, crop yield is influenced by several factors, including heavy metal contamination concentrations, nutrient elements in the soil, and microbial communities. These factors also have some mechanisms of action among each other. Currently, it is difficult to quantify the effects of these mechanisms of action on crop yield. Therefore, it has become a trend to use machine learning models to simulate and assess soil contamination levels (Wang et al., 2021; Liu et al., 2023). Based on the powerful data analysis methods of machine learning, and integrating the data-driven method (statistical analysis) with the model-driven method (causal analysis), it is possible to link heavy metal contamination with crop yield. It would be an effective



technical tool to explore the effect mechanism of remediated heavy metal contamination on nutrient elements, microorganisms and crop yield. The analysis diagram is shown in Figure 1.

Based on the excellent predictive performance of machine learning models, it is feasible to build a model to predict crop yield. Typical machine learning model structures include supervised learning represented by regression analysis and statistical classification, unsupervised learning represented by generating adversarial network (GAN) and clustering, and reinforcement learning (LeCun et al., 2015; Castaldo et al., 2016; Wang et al., 2020). Currently, deep learning represented by neural network is rapidly developing, which would be more efficient to integrate with supervised learning, unsupervised learning, and reinforcement learning to classify data and identify features. Due to the differences in the datasets, the prediction performance of each model behaves differently. During the model construction process, multiple models are usually selected for simultaneous parameter optimization and prediction, and the model with the best prediction performance is finally selected. Besides, a sufficient amount of data is also one of the important factors to support the success of model prediction. For example, Jhajharia et al. integrated 3,664 sets of yield data of various crops with soil type and rainfall of agricultural land in the Rajasthan region, and the results confirmed that crop yield could be successfully predicted using random forest model ( $R^2 = 0.963$ ,  $RMSE = 0.035$ ) (Jhajharia et al., 2023). Iniyar et al. counted data on precipitation, humidity, temperature, area, soil type, crop type, season, and yield for the last 18 years in the Maharashtra region. After training eight machine learning models such as linear regression, ridge regression, and gradient

boosting, it was found that the long short-term memory network showed the best prediction performance with 86.3% accuracy (Iniyar et al., 2023). Panigrahi et al. successfully used linear regression, decision tree regression, and other models to predict the yield of three different crops of corn, peanuts, and Bengal beans based on the monthly minimum and maximum temperature and annual rainfall in the Telangana (Panigrahi et al., 2023). Therefore, it is feasible to predict the yield of various crops using machine learning models, but the availability of sufficient data support from research institutions or government may be the key to it.

Machine learning models can not only predict crop yields, but also analyze the effect of PSM on remediation of heavy metal contamination or post-remediation soil properties on crop yields through interpretable algorithms such as feature importance, partial dependence, individual conditional expectation, and Shapley additive explanation. For example, by analyzing the partial dependence of a certain heavy metal ion, the concentration threshold for that heavy metal to affect crop growth is determined. Alternatively, using the characteristic importance algorithm, it is possible to obtain the importance weights for the effects of various types of heavy metals on crop yield. Such weighting factors, which can be introduced into the formula for calculating the risk level of heavy metal contamination, make the assessment results more informative. In addition, the joint application of machine learning and high-resolution aerial imaging technology has been shown to be feasible. For example, feature information is obtained based on high-resolution aerial imaging technology, and then machine learning models are used to predict the heavy metal concentrations at unknown points to

finally assess the soil contamination risk levels. The accuracy of this combined application of techniques to assess contamination levels was verified to be significantly better than the traditional kriging interpolation and inverse distance weight interpolation methods (Jia et al., 2021). In conclusion, machine learning models can simultaneously combine soil quality and crop yield so that the two are interlinked, which means that the impact of soil quality on crop growth can be analyzed along with predicting crop yield, correcting adverse factors that affect yield and continuously improving agricultural land quality. Thus, machine learning can be used as an effective technical tool to predict and evaluate the impact of phosphorus dissolving microbially remediated soils on crop yields.

## 5 Discussion

As an efficient and fast data analysis and processing method, machine learning has often been applied to solve various agricultural environmental problems in recent years. Its powerful prediction and analysis capability saves a lot of labor cost and time cost for research, and the application of machine learning in agricultural survey technology will be more promising in the future with the advancement of technology and the increase of data. However, the application direction of the technology is currently more limited. In the future, artificial intelligence, machine learning, and computer vision can be used to identify the growth status of crops and prevent them from being infected with toxic pests to affect their yield. On the other hand, machine learning is essentially a data-driven method, there is currently less training data available to researchers. A large amount of open-source data is also necessary for training models in future research. In this context, data-driven method and model-driven method can complement each other, which includes: 1) introduce casual analysis measures to researches that usually relied entirely on statistical analysis to solve data dependency and improve the analysis applicability and accuracy; 2) introduce statistical analysis measures to researches that usually relied entirely on casual analysis, to improve the analysis efficiency.

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In conclusion, machine learning can be used to achieve accurate analysis and prediction of agricultural data, improve the efficiency and quality of agricultural production, and promote the development and upgrading of the agricultural industry.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Author contributions

JW and FZ wrote the manuscript, collected the data, conceived the idea and revised the manuscript. JW led the project.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Phosphate solubilizing microorganisms as a driving force to assist mine phytoremediation

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## KEYWORDS

phosphate solubilizing microorganisms, mine remediation, soil nutrients, plant growth, promotion and synergy

## 1 Phosphate solubilizing microorganisms (PSMs) are partners in promoting plant growth

PSMs are a group of beneficial microorganisms that mediate soil phosphorus cycling and biological utilization. The primary function of these microorganisms is to release phosphorus from insoluble phosphorus compounds to promote the utilization of P by plants (Chen et al., 2019). PSMs category is very wide, including fungi, bacteria, arbuscular mycorrhizal fungi, actinomycetes, vesicular arbuscular mycorrhizae, etc. Among them, *Penicillium*, *Aspergillus* and *Bacillus* are representatives of fungi, while *Enterobacter*, *Serratia* and *Pseudomonas* are representatives of bacteria (Zaidi et al., 2016; Rawat et al., 2021). In the solubilization mechanism of soil phosphorus by PSM, mineralization of organic phosphorus by biological enzymes (Phytases) (Yadav and Tarafdar, 2011) and solubilization of inorganic phosphorus by organic acids are now widely accepted mechanisms (Chen et al., 2023). Furthermore, some PSMs can also release phosphorus sources from the environment by secreting inorganic acids, such as HCl and H<sub>2</sub>SO<sub>4</sub> (Etesami et al., 2021; Rawat et al., 2021). While PSMs enhance plant growth primarily by providing soluble phosphorus to plants, they also improve the overall performance of plants by providing a variety of other growth-promoting substances. On the one hand, microorganisms produce antibiotics, hydrogen cyanide, iron carriers, antifungal compounds (such as PAL, phenolic compounds and flavonoids) and other biocontrol agents to enhance plant resistance to diseases and pests, and indirectly promote plant growth (Alori et al., 2017). On the other hand, phytohormones secreted by PSM, such as Indole-3-Acetic Acid (IAA), cytokinin, and gibberellin acid, also play an important role in regulating plant growth. Among them, IAA is the plant growth regulator most related to the physiological activity of plants, which has been widely studied. It promotes plants' ability to absorb nutrients by stimulating root growth, expanding root surface area, and changing plant root morphology (Etesami et al., 2021). The promotion of plant growth by PSMs makes inoculation with PSMs a widely accepted and environmentally friendly method for improving agricultural productivity. Additionally, the soil phosphorus dissolved by the PSMs often exceeds the demand of plants (Richardson and Simpson, 2011; Raymond et al., 2021), which allows potential excess phosphorus to be used in other environments. This is particularly relevant for ecosystems that rely on plant assistance for their composition, such as for seedling planting, environmental restoration, and landscape renovation. In these cases, PSMs hold significant promise for playing a crucial role in the future.

## 2 Phytoremediation is a low-cost and sustainable technology for mine remediation

Mining is a temporary industrial activity, which leads to drastic changes in the natural environment, such as altering the landscape and terrain, destroying vegetation, eliminating soil microorganisms and animals, producing a large amount of mine waste, and endangering both the aquifer and water source supply area (de Moura et al., 2022). In most cases, mining activities affect the resilience of the ecosystem and it is almost impossible to regenerate through natural regeneration. Therefore, it is necessary to use restoration techniques to restore ecosystems and protect biodiversity (Menegaki and Kaliampakos, 2012). Studies have shown that bioremediation techniques using plants and/or microorganisms are cheaper and more environmentally friendly than conventional physical and chemical techniques (Gong et al., 2018). However, one of the main challenges in the rehabilitation of mine sites is the re-establishment of self-sustaining vegetation. Due to the lack of vegetation, the erosion in these areas is intensified, resulting in the pollution of the surrounding areas. In addition, groundwater may be contaminated by leaching. The spread of this contamination may pose a threat to human health if it enters the food chain through drinking water and crops. However, due to the serious degradation of the mining area, the establishment and development of future plants are hindered and recovery is affected.

It is well known that soil degradation caused by mining activities mainly includes: 1) dissolution of heavy metals in waste forms high-concentration pollution stress; 2) Lack of organic matter and sandy structure and poor water storage capacity; 3) Loss of large amounts of nutrients such as N, P, and K; and 4) The functional homogeneity and poor diversity of microbial community of the native soil (Wong, 2003; Sheoran et al., 2011; Lebrun et al., 2021). Among them, heavy metals have negative effects on plant cell growth, root development, and photosynthesis by inhibiting enzyme function, disrupting the nucleic acid structure, and interfering with plant nutrient absorption (Etesami, 2018). In addition, phosphorus can form complexes with metal ions in the soil, making it mostly unavailable for plants to absorb (Rawat et al., 2021). These factors combine to make phytoremediation a difficult process to achieve in abandoned mines. Therefore, reducing heavy metal stress and regulating soil properties are the basis for improving phytoremediation in mining areas. In addition to the introduction of heavy metal resistant plant species or breeding mechanisms to improve the heavy metal tolerance of traditional plant species, the addition of soil amendments (e.g., mycorrhizal agents, biochar, phosphate minerals, etc.) can also help to mitigate the negative effects.

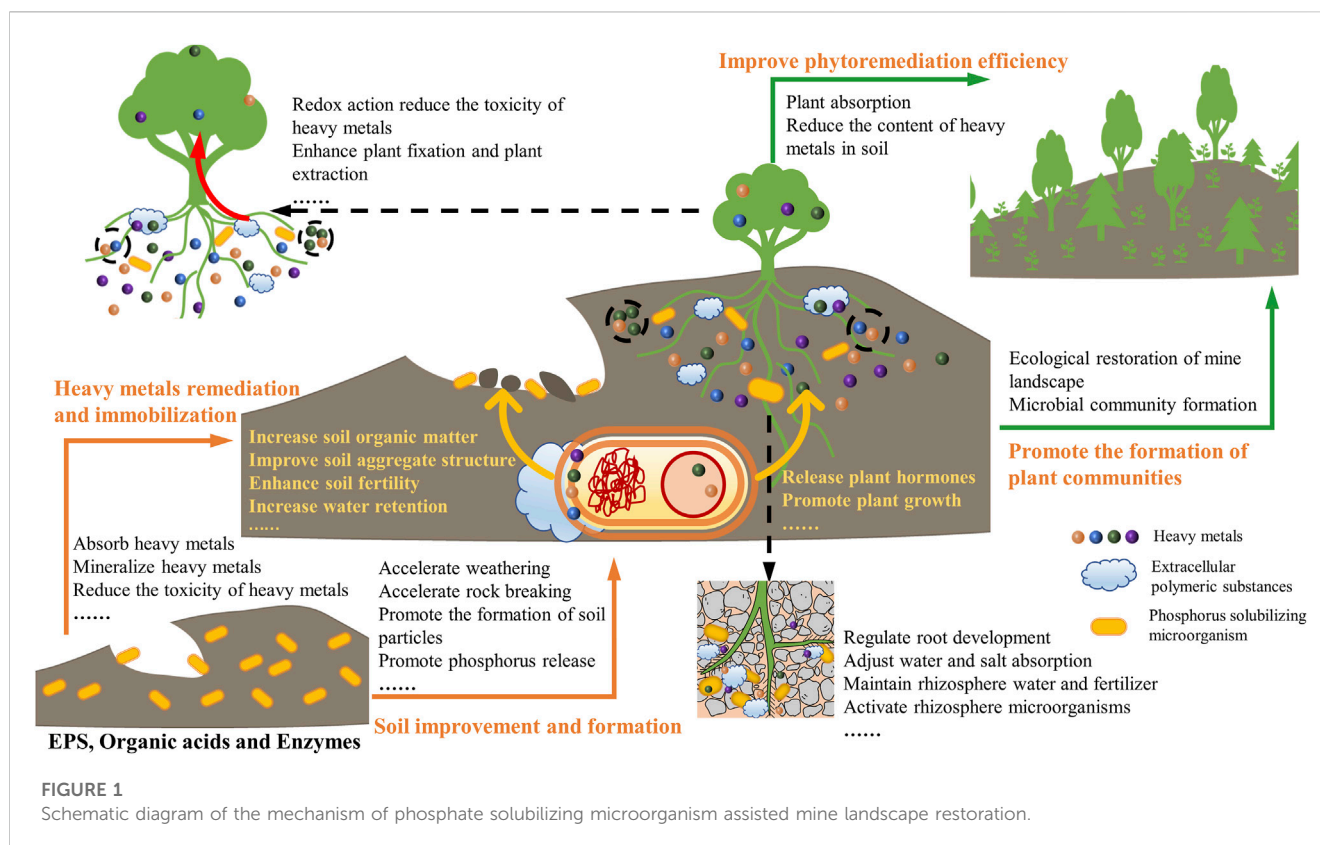
## 3 PSMs as potential regulators in mine remediation

The specific nature of pollution in mining sites often makes it difficult to achieve phytoremediation alone. Currently, most reclamation and remediation strategies used in abandoned mine sites are based on soil modification with chemicals, combined with plants for long-term remediation. However, these measures are often unsustainable because they tend to ignore the balance and enrichment effects of plant-associated microbes in the mine site.

Microbial activities in abandoned mine sites are mainly in the topsoil (20–30 cm), water, tailings and sediments, rhizosphere environment of plants, and phyllosphere environment of plants (Thavamani et al., 2017). Although some of the native microorganisms in the mine site are able to evolve new resistance mechanisms to cope with the pressure of toxic pollutants, the abundance and diversity of microbial communities are bound to be greatly reduced, especially the microbial flora associated with plant growth promotion. This phenomenon also makes it difficult for new plants to settle in the environment. Therefore, in order to develop sustainable remediation methods, more attention should be paid to understanding and utilizing the role of plant-microorganism interactions in mine sites.

PSMs, which possesses both heavy metal remediation and plant growth promotion functions, may be an extremely promising option. The main advantage of PSMs is their better rhizosphere ability, such as the ability to colonize, grow, and develop faster in rhizosphere soil. The process of PSMs promoting plant remediation of mine pollution is mainly divided into two aspects: soil improvement and formation, and plant growth and protection (Figure 1).

- (1) Soil improvement and formation: PSMs accelerate the weathering and decomposition of soil layers, as well as the formation of natural soil, and creates an environment for plants to take root (Tian et al., 2021; Yi et al., 2021). At the same time, functional microorganisms can change soil structure and nutrient cycling, increasing soil aggregate stability (Bruneel et al., 2019; da Silva et al., 2022; Thavamani et al., 2017).
- (2) Plant growth and protection: Firstly, PSMs play an important role in promoting plant growth. It promotes nutrient circulation, improves the plant defenses against disease and pests, and increases plant growth by secreting plant auxin, dissolving phosphorus, fixing nitrogen, and other mechanisms. Secondly, the efficient immobilization function of PSMs for heavy metals is key to their assistance in mine phytoremediation. The phosphate released by PSMs can solidify and stabilize the free heavy metal ions (Lai et al., 2022). Furthermore, PSMs can absorb, accumulate, and complex heavy metal ions through cells (internal and surface) and by the secretion of organic substances (extracellular polymer, glutathione, etc.). Meanwhile, PSMs reduce the toxicity of heavy metals through its own redox, methylation, and other metabolic effects. In addition, PSMs directly promote the phytoremediation of heavy metals by improving the transport ability of metal ions in the rhizosphere (phytoextraction) or reducing the metal migration ability (phytostabilization) (Ahemad, 2015; Gupta and Kumar 2017). The two key mechanisms of PSMs make them an efficient growth promoter and an immobilizer of heavy metals in mine sites. In conclusion, in the joint remediation system of PSMs and plants, on the one hand, plants can immobilize heavy metals in soil or themselves, while PSMs can transform heavy metals into a less potentially toxic, lower bioavailable, and/or less mobile form than the initial form. At the same time, PSMs interact with plants to improve their growth by releasing P, plant auxin, pest inhibitors, etc., making the phytoremediation process more effective. On the other hand, vegetation coverage stabilize the soil, reduce



erosion and leaching, and enrich the soil structure, thereby providing a cyclic gain effect for the expansion and reproduction of PSMs.

## 4 Prospect

PSMs play an important role in the establishment and development of plants and ecosystem functions in mine remediation. The use of PSMs in a degraded environment with high heavy metal pollution can become the driving force for the successful restoration of the transboundary ecosystem. In addition, PSMs have great potential to inoculate microorganisms into mine soil to improve plant growth and reduce metal toxicity. Although PSMs have not been widely promoted for use in mine remediation at present, the prospects for their application are self-evident. Therefore, the engineering application of PSMs in actual mines should be encouraged and promoted in future research. More attention should be paid to explore whether complex and rarely heavy metal species will have more severe stress effects and differences in remediation mechanisms for PSMs in special mining environments, as well as developing a combined remediation technology of PSMs with physical and chemical remediation techniques for mines.

## Author contributions

FC, JM, QY, and ZY conceived and wrote the paper. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors contributed to the article and approved the submitted version.

## Conflict of interest

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# Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering

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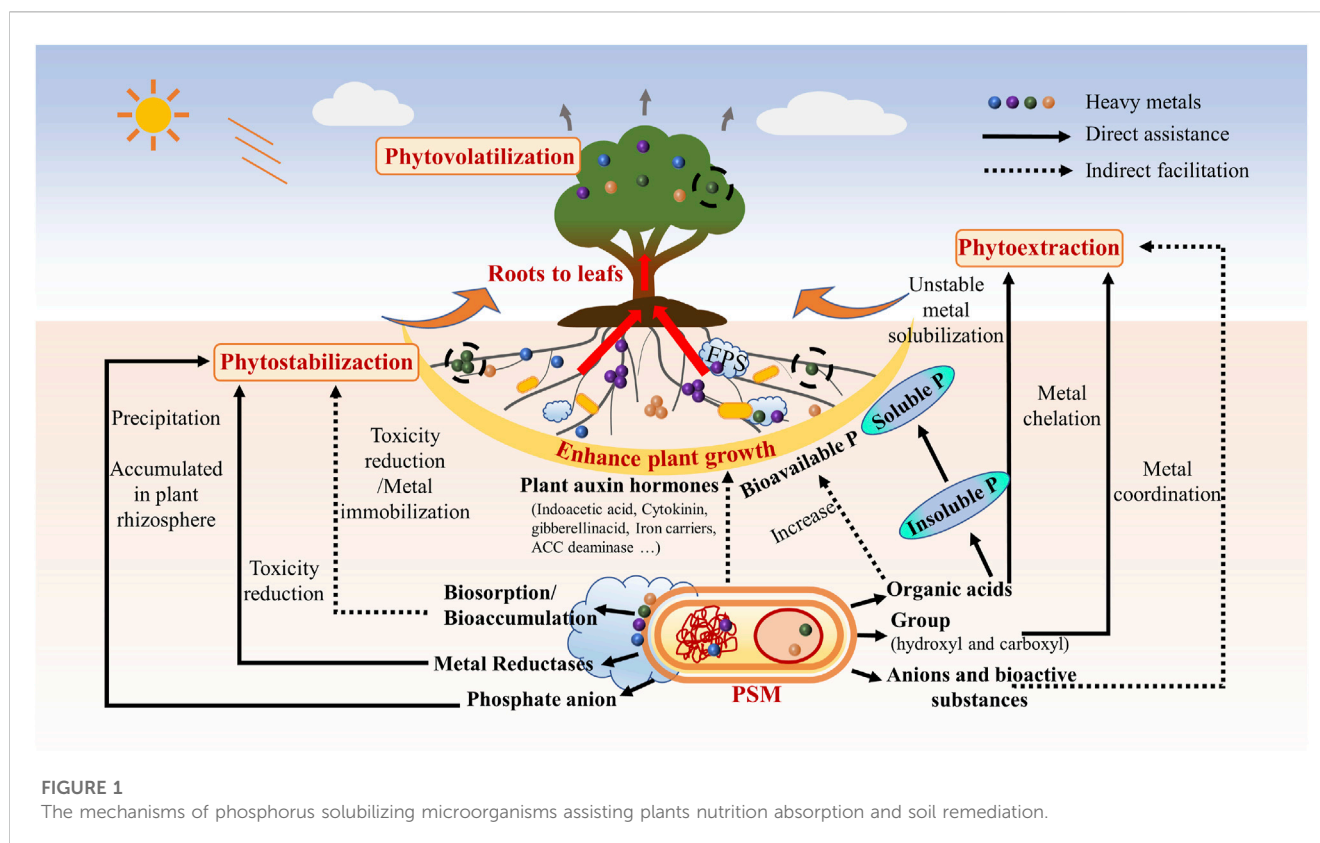
Phosphate solubilizing microorganisms (PSMs) are known as bacteria or fungi that make insoluble phosphorus in soil available to plants. To date, as beneficial microbes, studies on PSMs indicated they have potential applications in agriculture, environmental engineering, bioremediation, and biotechnology. Currently high cost and competition from local microbe are the most important factors hindering PSMs commercialization and application as for instance biofertilizer, soil conditioner or remediation agent, etc. There are several technical strategies can be engaged to approach the solutions of these issues, for instance mass production, advance soil preparation, genetic engineering, etc. On the other hand, further studies are needed to improve the efficiency and effectiveness of PSMs in solubilizing phosphates, promoting plant growth, soil remediation preferably. Hopefully, PSMs are going to be developed into ecofriendly tools for sustainable agriculture, environment protection and management in the future.

## KEYWORDS

phosphorus solubilizing microorganisms, biofertilizer, environment remediation, commercialization, cost-effectiveness, frontier research

## 1 Introduction

Phosphate solubilizing microorganisms (PSMs) are bacteria or fungi that are capable of breaking down insoluble forms of phosphorus, such as phosphates, in the soil and making it available to plants as a soluble form that can be easily absorbed (Figure 1) (Rawat et al., 2020). They are huge category of beneficial microbe including many genera for instance *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Aspergillus* spp., *Rhizobium* spp., *Fusarium* spp., *Trichoderma* spp., *Penicillium* spp., *Serratia* spp., *Micrococcus* spp., *Stenotrophomonas* spp., *Acinetobacter* spp., and *Agrobacterium* spp. (Rodríguez and Fraga, 1999). This process increases the overall availability of phosphorus to plants, leading to improved plant growth and crop yields. PSMs are commonly found in soil and rhizosphere, and play an important role in nutrient cycling and soil fertility (Beheshti et al., 2022). PSMs have had several applications in biotechnology. For instance, in the pharmaceutical and food industries, PSMs are used to produce antibiotics, vitamins, and other bioactive compounds (Sekurova et al., 2019); furthermore, PSMs have the potential to play a role in the development of new and efficient bioprocesses for the sustainable production of biofuels and other bio-based products



(Osanai et al., 2015). The most well-known uses of PMSs are in agricultural and environmental engineering. For their outstanding nature described above, PMS are used for improving soil fertility in agriculture, enhancing plant growth and tolerance against stress of instance salinity (Luo et al., 2022), nutrient deficiency (He et al., 2022; 2021; Chen et al., 2022a) etc.; and hence increasing crop yields (Chen et al., 2022b); they can also be used for bioremediation along or combining with plants, to remove for instance heavy metals from contaminated soil and water (Pinilla et al., 2008; Chen et al., 2019a).

## 2 Potential uses of PSMs in agriculture and environmental engineering

There is great potential and expectation for the future of PSMs in agriculture and environmental engineering. As the world's population continues to grow, the demand for food and other resources will increase, putting pressure on the world's finite resources, including phosphates. PSMs have the potential to help address this challenge by improving soil fertility and reducing the need for chemical fertilizers (Sarmah and Sarma, 2022; Tian et al., 2022).

PSMs are an emerging biotechnology tools in agriculture and environmental engineering, but the application is still in its early stages of development and not yet for commercialization. While there have been several studies demonstrating the potential of PSMs for enhancing plant growth, improving soil fertility, and detoxifying contaminated environments, much of the research is still in the laboratory or experimental stage (Chen et al., 2020; 2019b; Lai et al., 2022a).

### 2.1 For agriculture

PSMs can regulate plant metabolism by providing an increased availability of soluble phosphorus. This increased availability of phosphorus can lead to changes in plant growth and metabolism (Rodríguez and Fraga, 1999), such as an increased rate of photosynthesis, improved root development, and an enhanced ability to defend against stressors such as drought or disease (Billah et al., 2019; Kour et al., 2020). PSMs can enhance plant endogenous gene expression and help plants cope with abiotic stress by providing an increased availability of soluble phosphorus. This increased availability of phosphorus can activate signaling pathways and trigger the expression of stress-responsive genes in plants, which can enhance their ability to tolerate and resist environmental stressors such as drought, salinity, heavy metal toxicity, and temperature extremes. For example, research has shown that PSMs can increase the expression of genes involved in the regulation of water uptake, antioxidant defense, and heat shock response, which can help plants better cope with abiotic stress (Paul et al., 2017). In addition, PSMs can also stimulate the production of phytohormones such as auxins, cytokinin, gibberellins et al., which can enhance plant growth and stress tolerance (Figure 1).

### 2.2 For environmental engineering

PSMs can play a role in remediation of heavy metal pollution in soil by indirectly reducing the bioavailability of heavy metals in the soil, as showed in Figure 1. When PSMs solubilize phosphates, they

**TABLE 1** Potential uses and benefits of applying PSMs in agriculture and environment engineering.

Agriculture	Environmental engineering
Improve soil fertility	Remove and recover phosphorus from wastewater
Enhance plant growth	Improve water quality
Increase nutrient uptake	Reduce eutrophication
Boost plant immunity	Enhance bioremediation of contaminated soils
Reduce the need for chemical fertilizers	Improve soil quality in mine reclamation
Improve crop yields	Help plants establish on degraded land
Help plants tolerate stress	Enhance microbial diversity in ecosystems
Increase soil organic matter	Mitigate the impact of agricultural runoff
Improve soil structure	Improve soil carbon sequestration
Reduce soil erosion	Increase sustainability of land use practices

create a competition for metal ions, which can reduce the uptake of heavy metals by plants and lower their concentration in the soil alone or combining with other species or materials (Lai et al., 2022b; Feng et al., 2022; Chen et al., 2023). In addition, some PSMs have been shown to produce organic acids, such as citric acid, that can chelate heavy metals and make them less available for uptake by plants (Direct assistance, Figure 1).

Another way PSMs can help remediate heavy metal pollution in soil is by promoting the growth of plants that can tolerate high levels of heavy metals. This is known as phytoremediation, and it involves using plants to remove heavy metals from the soil by absorbing and accumulating them in their tissues (Indirect facilitation, Figure 1). PSMs can help improve the growth and health of these phytoremediation plants by providing them with an available source of phosphates, which are essential for plant growth and development.

As all the function and potential use of PMS, it worth to note that here is still a need for further research to fully understand the mechanisms by which PSMs interact with plants and the environment; and to develop cost-effective and scalable methods for their application in agriculture and environmental engineering. Additionally, there are challenges associated with the large-scale production, formulation, and storage of PSMs, which must be overcome in order to make their application more practical and widespread.

The potential uses of PSMs in agriculture and environmental engineering were summarized in Table 1.

### 3 The restraining factor in PSMs application and commercialization in agricultural and environmental engineering, and the promising solutions

There are several key challenges associated with the application of PSMs in crop fertilization and environmental engineering: 1) there is currently a lack of standardization in the production and application of PSMs as biofertilizers or soil remediation promoter, which can affect their efficacy and consistency; 2) PSMs are sensitive

to soil conditions such as pH, salinity, and heavy metal toxicity, and may not be able to survive or function effectively in all soils; PSMs are often introduced into soil as suspensions or inoculants, but they may not persist in the soil for long periods of time, limiting their effectiveness over the long term; 3) PSMs may face competition from other microorganisms in the soil for nutrients and space, which can limit their ability to establish and grow in soil; 4) the production and application of PSMs as biofertilizers can be expensive, and the cost of this technology may be a barrier for widespread implementation in agriculture. These challenges highlight the need for further research to optimize the production and application of PSMs as biofertilizers, and to develop new and more cost-effective methods for enhancing soil fertility and crop productivity. Currently, high cost and competition from local microbe are the most important factors hindering PSMs commercialization and application.

There are several ways to reduce the cost of Phosphate Solubilizing Microorganisms (PSMs). Some of these were listed and interpreted below.

- 1) Mass production of PSMs: Mass production of PSMs using low-cost and efficient methods, such as fermentation, can help to reduce the cost of producing these microorganisms on a large scale.
- 2) Optimizing application methods: Developing more efficient and cost-effective methods for applying PSMs to soil, such as using granules (Jokkaew et al., 2022), liquid formulations (Goljanian-Tabrizi et al., 2016), or seed treatments, can help to reduce the cost of PSMs application.
- 3) Selecting low-cost PSMs (Lobo et al., 2019): Selecting PSMs that are naturally occurring and widely distributed, or that can be easily isolated and mass-produced, can help to reduce the cost of PSMs application.
- 4) Using natural sources of phosphates: Encouraging the use of natural sources of phosphates, such as rock minerals and guano, can help to reduce the need for costly commercial fertilizers.
- 5) Promoting the use of integrated nutrient management: Encouraging the use of integrated nutrient management practices, such as the use of cover crops, composting, and intercropping, can help to reduce the dependence on commercial fertilizers and increase the efficiency of PSMs application.

To avoid competition with local soil microorganisms for instance plant pathogens, decomposer, predatory microorganisms *et al*, the following factors should be optimized in the application of PSMs.

- 1) Soil preparation: To enhance the survival and persistence of PSMs, the soil should be prepared prior to their introduction. This may involve amending the soil with organic matter, adjusting the pH, or reducing soil salinity levels.
- 2) Inoculum preparation: The PSM inoculum should be prepared in a way that optimizes their survival and growth. This may involve selecting PSMs with high tolerance to environmental stress, such as heavy metal toxicity or salinity, or formulating the inoculum to enhance their survival and persistence in the soil (Johri et al., 1999).
- 3) Timing of application: PSMs should be applied at a time that minimizes competition with other soil microorganisms. For example, application during periods of low microbial activity, such as early in the growing season, may reduce competition and enhance the survival and growth of PSMs.
- 4) Selection of PSMs: PSMs should be selected for their ability to compete with local soil microorganisms. This may involve selecting PSMs with high competitiveness and/or selecting PSMs that are able to produce antibiotics or other metabolites that inhibit the growth of other soil microorganisms.
- 5) Microbial community management: The soil microbial community should be managed in a way that enhances the competitiveness of PSMs. This may involve applying compost, mulch, or other organic matter to the soil, or using other microorganisms or biostimulants to enhance the growth and competitiveness of PSMs. These factors should be optimized to ensure that PSMs are able to establish and persist in the soil, and to minimize competition with local soil microorganisms.

## 4 The current Frontier research areas in the field of PSMs

**Microbial ecology:** Researchers are working to understand the interactions between PSMs and other soil microorganisms, and the role that PSMs play in soil health and fertility. This research includes the study of microbial competition and cooperation, as well as the development of methods for enhancing the survival and persistence of PSMs in the soil (Nacoon et al., 2020).

**Metagenomics:** Metagenomic were used to approaches the diversity and function of PSMs in soil, and to identify new PSMs with enhanced abilities for solubilizing phosphates and improving soil fertility.

**Biostimulants:** Researches were carried out to Investigate the use of PSMs and other microorganisms as biostimulants to enhance plant growth and productivity, as well as the ability of plants to cope with environmental stress (Yahya et al., 2022).

**Synthetic biology:** Researchers are developing new approaches for engineering PSMs to enhance their abilities for solubilizing phosphates and improving soil fertility. This includes the use of synthetic biology to produce new strains of PSMs with improved abilities, as well as the use of CRISPR-Cas9 technology to engineer existing PSMs (Liu et al., 2020).

**Soil remediation:** Researchers are investigating the use of PSMs for soil remediation, with a focus on removing heavy metals and other contaminants from contaminated soils (Song et al., 2022). This

research includes the development of new methods for applying PSMs to contaminated soils, as well as the study of the mechanisms by which PSMs remove contaminants from soil.

## 5 Prospect

In the future, it is expected that research into PSMs will continue to advance, with a focus on improving the efficiency and effectiveness of PSMs in solubilizing phosphates and promoting plant growth. This may involve the development of new strains of PSMs through synthetic biology and other techniques, as well as the optimization of existing PSMs for different environmental conditions and applications. In addition, it is expected that the use of PSMs will become increasingly widespread in agriculture and environmental engineering, as farmers and environmental engineers seek more sustainable and environmentally friendly solutions to the challenges posed by declining soil fertility and limited resources.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

## Author contributions

WQ and XL- Conceptualization; WQ and CW- Writing—original draft; CW, WQ, GP, and XL- Writing—review and editing; CW and XL- Funding acquisition.

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