RESEARCH ARTICLE

Application of microbial-assisted phytoremediation technology in heavy metal pollution soil

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The remediation of heavy metal pollution (HMP) soil is crucial for ecological environment security and sustainable utilization of land resources. Plant restoration technology is widely used in this field, but there are also problems such as low efficiency and a long cycle of single plant restoration. To enhance the efficiency of plant remediation of HMP soil, this study selected a mining area in Chenzhou, Hunan, China to investigate microbial assisted enhanced plant remediation technology using *Zoysia japonica* ''Lan Yin No. 3'' and *Bermuda grass* ''Tifdwarf Bermudagrass 328'' as restorative plants. *Bacillus cereus* was screened as a cadmium-tolerant strain. Single factor experiments and pot experiments were conducted to analyze the effects of different factors on the growth and adsorption characteristics of bacterial strains, as well as the practical application effects of plants under different numbers of active bacteria. The results showed that the optimal growth conditions for this strain were a temperature of 30℃, a pH of 7, a sodium chloride concentration of 2%, and an initial cadmium ion concentration (CIC) of 0 to 40 mg/L. Within the optimal conditions, the OD_{600} values of the strain were all above 0.6, indicating stable growth and active metabolism. As the number of viable bacteria increased, the cadmium concentration in the soil decreased. The removal rate of the A5 group with 30 mL of aseptic water and 10^8 CFU/mL viable bacterial counts in *Zoysia japonica* reached 45.75%, while the B5 group with 30 mL of aseptic water and 10^8 CFU/mL viable bacterial counts in *Bermuda grass* reached 42.17%. When *Zoysia japonica* reached 10^4 CFU/mL and *Bermuda grass* reached 10^2 CFU/mL, the plant enrichment factors were optimal at 0.967 and 0.588, respectively. Microbial-assisted plant remediation technology could effectively reduce cadmium content in soil, enhance plant remediation capacity, and provide a practical reference for efficient remediation of HMP soil.

Keywords: heavy metals; soil; microorganism; botany; *Zoysia japonica*; *Bermuda grass*; *Bacillus cereus*.

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Introduction

Soil is the core carrier of material cycling and energy flow in ecosystems and the fundamental resource for ensuring food security and ecological balance [1, 2]. However, with the acceleration of industrialization and urbanization, heavy metal pollution (HMP) has become a severe challenge to the global soil environment. Among them, cadmium as a highly toxic heavy metal has the characteristics of strong mobility and significant biological enrichment effect. Cadmium can enter soil through mining activities, sewage irrigation, and atmospheric deposition, causing serious damage to soil physical and chemical properties, microbial community structure, and plant growth [3, 4]. HMP soil not only threatens the stability of terrestrial ecosystems but may also accumulate in the human body through the food chain, causing health risks such as chronic poisoning. Therefore, carrying out HMP soil remediation has become an urgent need to ensure ecological and environmental security.

The commonly used technologies for addressing HMP issues include soil remediation, electric remediation, leaching, stabilization, plant remediation, and microbial remediation [5]. Liu *et al*. used composite white rot fungi combined with immobilization technology to remediate cadmium pollution, addressing the issue of insufficient exploration of the interaction between microorganisms and plants in cadmium pollution remediation. The study also introduced mixed fermentation technology and applied immobilized mixed bacteria to soybean cultivation in simulated cadmium-contaminated soil with the adsorption rate of the strain in cadmium-contaminated solution reaching 87.33% [6]. Jiang *et al*. clarified the mechanism of microbial action in lead-contaminated soil remediation and summarized the connection between microorganisms and plants in soil lead pollution remediation and found that cations exchanged ions with heavy metals during their interaction [7]. Zhang *et al*. proposed a combined remediation method using plants such as *Metasequoia* and *Miscanthus fragrans* planted in mildly zinc-polluted areas to assess the efficacy of plant combinations in the remediation of zinc-polluted soil. The study continuously monitored key parameters such as soil pH and total zinc and found that the total zinc removal rate of the selected combination was between 8.89% and 26.24%, and the removal rate of *Taxodium* + *Ryegrass* was the highest [8]. Other researchers proposed a method for remediation of soil pollution in abandoned mining areas by combining municipal sludge and plant remediation techniques to explore the effect of different factors on the distribution of heavy metals. The results showed that heavy metals in non-rhizosphere soil would enter the rhizosphere environment with the plant growth, causing a rise in heavy metal content in rhizosphere soil [9]. Current research on HMP soil remediation is relatively abundant, and the technologies used are also diverse. However, these methods have certain problems such as the high cost of the guest soil method, the possibility of secondary pollution introduced by the leaching method, low efficiency of single plant remediation, and significant environmental impact on single microbial remediation. Meanwhile, the adaptability of microbial plant collaborative repair and the survival stability of functional microorganisms in complex soil environments have not been fully resolved in existing research, which restricts the large-scale application of remediation technology and becomes a key bottleneck that urgently needs to be overcome in the field of HMP soil remediation.

To improve the plant remediation efficiency of HMP soil, compensate for the shortcomings of single remediation techniques, and solve the compatibility and stability issues in microbial plant collaborative remediation, this study adopted *Zoysia japonica* “Lan Yin No. 3” and *Bermuda grass* “Tifdwarf Bermudagrass 328” as a restoration plant and introduced cadmium-tolerant strains. Single factor experiments were employed to determine the optimal conditions of time, initial cadmium ion concentration (CIC), temperature, pH value, and sodium chloride concentration for bacterial strain growth and adsorption characteristics. Pot experiments were conducted on HMP soil in mining areas with different concentrations of active bacteria. The soil cadmium content, plant growth, and enzyme activity were monitored to verify the actual effect of microbial-assisted plant remediation. This study explored the optimal growth and adsorption conditions for bacterial strains, reduced the impact of the environment on microorganisms, and improved the application effect of microbial-assisted plant remediation technology. The combination of microbial and plant remediation techniques broke through the limitations of single remediation techniques in terms of efficiency and stability and achieved an improvement in remediation efficiency. This study clarified the optimal synergistic conditions between cadmium-resistant strains and remediation plants and provided an experimental basis for parameter optimization of microbial-assisted phytoremediation technology. It enriched the theoretical system of HMP soil collaborative remediation and provided a technical framework for similar research in the future.

Materials and methods

Experimental area and plants

This study selected a mining area in southern China (Chenzhou, Hunan, China) with the geographical coordinates of 25°45′N-26°15′N and 112°13′E-113°01′E and an area of approximately 100 hectares. The mining activity has been carried out since 1975. The region belongs to the subtropical monsoon climate with the average annual temperature about 18.5°C and the average annual precipitation about 1,500 mm. Due to long-term mining activities, the heavy metal content in the soil has been significantly increased with the average cadmium content in the surface soil as 0.336 mg/kg, which was 111 times higher than the national soil background value. About 68.9% of the soil samples in the mining area have been contaminated with varying degrees of cadmium. Two common types of restoration plants, *Zoysia japonica* “Lan Yin No. 3” (Hunan Jielu Ecological Grass Industry Development Co., Ltd., Changsha, Hunan, China) and *Bermuda grass* “Tifdwarf Bermudagrass 328” (Yuxi Bermuda Lawn Planting Base, Yuxi, Yunnan, China) were selected for this research. “Lan Yin No. 3” has a well-developed root system and is resistant to acidic soil, which can activate cadmium ions through the synergy of root exudates and microorganisms and exhibits stability in acidic polluted environments in southern mining areas [10, 11]. “Tifdwarf Bermudagrass 328” can quickly construct and repair vegetation layers and has an outstanding ability to enrich and transport cadmium [12].

Screening and determination of cadmium resistant bacterial strains

To isolate and identify bacterial strains, 10 g of soil from 0 - 30 cm in sampling point of mining area was mixed with 90 mL of sterile physiological saline to prepare a suspension. After gradient dilution to 10^{-6}, 0.1 mL of suspension was inoculated into LB medium containing 10 g peptone, 5 g beef extract, and 40 mg/L cadmium chloride and incubated at 30°C in DHP-9052 constant temperature incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) for 48 hours. Single colonies were selected and purified three times by streaking. After re-screening, strains with optical density at 600 nm wavelength (OD_{600}) ≥ 0.3 at a cadmium concentration of 160 mg/L were retained. The bacterial cells were collected by centrifugation, and the bacterial genomic DNA was extracted using the Tiangen Deoxyribonucleic Acid Extraction Kit (Tiangen Biochemical Technology (Beijing) Co., Ltd., Beijing, China) following manufacturer’s instructions. Polymerase chain reaction (PCR) amplification was then performed using bacterial 16S rDNA universal primers 27F (5'-AGG TTT GAT CCT GCT CAG-3') and 1492R (5'-GTT ACC TTG TTA CGA CTT-3') synthesized by Bioengineering (Shanghai) Co., Ltd. (Shanghai, China). The PCR reaction contained 12.5 µL of 2× Taq PCR MasterMix (Beijing Quanshi Gold Biotechnology Co., Ltd., Beijing, China), 1 µL of each 10 µmol/L primers, 2 µL of template DNA, and 8.5 µL of sterile deionized water. The reaction mixture was pre-denatured at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 minute and a final extension at 72°C for 10 minutes. The PCR products were sent to Biotechnology (Shanghai) Co., Ltd. (Shanghai, China) for sequencing. The sequencing results were compared with the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) nr database using BLASTn program for bacterial strain identification. Gram staining was then performed for additional verification of the isolated bacterial strains using Gram staining kit (Beijing Soleibao

Technology Co., Ltd., Beijing, China) following manufacturer's instructions.

Determination of the optimal condition for bacterial strain culture

The single factor experiment selected five influencing factors including time, Initial cadmium ion solution concentration (ICISC), temperature, pH value, and sodium chloride concentration [13, 14], which covered 6 time periods of 10, 20, 30, 40, 50, 60 hours, 6 ICISC of 0, 40, 80, 120, 160, 180 mg/L, 6 temperatures of 20, 25, 30, 35, 40, 45℃, 6 pH values of 4, 5, 6, 7, 8, 9, and 6 sodium chloride concentrations of 2, 4, 6, 8, 10, 12%. A Genesys 150 UV-visible spectrophotometer (Thermo Fisher Scientific (China) Co., Ltd., Shanghai, China) was used to determine the bacterial growth and its influencing factors. In the analysis of adsorption characteristics, this study considered the effects of temperature, bacterial inoculation volumes of 1, 3, 5, 7, 9, 11%, and pH values on the bacterial adsorption rate of cadmium ions, which was tested by using the JC-KJB01 Soil Heavy Metal Rapid Detection Kit (Qingdao Jingcheng Instrument Co., Ltd., Qingdao, Shandong, China) following manufacturer's instructions. The isothermal adsorption test only considered the influence of different ICISCs. The cadmium adsorption rate (A_e) was calculated as follows [15, 16].

$$A_e = \frac{(B_0 - B_e) \times C}{D} \quad (1)$$

where B_0 and B_e were the concentrations of cadmium in the initial and equilibrium solutions. C was the solution volume. D was the solution mass. The isothermal adsorption model adopted Langmuir model and Freundlich model. Among them, the Langmuir model was built on the monolayer adsorption theory, which had the advantages of clear physical meaning and the ability to judge adsorption feasibility and was expressed as follows [17, 18].

$$\frac{B_e}{A_e} = \frac{1}{A_m K_L} + \frac{B_e}{A_m} \quad (2)$$

where A_m was the maximum adsorption capacity. K_L was the Langmuir adsorption constant, which was related to the affinity between the adsorbent and the adsorbate. The Freundlich model was an empirical model that had the advantages of not requiring the assumption of a single molecular layer, reflecting adsorption strength, and having a wide fitting range as follows [19, 20].

$$A_e = K_F B_e^{1/n} \quad (3)$$

where K_F was the Freundlich adsorption constant, which was correlated to the adsorption capacity. $1/n$ was the adsorption strength factor.

Pottery experiment

The pot experiment was conducted on June 15, 2024, with a duration of 90 days in an experimental greenhouse in Forestry Science Research Institute (Chenzhou, Hunan, China) near the mining area. 5 kg of soil collected from the mining area was placed in 32 plastic pots with dimensions of 40 × 20 × 15 cm^3 and applied bottom fertilizer. *Zoysia japonica* "Lan Yin No. 3" and *Bermuda grass* "Tifdwarf Bermudagrass 328" seeds were then sown. During the experiment, the soil moisture content in the potted plants was maintained within the range of 60% and 70%. All potted plants had sufficient sunlight. The germinated seeds were moved to the culture medium and introduced with different concentrations of viable bacteria. Five sets of experiments were conducted on both *Zoysia japonica* (A1 to A5) and *Bermuda grass* (B1 to B5) with 30 mL of aseptic water being added to each plant with viable bacterial counts of 0, 10^2, 10^4, 10^6, and 10^8 CFU/mL, respectively. After the 90-day experiment, soil samples and plant samples were collected, respectively. The soil indicators including cadmium concentration, pH, organic matter (OM), total nitrogen (TN), and available phosphorus (AP) were measured. The plant indicators including plant enzyme activity and cadmium accumulation in lawn plants were also measured.

Table 1. Information on sampling points.

Sampling point	Nitrogen-all (g/kg)	Rapid available phosphorus (AP) (mg/kg)	Potential of hydrogen (pH)	Organic matter (OM) (g/kg)	Cation exchange capacity (cmol/kg)	Cadmium (mg/kg)
I	0.43	6.92	5.02	9.15	12.5	1.62
II	1.10	7.15	4.01	24.02	14.3	2.25
III	0.48	5.05	4.83	11.56	11.8	2.31
IV	0.55	5.78	4.62	12.34	12.1	2.38
V	0.41	4.71	4.45	9.58	13.2	2.47
VI	1.09	9.12	5.12	22.76	13.8	0.92
VII	0.52	6.91	6.08	12.95	12.7	1.35
VIII	0.46	5.34	4.77	10.33	11.9	1.22

Statistical analysis

Student t-test was employed to compare the results between groups. *P* values less than 0.05 were defined as the statistically significant difference between groups.

Results and discussion

Influencing factors and adsorption characteristics of cadmium resistant bacterial strains

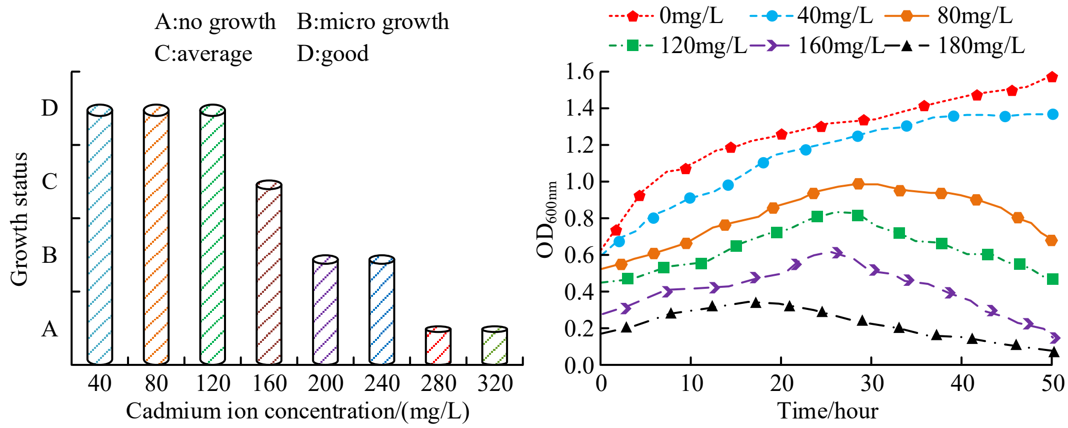
The results demonstrated that, among the 8 sampling points, the cadmium concentration at sampling point V reached 2.47 mg/kg, significantly higher than those at other sampling points (Table 1). Because high concentrations of cadmium can simulate "severely polluted scenarios", the remediation system faces more significant challenges in terms of cadmium bioavailability, making it easier to observe the differences produced by different remediation measures. Therefore, this study selected sampling point V as the soil resource for the pot experiment.

(1) Selection of cadmium tolerant bacterial strains and analysis of the influence of different initial cadmium ion solution concentrations

The selection results of cadmium-resistant strains and the analysis of the influence of different ICISCs demonstrated that, when the CIC was 40 mg/L, the growth of the strain was good. As the CIC increased, the growth of the strain gradually deteriorated from good growth to no growth (Figure 1a). The isolated bacterial strain showed a homology of ≥ 99% with the sequence of the *Bacillus cereus* standard strain in BLASTn and was identified as *Bacillus cereus*, which was a Gram-positive, short rod-shaped bacterium. As the initial CIC increased, the growth of the strain was more inhibited. Specifically, the strain could grow well at low concentrations of 0 and 40 mg/L, while at high concentrations of 160 and 180 mg/L, the growth of the strain was significantly inhibited but did not stop. When the concentration exceeded 40 mg/L, the OD_{600} value sharply decreased with the increase of concentration, showing a significant difference with the growth level in the range of 0 to 40 mg/L ($P < 0.05$). This further confirmed that 0 to 40 mg/L was the key concentration range for the strain to avoid cadmium ion stress and maintain efficient growth (Figure 1b). Overall, the initial CIC influenced the growth of bacterial strains, and the strains had strong tolerance to it.

(2) The effect of temperature on strain generation and adsorption

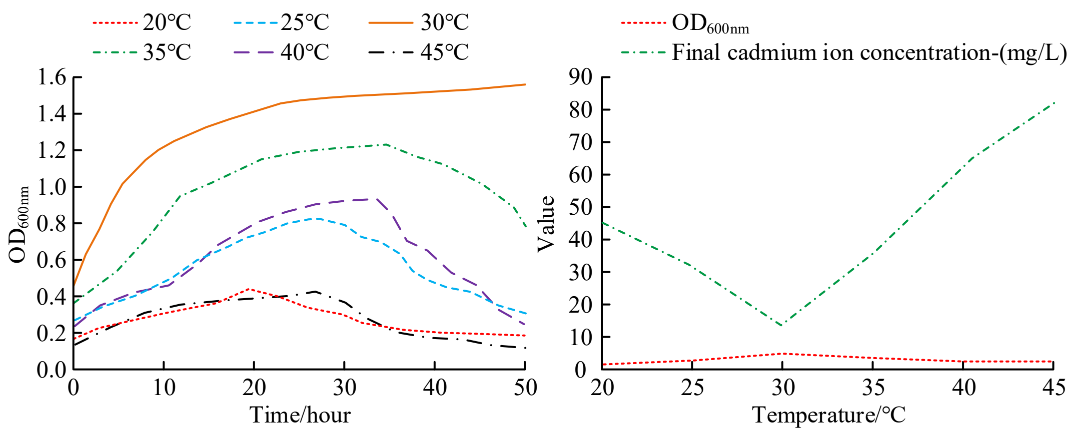
The effect of temperature on strain generation and adsorption showed that the growth of the strain under 30℃ conditions was significantly better than that under other temperature conditions, while the growth of the strain under other temperature conditions was inhibited. Specifically, under the condition of 20℃, the strain grew slowly, and the OD_{600} value did not change significantly throughout the entire



(a) Selection results of cadmium resistant bacterial strains

(b) The influence of initial cadmium ion solution concentration

Figure 1. Selection of cadmium tolerant bacterial strains (**a**) and analysis of the influence of different ICISCs (**b**).



(a) The effect of temperature on the growth of bacterial strains

(b) The effect of temperature on bacterial adsorption

Figure 2. Temperature influence on the generation (**a**) and adsorption (**b**) of bacterial strains.

cultivation process with a maximum of 0.45 and a minimum of 0.17, respectively. At 30℃, the OD_{600} value of the strain increased rapidly with a maximum value of 1.55 (Figure 2a). When the temperature reached the optimal 30℃, the final CIC reached its minimum value of 13.12 mg/L, demonstrating good adsorption capacity. When the temperatures were 20, 25, 35, 40, and 45℃, the CICs were 45.87, 30.99, 38.16, 65.19, and 83.99 mg/L, respectively. In addition, there were significant differences in the OD_{600} values and cadmium concentrations between the 30℃ group and other temperature groups ($P < 0.05$) (Figure 2b). Overall, 30℃ was the most suitable temperature for the bacterial strain to adsorb cadmium ions, at which point the strain grew better and ultimately had the lowest CIC.

(3) The effect of pH on bacterial strain generation and adsorption

The effect of pH on bacterial strain generation and adsorption demonstrated that the growth of the strain reached its optimum at pH 7 with a corresponding maximum OD_{600} value of 1.75 and the fastest growth rate, which indicated that a neutral environment was most suitable for the growth of bacterial strains. When the pH deviated from 7, the growth of the strain was

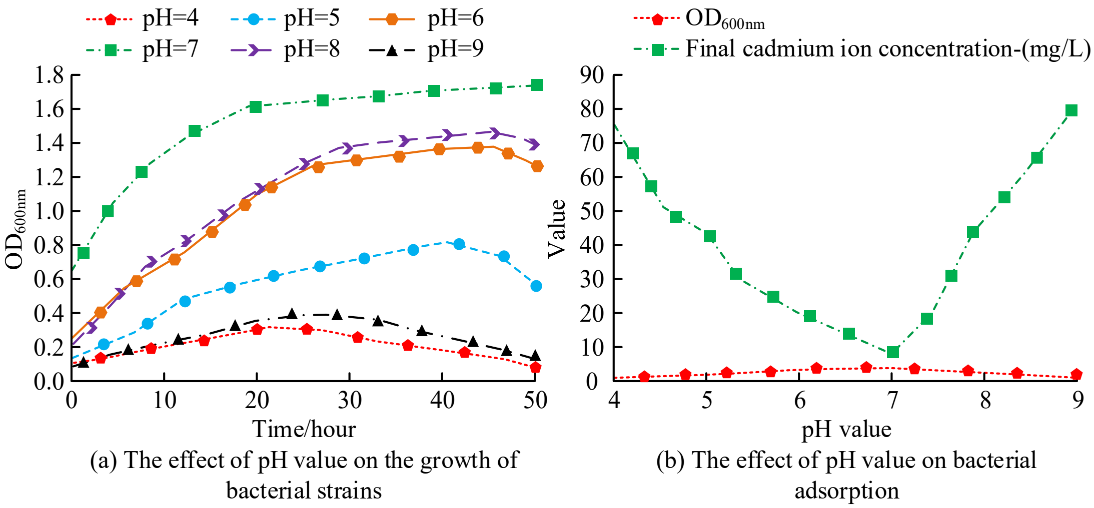
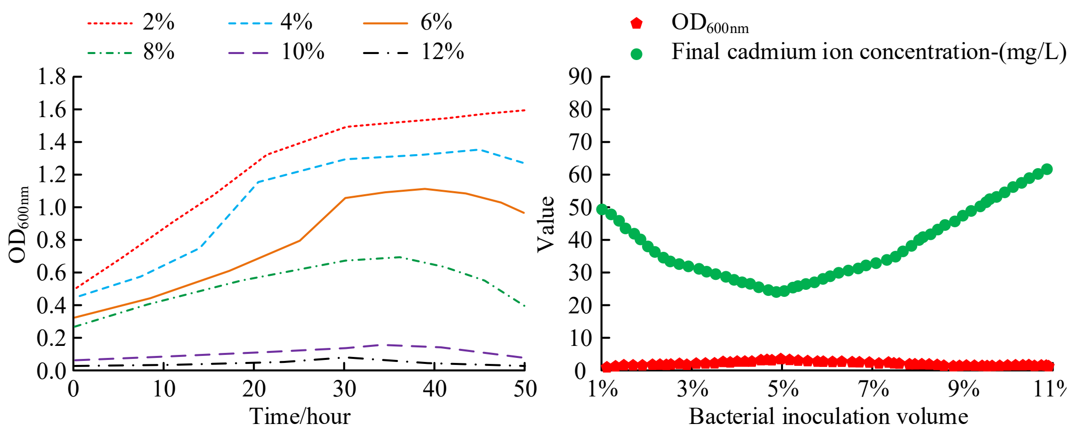


Figure 3. pH influence on bacterial strain generation (**a**) and adsorption (**b**).

inhibited to some extent either in acidic or alkaline (Figure 3a). In terms of the pH influence on the adsorption characteristics of bacterial strains, the OD_{600} value and final CIC were more advantageous when the pH value was 7 with respective values of 1.48 and 8.56 mg/L. In acidic or alkaline environments, the final CIC of the strain was significantly higher than that under neutral conditions (pH 7) in cadmium concentrations ($P < 0.05$), which further confirmed the optimal neutral environment (Figure 3b). Overall, pH value of 7 was most favorable for the growth of the strain and the removal of cadmium ions.

(4) The effects of sodium chloride concentration and bacterial inoculation on bacterial strains

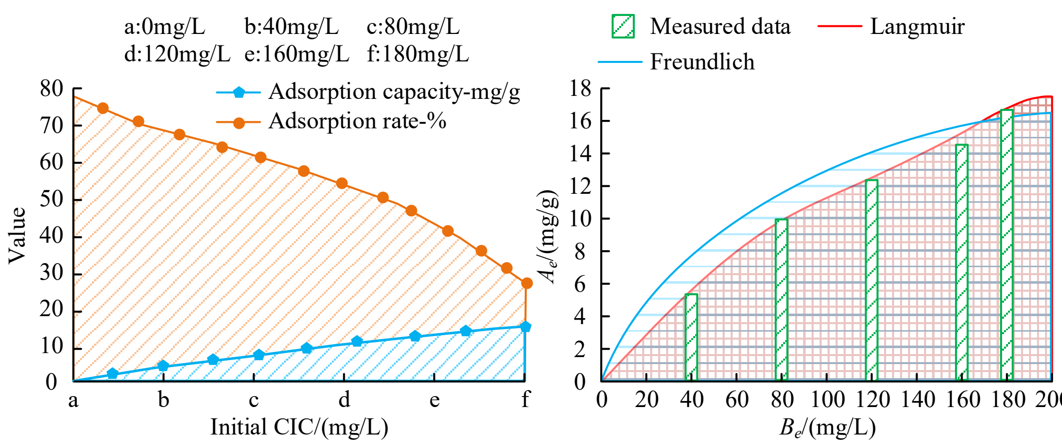
The effects of sodium chloride concentration and bacterial inoculum size on bacterial strains showed that as the sodium chloride concentration increased, its effect on bacterial growth gradually weakened, leading to a gradual deterioration in the growth of the bacterial strains. When the time was all 30 hours, the OD_{600} values corresponding to concentrations of 2, 4, 6, 8, 10, and 12% were 1.50, 1.32, 1.07, 0.66, 0.13, and 0.08, respectively. When the concentration of sodium chloride was 2%, the OD_{600} value of the strain was higher, and the formation state was better (Figure 4a). With high concentrations of sodium chloride, the growth was inhibited, which might be due to the high concentration of sodium chloride causing an increase in osmotic pressure, that in turn, affected the water balance and metabolic activity of the strain. With the rise of inoculation amount, the OD_{600} value first risen and then decreased in the influence of bacterial inoculation amount on the adsorption characteristics of the strain. The inflection point corresponded to an inoculation amount of 5%, indicating that this strain grew most vigorously at an inoculation amount of 5%. In addition, the final trend of CIC decreased first and then increased with the rise of inoculation amount, and the corresponding turning point was also 5%, indicating that the adsorption effect of the strain was better at this time. The OD_{600} value and final CIC corresponding to a 5% bacterial inoculation amount were 1.38 and 24.56 mg/L, respectively. Further, the OD_{600} value of the 2% sodium chloride group was significantly higher than that of other concentration groups ($P < 0.05$), and the difference between the 5% inoculation group and other groups was also significantly different ($P < 0.05$) (Figure 4b). Therefore, both of which were optimal conditions. Overall, the concentration of sodium chloride should be 2%, while the inoculation number of bacterial cells should be 5%.



(a) The effect of sodium chloride concentration on the growth of bacterial strains

(b) The effect of bacterial inoculation volume on bacterial adsorption

Figure 4. The influence of sodium chloride concentration (**a**) and bacterial inoculation number (**b**) on bacterial strains.



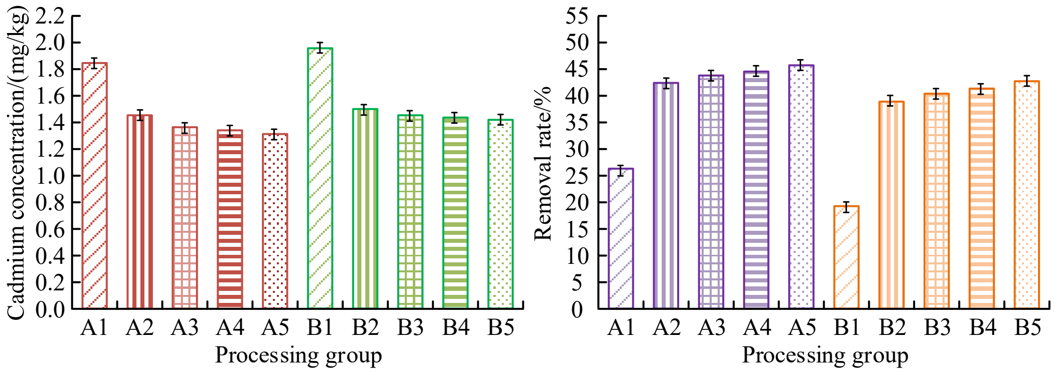
(a) Adsorption capacity and adsorption rate of bacterial strains under different initial CICs

(b) Isotherm adsorption model

Figure 5. The result of isothermal adsorption.

(5) The isothermal adsorption results

The isothermal adsorption results demonstrated that as the initial CIC increased, the adsorption capacity of the bacterial cells for cadmium ions also increased synchronously, while the adsorption rate decreased in the opposite direction. This trend was mainly due to the large number of available adsorption sites on the surface of the strain at low concentrations, which enabled cadmium ions to be quickly and efficiently adsorbed, resulting in a higher adsorption rate. However, as the CIC increased, the available adsorption sites gradually decreased, leading to a slower increase in adsorption capacity. Meanwhile, due to limitations in mass transfer and competitive adsorption between cadmium ions, the adsorption rate also significantly decreased. The maximum values of adsorption capacity and adsorption rate were 16.23 mg/g and 78.55%, respectively (Figure 5a). As the CIC in the solution increased, the adsorption rate of the strain also increased, but the rate of increase gradually slowed down and eventually approached



(a) Cadmium concentration in soil under different treatments

(b) Removal rate of cadmium in soil under different treatments

Figure 6. Soil cadmium concentration under different treatments.

saturation. The Langmuir model and the Freundlich model could both describe the adsorption process well and demonstrate that the strain had a good adsorption effect on cadmium ions. In addition, there were significant differences in adsorption capacity and adsorption rate among different initial cadmium concentration groups (P < 0.05), and the adsorption rate of the low concentration group was significantly higher than that of the high concentration group (P < 0.05) (Figure 5b).

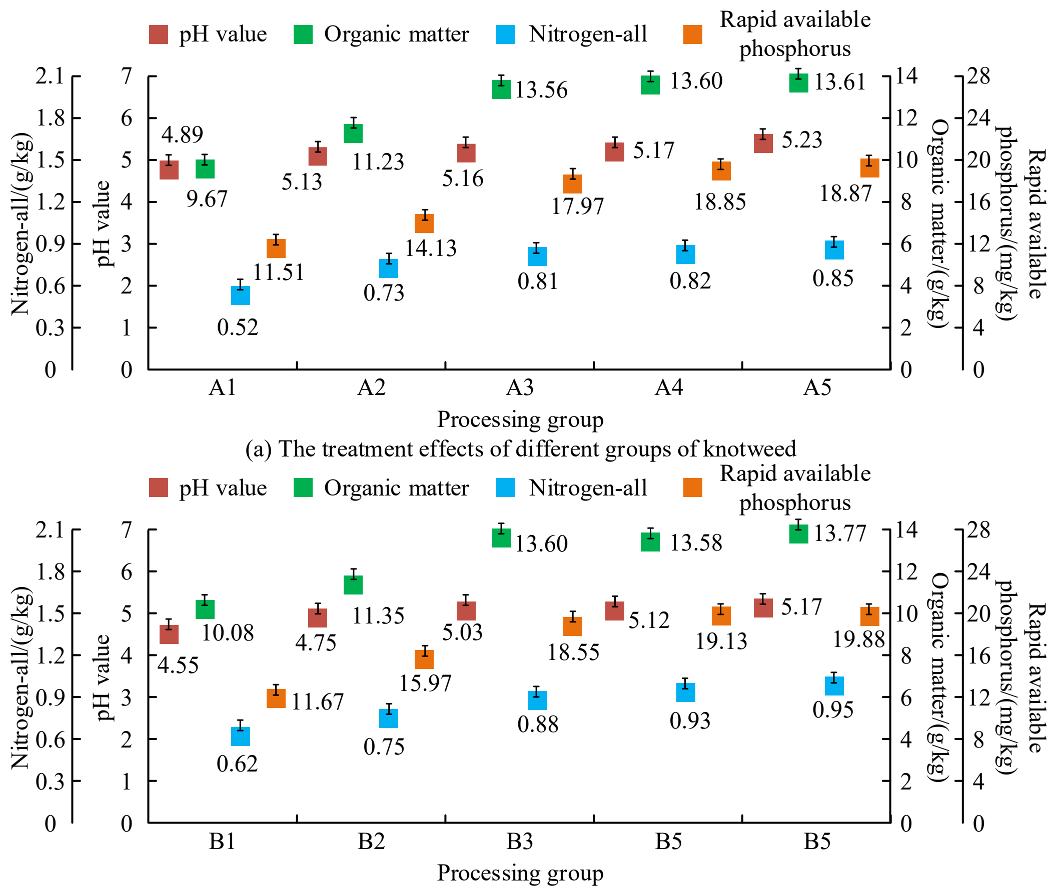
Application effect of cadmium resistant bacterial strains

(1) The cadmium concentration in soil under different treatments

The results showed that both *Zoysia japonica* and *Bermuda grass* plant remediation could significantly reduce cadmium concentration in soil. In *Zoysia japonica*'s A1 to A5, the corresponding soil cadmium concentrations were 1.82, 1.43, 1.38, 1.36, and 1.34 mg/kg, respectively, while *Bermuda grass*'s B1 to B5 groups corresponded to soil cadmium concentrations of 1.98, 1.51, 1.47, 1.45, and 1.43 mg/kg, respectively. As the number of viable bacteria increased in the *Zoysia japonica* and *Bermuda grass* treatment groups, the removal rate of cadmium in the soil also improved, indicating the positive role of bacterial strains in improving remediation efficiency in microbial-assisted plant remediation technology (Figure 6a). In terms of specific removal rates, the values corresponding to groups A1 to A5 in *Zoysia japonica* were 26.32, 42.11, 44.13, 44.94, and 45.75%, while the values corresponding to groups B1 to B5 in *Bermuda grass* were 19.84, 38.87, 40.49, 41.30, and 42.17%, respectively (Figure 6b). There was a significant difference in soil cadmium concentration and removal rate between the high and low viable bacterial arrays of *Zoysia japonica* and *Bermuda grass* (P < 0.05), indicating a significant synergistic effect of the bacterial strains.

(2) The soil pH, OM, TN, and AP under different treatments

The results of soil pH, OM, TN, and AP under different treatments demonstrated that different groups in A1 to A5 of *Zoysia japonica* were able to increase the soil pH to a certain extent, gradually approaching the neutral value of 7. The pH values corresponding to A1 to A5 were 4.89, 5.13, 5.16, 5.17, and 5.23, respectively. OM corresponding to A1 to A5 increased by 0.09, 1.65, 3.98, 4.02, and 4.03. In addition, the TN and AP in the soil increased in different groups (Figure 7a). Different groups from B1 to B5 of *Bermuda grass* improved soil pH, OM, TN, and AP. There were significant differences observed in pH, OM, and other indicators between the treatment groups of *Zoysia japonica* and *Bermuda grass* and the initial soil (P < 0.05), indicating a significant soil improvement effect (Figure 7b). The



(a) The treatment effects of different groups of knotweed

(b) The treatment effect of different dog tooth root groups

Figure 7. Soil pH, OM, TN, and AP under different treatments.

treatment of *Zoysia japonica* and *Bermuda grass* in different groups not only increased the pH value of the soil, making it closer to neutrality, but also increased the content of OM, TN, and AP in the soil, which further confirmed the potential of microbial-assisted plant remediation technology in improving the soil environment and promoting plant growth.

(3) The plant enzyme activity under different treatments

The results of plant enzyme activity under different treatments showed that, from A1 to A5 in *Zoysia japonica*, the corresponding leaf malondialdehyde demonstrated a decreasing trend, while the activities of superoxide dismutase, peroxidase, and catalase showed an increasing trend. In addition, the contents of malondialdehyde in the leaves of these five groups were 8.23, 8.18, 5.99, 5.08, and 5.03 mmol/g FW, respectively (Figure 8a). In the B1 to B5 groups of *Bermuda grass*, it had the same effects as *Zoysia japonica* on different indicators with the optimal group in *Bermuda grass* corresponding to leaf malondialdehyde, superoxide dismutase activity, peroxidase activity, and catalase activity as 7.02 mmol/g FW, 489.04 U/g FW, 70.13 µg/g FW/min, and 1,001.25 U/g FW/min, respectively (Figure 8b). There were significant differences observed in the levels of malondialdehyde and enzyme activity between the high activity bacterial arrays of *Zoysia japonica* and *Bermuda* grass and the A1/B1 group ($P < 0.05$), indicating that the strains could alleviate plant stress. By increasing the number of viable bacteria, the malondialdehyde contents in the leaves of *Zoysia japonica* and *Bermuda grass* were reduced, while the activities of

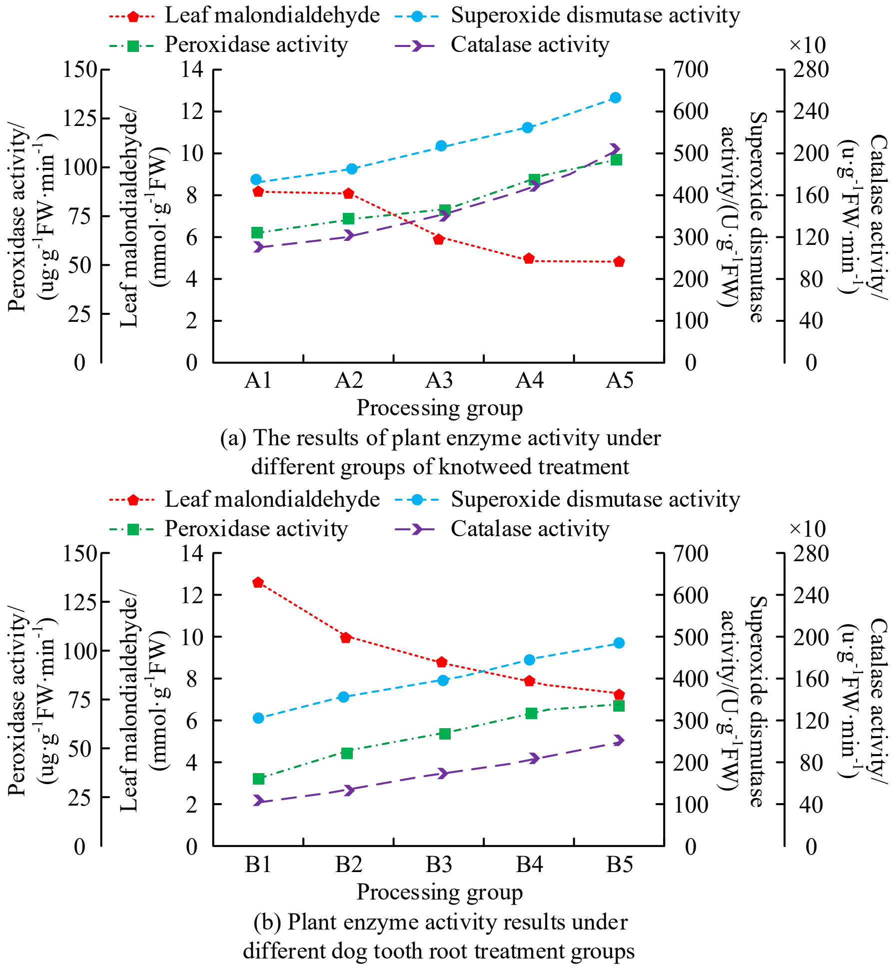


Figure 8. Plant enzyme activities under different treatments.

superoxide dismutase, peroxidase, and catalase were increased, which indicated that introducing strains could effectively enhance the remediation ability of plants in the HMP environment.

(4) The comparison of cadmium accumulation in lawn plants under different treatments

The comparison of cadmium accumulation in lawn plants under different treatments showed that, in the A1 to A5 groups of *Zoysia japonica*, the accumulation of cadmium in the aboveground and root systems of plants in group A3 was higher at 518 and 782 mg/kg with the corresponding number of active bacteria of 10^4 CFU/mL. In addition, in the B1 to B5 groups of *Bermuda grass*, the B3 group had a higher accumulation of cadmium in both aboveground and root systems with an active bacterial count of 10^4 CFU/mL (Figure 9a). The results indicated that, under moderate conditions of active bacterial count, plants could achieve optimal accumulation of cadmium, which might be because moderate microbial assistance promoted the absorption and transport of cadmium by plants, thereby improving the efficiency of plant remediation. The trends of enrichment coefficients for the *Zoysia japonica* and *Bermuda grass* groups were basically the same as increasing first and then decreasing with only different turning points. *Zoysia japonica* had a turning point at A3 with an active bacterial count of 10^4 CFU/mL, while *Bermuda grass* had a turning point at B2 with active bacterial count of 10^2 CFU/mL. In addition, the maximum

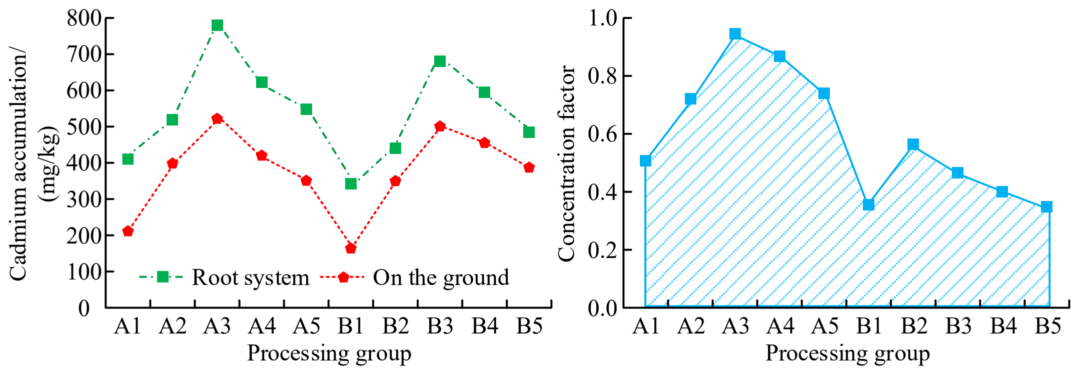


Figure 9. Accumulation of cadmium in lawn plants under different treatments.

enrichment coefficients for *Zoysia japonica* and *Bermuda grass* were 0.967 and 0.588, respectively. There were significant differences in cadmium accumulation and enrichment coefficient between the A3 group of *Zoysia japonica* and the B2 group of *Bermuda grass* compared to other groups with the optimal moderate bacterial count ($P < 0.05$) (Figure 9b). The number of active bacteria had a significant impact on the efficiency of plant remediation, and a moderate number of bacteria could maximize the accumulation and enrichment of cadmium in plants.

Conclusion

This research demonstrated that the growth and adsorption of cadmium resistant strain, *Bacillus cereus*, was significantly influenced by multiple factors including time, ICISC, temperature, pH value, and sodium chloride concentration, which all affected the growth of bacterial strains. In the pot experiment, the results suggested that soil quality gradually improved with the increase of viable bacteria. As the number of live bacteria increased, the malondialdehyde content in the leaves of *Zoysia japonica* and *Bermuda grass* significantly decreased, while the activities of superoxide dismutase, peroxidase, and catalase increased. The cadmium accumulation in the aboveground and root systems of *Zoysia japonica* A3 group plants was higher, while *Bermuda grass* had higher levels in B2. The synergistic effect of microorganisms and plants could effectively enhance the remediation effect of HMP soil. However, this study was only conducted under potted conditions and did not involve the impact of complex field environments. Future work should conduct field experiments and optimize the remediation system by combining soil improvement measures.

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