Pak. J. Bot., 58(3): 791-799, March 2026.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2026-3(12)

EFFECTS OF PHOSPHATE-SOLUBILIZING BACTERIA ON ELEMENT CONTENT IN RHIZOMES AND RHIZOSPHERE SOIL OF *PARIS POLYPHYLLA* VAR. *YUNNANENSIS*

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Abstract

This study aimed to investigate the impact of phosphate-solubilizing bacteria on the contents of 5 heavy metals and 10 nutrients in the rhizomes and rhizosphere soil of *Paris polyphylla* var. *yunnanensis*. A pot experiment was conducted by inoculating three bacterial strains into the rhizosphere soil. Subsequently, the element content was determined by microwave digestion using inductively coupled plasma mass spectrometry. The results showed that the contents of N, P, Ca, Mg, Fe, Cu, and Zn in the rhizomes were higher in the 7 treatment groups than in the control (CK) group. Also, the contents of K, Ca, Mg, Na, Cu, and Zn in the rhizosphere soil were higher in these groups than in the CK group. Further, the Pb contents in rhizomes were higher in the treatment groups than in the CK group. Except for Cd, the contents of the other heavy metals in the rhizosphere soil were higher in the treatment groups compared with the CK group. The contents of Pb in rhizomes and Cd in the rhizosphere soil in the treatment groups slightly exceeded the standard limit. The enrichment analysis showed that the phosphate-solubilizing bacteria promoted the enrichment of P, Cu, Zn, K, Ca, and Mg but reduced the enrichment of heavy metals in the rhizomes of *Paris polyphylla* var. *yunnanensis.* The correlation analysis showed that the contents of As and Hg in rhizomes were negatively correlated, whereas the contents of other elements were positively correlated. The contents of Fe, Mn, and Zn in soil were positively correlated, whereas the contents of Pb and Hg were negatively correlated. Zn and Hg were positively correlated with Cr and Pb in rhizosphere soil. In conclusion, the application of phosphate-solubilizing bacteria can enhance the concentration of nutrient elements in rhizomes and rhizosphere soil and reduce the enrichment of heavy metals in *Paris polyphylla* var. *yunnanensis*, thereby maintaining soil fertility.

Key words: Nutrient element; *Paris polyphylla* var. *yunnanensis*; Phosphate-solubilizing bacteria; Soil heavy metals

Introduction

Paris polyphylla var. *yunnanensis* (*P. polyphylla* var. *yunnanensis*) is a perennial herb belonging to the Liliaceae family. Its rhizome is bitter in taste and used as medicine. It clears heat, detoxifies the body, cools the liver, and calms (Anon., 2020). Studies have shown that *P. polyphylla* var. *yunnanensis* contains various chemical components, such as steroidal saponins, flavonoids, and polysaccharides, with multiple effects including anti-inflammatory, analgesic, hemostatic, and tumor inhibitory (Wang *et al*., 2022). Its wild resources are mainly distributed in southwestern China, such as Yunnan, Guizhou, Guangxi, and Sichuan, with slow regeneration and low reproductive rate. However, given the excessive exploitation due to medicinal demand and the depletion of wild resources, the artificial cultivation of *P. polyphylla* var. *yunnanensis* has become one of the most effective ways to alleviate the contradiction between supply and demand. *P. polyphylla* var. *yunnanensis* is mainly cultivated via two modes: understory planting and intensive planting (Li *et al*., 2016). Fertilization can facilitate the symbiosis between the roots of *P. polyphylla* var. *yunnanensis* and AM (Arbuscular mycorrhizae) fungi, improve soil microenvironment, and promote the accumulation of photosynthetic pigments and saponins (Li *et al*., 2022). However, the extensive use of inorganic fertilizers can result in the imbalance of N and P cycles in the soil environment in which the medicinal plants grow, causing serious problems such as deterioration of the physical and chemical properties of soil, a decrease in the activity of beneficial enzymes, destruction of bacterial flora, excessive accumulation of heavy metals, and so on (Hu *et al*., 2021; Li *et al*., 2023).

Compared with traditional fertilizers, microbial fertilizers are more environmentally friendly and conducive to sustainable development. The microorganisms in the plant rhizosphere can regulate the soil microenvironment, resist heavy metal stress, increase the activity of beneficial enzymes, and enhance the resistance of the host to pests and diseases. Phosphate-solubilizing bacteria can transform insoluble phosphate into a form that can be easily absorbed by plants through phosphorus dissolution and mineralization (Cheng *et al*., 2023), thereby providing more available phosphate fertilizer for plant growth. Phosphate-solubilizing bacteria can increase phosphatase activity, reduce heavy metal accumulation, balance the microbial community structure (Yang *et al*., 2023; Hu *et al*., 2022), and regulate the physical and chemical properties of soil, thus promoting the absorption of calcium, magnesium, and iron in plants

Received: 15-01-2025 | Revised: 05-05-2025 | Accepted: 15-05-2025 | Online: 15-12-2025

(Feng *et al*., 2020) and having a positive impact on plant photosynthesis. *Bacillus* spp., are widely distributed in soil and a valuable group of phosphate-solubilizing bacteria. *Bacillus cereus* CLY07 isolated from the rhizosphere soil of *Taxus chinensis* could decompress inorganic phosphorus and produce IAA (Indole-3-acetic acid) and siderophores (Feng *et al*.,2022). Wang *et al.* (Wang *et al*., 2022) mixed *B. aryabhattai* SK1-7 and cow manure into the planting soil of grapes and found an increase in the weight of grape ears and fruit grains. This indicates immense potential of *Bacillus* in promoting plant growth.

The effect of phosphate-solubilizing bacteria on *P. polyphylla* var. *yunnanensis* and its rhizosphere soil environment have rarely been investigated at home and abroad. *P. polyphylla* var. *yunnanensis* absorbs both nutrient elements and heavy metals, and hence can be used to assess the nutrient level of the environment in which it grows. This study was performed to explore the mechanism of phosphate-solubilizing bacteria in regulating the nutrient level of rhizosphere soil and clarify the enrichment ability of *P. polyphylla* var. *yunnanensis* under the influence of these bacteria. The rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis* were examined. Also, the contents of 10 nutrient elements and 5 heavy metals in the samples were determined by applying phosphate-solubilizing bacteria. The effect of phosphate-solubilizing bacteria on the nutrient level of the soil environment was preliminarily evaluated, providing a foundation for artificial planting and the development of microbial fertilizers.

Materials and Methods

Experimental materials and cultivation conditions: The bacterial strains used in this study were provided by Associate Professor Huihui Du, College of Biology and Food Engineering, Chongqing Three Gorges University, Chongqing, China. They were *B. cereus* Y1-1, *B. aryabhattai* Z6-1, and *B. aryabhattai* Z3-4. The 4-year-old seedlings used in this study were provided by Shanyang Town, Yongping County, Dali Prefecture, Yunnan Province and identified as *P. polyphylla* var. *yunnanensis* by Associate Professor Fajian Yang of Baoshan College of Traditional Chinese Medicine.

The experiment was conducted at Anshun College, Anshun City, Guizhou Province, in a greenhouse using a potted method. The planting soil was a mixture of organic fertilizer, garden soil, and river sand mixed in a ratio of 1:1:2. The soil was sterilized at 121℃ for 2 h and placed for 7 days. Then, the planting soil was placed in a pot with a bottom diameter of 18 cm, a top diameter of 19.8 cm, and a height of 20 cm. A total of seven treatment groups from S1 to S7 were set up, including the inoculation of single bacterial strains and the mixed inoculation of two and three bacterial strains. A control (CK) group was set up, with conditions consistent with those in the treatment group, except that no bacterial solution was inoculated. A total of 10 pots for each group were set up, with 5 seedlings planted in each pot.

Each bacterial strain was cultured in a liquid medium and diluted with normal saline to a concentration of 10^6 CFU·mL^{-1} (Colony Forming Unit, CFU). The bacterial suspension was inoculated two times, and the plants were harvested in October of the following year. *B. cereus* Y1-1 (300 mL) was applied to the S1 group, *B. aryabhattai* Z6-1 (300 mL) was applied to the S2 group, *B. aryabhattai* Z3-4 (300 mL) was applied to the S3 group, and a combination of *B. cereus* Y1-1 (150 mL) and *B. aryabhattai* Z6-1 (150 mL) was applied to the S4 group. A combination of *B. cereus* Y1-1 (150 mL) and *B. aryabhattai* Z3-4 (150 mL) was applied to the S5 group, a combination of *B. aryabhattai* Z6-1 (150 mL) and *B. aryabhattai* Z3-4 (150 mL) was applied to the S6 group, and a combination of *B. cereus* Y1-1 (100 mL), *B. aryabhattai* Z6-1 (100 mL), and *B. aryabhattai* Z3-4 (100 mL) was applied to the S6 group. Distilled water (300 mL) was applied to the CK group.

Instruments: The instruments used in this study were as follows: a single-coupled multipurpose electric furnace (Beijing Kewei Yongxing Instrument Co., Ltd.), a 4010 microwave digester (Shanghai Yiyao Instrument Technology Development Co., Ltd.), an EXPEC-7000 inductively coupled plasma mass spectrometer (Hangzhou Concentrating Technology Co., Ltd.), and an ME204E analytical balance (METTLER Toledo Instrument Shanghai Co., Ltd.).

Determination method: The root-shaking method was used to collect the rhizosphere soil (Tong *et al*., 2019). For this, 0.2 g of rhizosphere soil was weighed, placed in a microwave digestion tank, mixed with 1 mL of hydrofluoric acid and 8 mL of concentrated nitric acid, covered tightly, and kept for 12 h. The blank solution was prepared using the same method. The digestion was carried out as described by Qiaojuan Lan (Lan *et al*., 2018). Then, 0.2 g of rhizome in each group was weighed, and the sample was processed using the method proposed by Guangjiao Zhou *et al.* (Zhou *et al*., 2018), followed by digestion as proposed by Hailing Li *et al*., (Li *et al*., 2021). The reference storage liquid was precisely measured, and the standard working liquid of various concentrations was prepared with 2% dilute nitric acid. The concentration of each element was measured by inductively coupled plasma mass spectrometry (ICP-MS). The nitrogen content was determined by the Kjeldahl method (Song, 2019).

Evaluation of bioconcentration factor of metal elements: The bioconcentration factor (BCF) of an element is the ratio of its concentration in the plant to that in the soil (Yang *et al*., 2018).

Limit standard of heavy metals: The limit standards for five heavy metals in medicinal materials are shown in Table 1 (Anon., 2020; Anon., 2017).

Evaluation of soil pollution status of *P. polyphylla var. yunnanensis*: In this study, the pH value of rhizosphere soil ranged from 7.00 to 7.45. The risk assessment of five heavy metals in the rhizosphere soil of *Paris polyphylla* var. *yunnanensis* is presented in Table 2 (Anon., 2018).

Data analysis: Excel 2010, SPSS 16.0, and Origin 2022 software were used for analyzing the data.

Table 1. Limit standards of five heavy metals (mg · kg^{-1})

Element	Limit standard	Element	Limit standard
Cd	≤1.0	As	≤2.0
Cr	≤2.0	Hg	≤0.2
Pb	≤5.0		

Table 2. Risk assessment of five heavy metals (mg · kg^{-1})

Element	6.5 < pH ≤ 7.5	pH > 7.5
Hg	≤2.4	≤3.4
Pb	≤120	≤170
Cd	≤0.3	≤0.6
As	≤30	≤25
Cr	≤200	≤250

Results

Effects of phosphate-solubilizing bacteria on the nutrient elements in the rhizomes of *P. polyphylla* var. *yunnanensis*

Nutrient element contents in the rhizomes of *P. polyphylla* var. *yunnanensis*: Significant differences in the concentration of 10 nutrient elements in rhizomes were observed among different groups after inoculation with various phosphate-solubilizing bacteria (Table 3). The total element content was higher in the 7 treatment groups compared with the CK group, with the S2 and S7 groups exhibiting the highest total content. The contents of N, Cu, P, Ca, Fe, Mg, and Zn in the S1-S7 groups were higher than those in the CK group. The content of N ranged from 2.65 to 3.45 g · kg^{-1}, with the highest in the S7 group. The content of P was 4.43-5.88 g · kg^{-1}, with the highest in the S2 group. The content of Ca was 8.11-10.14 g · kg^{-1}, with the highest in the S3 group. The content of Mg was 3.42-4.51 g · kg^{-1}, with the highest in the S2 group. The content of Fe was 0.50-0.94 g.kg^{-1}, with the highest in the S7 group. The contents of Cu and Zn were 0.33-0.66 and 0.28-0.41 g · kg^{-1}, respectively, with the highest in the S2 group.

The element contents in each group followed an increasing trend after inoculation compared with that in the CK group. The top three groups with the largest increase in the total nutrient element contents were S7, S2, and S6, with an increase of 26.89%, 25.13%, and 18.50%, respectively. Therefore, it was inferred that the inoculation of phosphate-solubilizing bacteria promoted the use of nutrient elements in the rhizomes.

Principal component analysis of nutrient elements in the rhizomes of *P. polyphylla* var. *yunnanensis*: Principal component analysis (PCA) was performed on 10 nutrient elements in the rhizomes. Three principal components with eigenvalues greater than 1 were extracted (Fig. 1A and Table 4), with a cumulative contribution rate of 81.53%. The eigenvalue of principal component 1 (PC1) was 5.01 and the contribution rate was 50.05%, primarily representing the concentrations of Na, Fe, Ca, Mg, Cu, Zn, and Mn in the rhizomes. The eigenvalue of principal component 2 (PC2) was 2.09 and the contribution rate was 20.89%, primarily representing the concentrations of K, P, and N in the rhizomes. The characteristic value of principal component 3 (PC3) was 1.06 and the contribution rate was 10.59%, primarily representing the concentrations of Zn in *P. polyphylla* var. *yunnanensis*. The principal component scores are shown in Figure 1B, wherein dots of various colors represent the scores for different groups of elements in the PCA. The direction and length of each arrow indicate the contribution and significance of the content of corresponding elements to the principal component. The longer the length, the greater is the relative influence. For PC1, PC2, and PC3, the S2, S7, and S1 groups had the highest score. The S2 group had the highest comprehensive score. The S2, PC1, and PC3 groups had the highest scores. The matrix coefficients of Na, Fe, Ca, Mg, Cu, Zn, and Mn in PC1 were larger, whereas the matrix coefficients of Zn in PC3 were the largest. These elements could be considered as the characteristic elements of rhizomes in *P. polyphylla* var. *yunnanensis*.

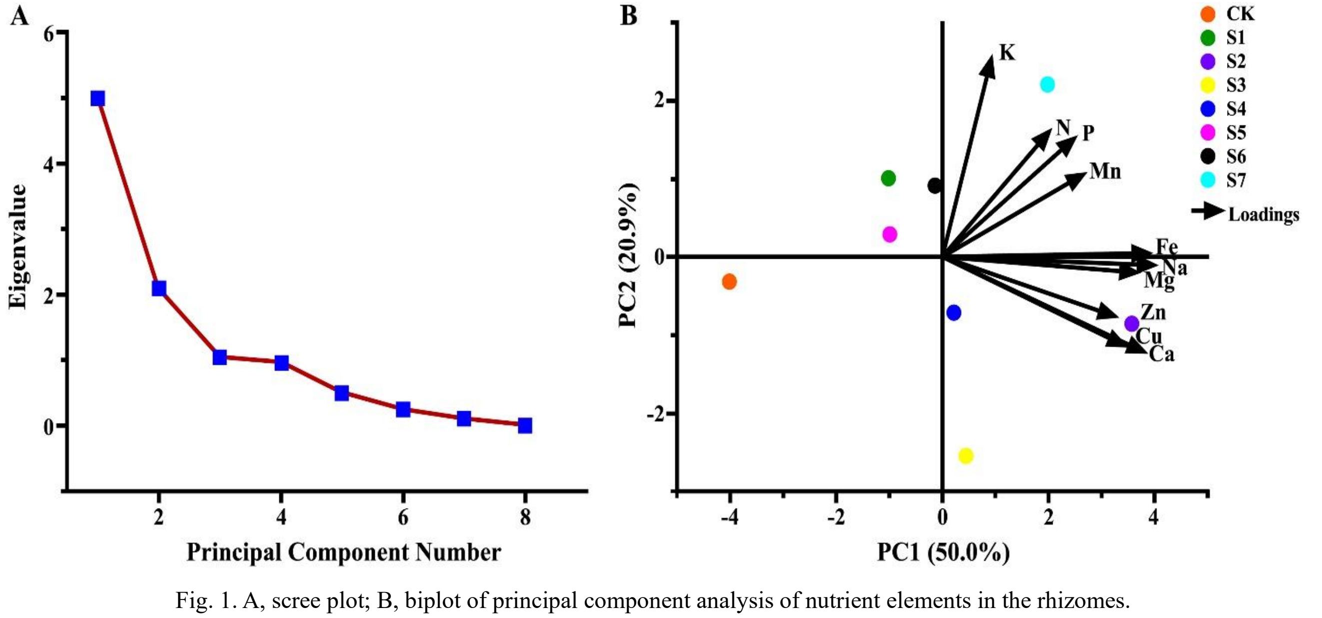

Fig. 1. A, scree plot; B, biplot of principal component analysis of nutrient elements in the rhizomes.

Table 3. Content of 10 nutrient elements in the rhizomes ($\bar{x} \pm s$, $n = 3$, $g \cdot kg^{-1}$).

	S1	S2	S3	S4	S5	S6	S7	CK
N	2.802 ± 0.092e	2.657 ± 0.137g	2.700 ± 0.043f	3.107 ± 0.108d	3.121 ± 0.040c	3.132 ± 0.073b	3.455 ± 0.040a	2.257 ± 0.051h
P	5.542 ± 0.074d	5.887 ± 0.025a	4.666 ± 0.015f	4.431 ± 0.003h	5.594 ± 0.018c	5.367 ± 0.046e	5.697 ± 0.012b	4.483 ± 0.153g
K	14.350± 0.055b	13.309 ± 0.040e	10.852 ± 0.053h	13.497 ± 0.042d	13.299 ± 0.019f	14.199 ± 0.006c	14.909 ± 0.014a	12.531 ± 0.200g
Ca	8.119 ± 0.033g	10.038 ± 0.076b	10.149 ± 0.048a	9.202 ± 0.015d	8.511 ± 0.018f	8.629 ± 0.017e	9.391 ± 0.057c	7.661 ± 0.081h
Mg	3.849 ± 0.100c	4.510 ± 0.016a	3.803 ± 0.009e	3.803 ± 0.007e	3.424 ± 0.008f	4.053 ± 0.058b	3.811 ± 0.019d	3.357 ± 0.131g
Na	0.284 ± 0.010h	0.836 ± 0.065b	0.636 ± 0.021c	0.586 ± 0.004d	0.472 ± 0.004f	0.495 ± 0.003e	0.849 ± 0.004a	0.299 ± 0.010g
Fe	0.598 ± 0.021f	0.932 ± 0.006b	0.841 ± 0.007d	0.761 ± 0.002e	0.502 ± 0.007g	0.873 ± 0.004c	0.947 ± 0.004a	0.453 ± 0.017h
Cu	0.370 ± 0.017e	0.669 ± 0.005a	0.443 ± 0.008bc	0.462 ± 0.003b	0.436 ± 0.002d	0.336 ± 0.004g	0.350 ± 0.003f	0.250 ± 0.101h
Mn	0.201 ± 0.005c	0.318 ± 0.011b	0.147 ± 0.011e	0.132 ± 0.003f	0.093 ± 0.007h	0.098 ± 0.004g	0.406 ± 0.007a	0.171 ± 0.021d
Zn	0.344 ± 0.010e	0.411 ± 0.005a	0.371 ± 0.005c	0.393 ± 0.002b	0.362 ± 0.004d	0.288 ± 0.004g	0.308 ± 0.007f	0.158 ± 0.030h

Note: Different letters in the same line indicate a significant difference ($p<0.05$), the same below

Table 4. Matrix of principal component analysis of nutrient elements in the rhizomes.

Element	PC1	PC2	PC3
N	0.449	0.577	0.328
P	0.560	0.551	0.201
K	0.207	0.924	0.149
Ca	0.851	-0.445	-0.110
Mg	0.822	-0.074	-0.011
Na	0.899	-0.037	-0.277
Fe	0.871	0.015	-0.251
Cu	0.769	-0.415	0.276
Mn	0.590	0.380	-0.587
Zn	0.735	-0.278	0.563
Eigenvalue	5.005	2.090	1.059
Contribution rate (%)	50.046	20.896	10.590
Cumulative contribution rate (%)	50.046	70.942	81.532

Table 5. Content of 10 nutrient elements in the rhizosphere soil ($\bar{x} \pm s$，$n = 3$，$g \cdot kg^{-1}$).

	S1	S2	S3	S4	S5	S6	S7	CK
N	8.883 ± 0.029a	5.596 ± 0.005f	8.092 ± 0.159d	4.259 ± 0.003h	4.910 ± 0.013g	8.515 ± 0.047c	8.646 ± 0.010b	6.466 ± 0.007e
P	1.052 ± 0.015b	1.110 ± 0.017a	0.945 ± 0.009d	0.769 ± 0.010h	0.844 ± 0.006g	1.001 ± 0.001c	0.912 ± 0.006f	0.933 ± 0.003e
K	11.297 ± 0.059h	11.793 ± 0.008c	11.398 ± 0.003g	11.417 ± 0.005e	12.214 ± 0.013b	11.424 ± 0.010d	12.261 ± 0.006a	11.404 ± 0.007f
Ca	8.713 ± 0.100d	9.819 ± 0.041b	4.056 ± 0.005h	9.402 ± 0.011c	10.606 ± 0.021a	6.294 ± 0.019f	6.599 ± 0.015e	4.081 ± 0.039g
Mg	2.239 ± 0.027d	2.319 ± 0.004b	1.723 ± 0.011h	2.022 ± 0.009g	2.250 ± 0.322c	2.388 ± 0.065a	2.070 ± 0.011e	2.056 ± 0.004f
Na	8.519 ± 0.005g	11.110 ± 0.019b	9.827 ± 0.023d	14.510 ± 0.004a	9.626 ± 0.013f	10.585 ± 0.008c	9.748 ± 0.015e	8.348 ± 0.018h
Fe	33.596± 0.006a	32.767 ± 0.008b	25.607 ± 0.013f	28.216 ± 0.025d	29.156 ± 0.012c	24.187 ± 0.028h	25.548 ± 0.015g	26.289 ± 0.021e
Cu	0.069 ± 0.003bc	0.070 ± 0.003b	0.058 ± 0.004d	0.062 ± 0.003cd	0.065 ± 0.003bcd	0.049 ± 0.003e	0.094 ± 0.002a	0.050 ± 0.002e
Mn	5.557 ± 0.016a	5.418 ± 0.014b	4.224 ± 0.011g	4.661 ± 0.004e	4.803 ± 0.014c	4.005 ± 0.004h	4.284 ± 0.064f	4.694 ± 0.064d
Zn	0.191 ± 0.021a	0.179 ± 0.011b	0.165 ± 0.028d	0.169 ± 0.004c	0.167 ± 0.009cd	0.144 ± 0.010e	0.134 ± 0.005g	0.137 ± 0.009f

Table 6. Matrix of principal component analysis of nutrient elements in the rhizosphere soil.

Rhizosphere soil element	PC1	PC2	PC3	PC4
N	-0.364	0.766	0.285	-0.113
P	0.335	0.828	0.078	0.167
K	0.093	-0.393	0.869	0.049
Ca	0.848	-0.433	0.153	0.167
Mg	0.515	0.141	0.227	0.797
Na	0.134	-0.751	-0.339	0.037
Fe	0.961	0.178	-0.050	-0.178
Cu	0.232	-0.128	0.818	-0.414
Mn	0.909	0.233	-0.068	-0.163
Zn	0.827	0.096	-0.344	-0.295
Eigenvalue	3.744	2.308	1.827	1.025
Contribution rate (%)	37.443	23.079	18.273	10.245
Cumulative contribution rate (%)	37.443	60.522	78.795	89.040

Effects of phosphate-solubilizing bacteria on nutrient elements in the rhizosphere soil of *P. polyphylla* var. *yunnanensis*

Nutrient element contents in the rhizosphere soil: As shown in Table 5, the nutrient element contents in the rhizosphere soil in each group showed significant differences after applying phosphate-solubilizing bacteria (p<0.05). The total concentration of nutrient elements was higher in the bacterial application groups compared with the CK group, with Fe, Na, K, and N having relatively high proportions. The concentration of K, Ca, Mg, Na, Cu, and Zn elements displayed an increasing trend in the S1-S7 groups compared with the CK group, The contents of each element were 11.29-12.26, 4.05-10.60, 1.72-2.38, 8.51-14.51, 0.04-0.09, and 0.13-0.19 g · kg^{-1}, respectively. The content of other nine elements, except K, was higher in the S1 group compared with the CK group, with the most significant increase in the content of Ca (53.16%). The content of other elements, except N, was higher than that in the CK group. The Ca content showed the most significant increase, reaching 140.60%. The contents of N, P, Na, Cu, and Zn displayed an increasing trend in the S3 group compared with the CK group, with the largest increase in the N content (25.15%) and the largest decrease in the Mg content (16.20%). The contents of N, Mn, Mg, and P were lower in the S4 group compared with the CK group; the N content was decreased by 34.13%. However, the contents of Na, K, Fe, Ca, Cu, and Zn showed an increasing trend; the Ca content showed the most significant increase (130.38%). Except for N and P, all other elements, except N and P, displayed an increasing trend in the S5 group compared with the CK group; the Ca content showed the most significant increase (159.89%). The N content in the S6 and S7 groups were increased most significantly, with an increase of 31.69% and 33.71%, respectively.

The total nutrient content in rhizosphere soil was higher than that in the CK group after applying phosphate-solubilizing bacteria. The top four groups with the most significant increases were S2, S1, S4, and S5, with an increase of 24.39%, 24.29%, 17.11%, and 15.79%, respectively. These bacteria probably promoted the cycling of soil elements.

Principal component analysis of nutrient elements in the rhizosphere soil of *P. polyphylla var. yunnanensis*: Four principal components satisfying the conditions were extracted based on the 10 nutrient elements in the rhizosphere soil. Their cumulative contribution rate of variance was 89.04% (Fig. 2A and Table 6). The characteristic value of PC1 was 3.744 and its contribution rate was 37.44%, primarily representing the contents of Fe, Mg, Mn, Ca, and Zn. The characteristic value of PC2 was 2.308 and its contribution rate was 23.08%, primarily representing the contents of N and P in soil. PC3 primarily represented the contents of K and Cu. The eigenvalue was 1.827 and its contribution rate was 18.27%. PC4 primarily represented the content of Mg in soil. The characteristic value was 1.025, and its contribution rate was 10.25%. The results of principal component scores are shown in Figure 2B. For PC1, PC2, PC3, and PC4, the S2, S1, S7, and S6 groups had the highest score, respectively. The S1 group achieved the highest comprehensive scores from PC1, PC2, and PC4. In PC1, the elements Fe, Mn, Ca, and Zn had relatively higher matrix coefficients. In PC2, N and P elements had relatively higher matrix coefficients. Further, in PC4, the matrix coefficient of Mg was relatively higher. These elements are considered as the typical elements in the rhizosphere soil of *P. polyphylla* var. *yunnanensis*.

Enrichment analysis of 10 nutrient elements in *P. polyphylla* var. *yunnanensis*: BCF is the ratio of a certain element content in the plant to that in the soil. Based on the enrichment coefficient, the enrichment degree can be divided into extremely strong enrichment (BCF > 5), strong enrichment (BCF 2-5), enrichment (BCF 1.5-2), weak enrichment (BCF 1.2-1.5), and non-enrichment (BCF < 1.2) (Qu *et al*., 2021). As shown in Figure 3, the farther the distance from the origin, the greater the enrichment factor. *P. polyphylla* var. *yunnanensis* had the strongest enrichment ability for P and Cu in the soil (strong enrichment or extremely strong enrichment). Except for the S1 and CK groups, the other 6 treatment groups showed a strong enrichment for Zn. Most treatment groups displayed a weak enrichment for K, Ca, and Mg. Neither the treatment groups nor the CK group showed enrichment for Fe, N, Na, Mn, and K. The enrichment coefficients of nutrient elements (except Ca) were higher in the 7 treatment groups compared with the CK group, indicating that these bacteria might promote the absorption and enrichment of nutrient elements in the soil of *P. polyphylla* var. *yunnanensis*.

Effects of phosphate-solubilizing bacteria on heavy metals in rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis*

Heavy metal element contents in rhizomes and rhizosphere soil: The contents of 5 kinds of heavy metals in rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis* are shown in Table 7. In rhizomes, the Pb content in the S1-S7 groups was 3.37-6.15 g · kg^{-1}, which was better than that in the CK group. The Cd content was 0.14-0.93 mg · kg^{-1}. The content of As, Hg, and Cr was 0.68-1.78, 0.04-0.15, and 0.83-1.92 mg · kg^{-1}, displaying a decreasing trend compared with that in the CK group. The Pb content in the rhizosphere soil was 21.77-29.18 mg · kg^{-1}; the contents in the S1, S4, and S7 groups were less than that in the CK group. The Cd content in the treatment groups was 0.31-0.78 mg · kg^{-1}, which was higher than that in the CK group. The As, Hg, and Cr contents were 16.89-23.19, 1.05-1.71, and 36.42-66.42 mg · kg^{-1}, respectively, displaying a decreasing trend compared with the CK group.

The Pb content in the rhizomes of *P. polyphylla* var. *yunnanensis* showed an increasing trend compared with that in the CK group. The S6 group displayed the most significant increasing trend (88.68%), whereas the contents of Cd, Hg, and Cr showed a decreasing trend compared with that in the CK group on the whole. The top three groups with the most significant decreases were the S3 (80.19%), S5 (66.90%), and S7 (51.56%) groups. The contents of As, Hg, and Cr in the rhizosphere soil displayed a decreasing trend compared with that in the CK group. The top three groups with the most significant decreases were the S7 (19.78%), S6 (18.03%), and S5 (41.37%) groups. The Cd content showed an increasing trend compared with that in the CK group. The Cd content in the rhizosphere soil displayed the largest increase in the S2 group (227.39%) compared with the CK group. The Pb content in the rhizomes in the S1, S2, S4, S6, and S7 groups exceeded the limit standard. Also, the Cd content in the rhizosphere soils in the S1, S2, S4, S5, S6, and S7 groups exceeded the risk-based screening value.

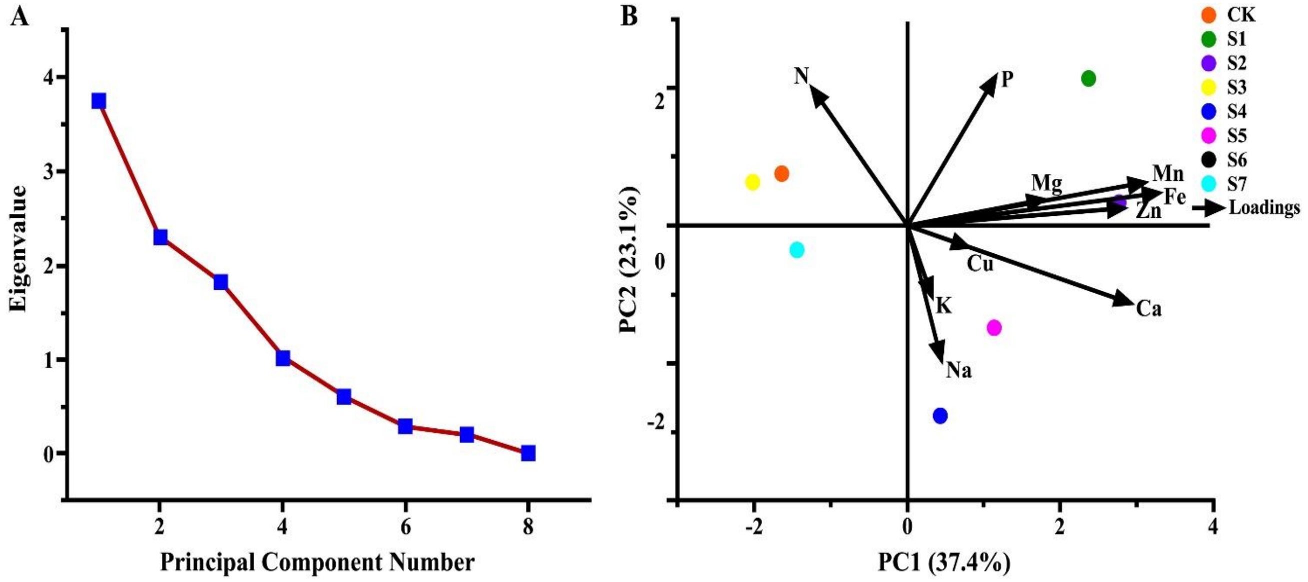

Fig. 2. A, scree plot; B, biplot of principal component analysis of nutrient elements in the rhizosphere soil.

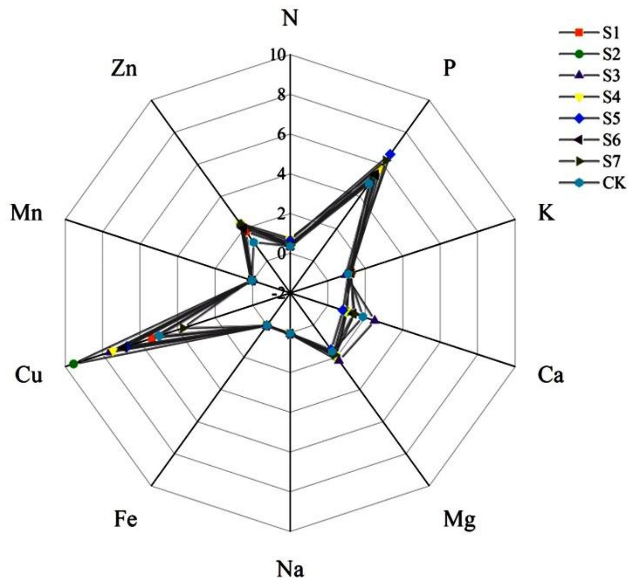

Fig. 3. Enrichment factor of 10 nutrient elements in the soil of *P. polyphylla* var. *yunnanensis.*

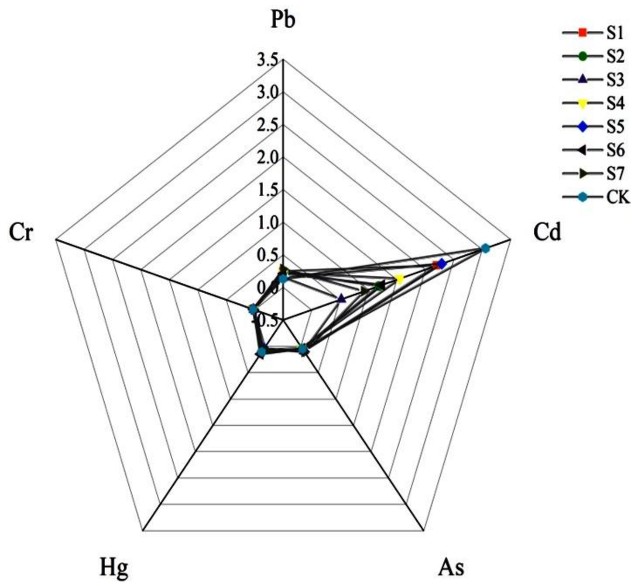

Fig. 4. Enrichment factor of five heavy metals in *P. polyphylla var. yunnanensis*.

Enrichment analysis of five heavy metals: The enrichment coefficients of 5 heavy metal elements in *P. polyphylla* var. *yunnanensis* rhizomes are shown in Figure 4. The enrichment of Cd in the group with bacterial suspension was weaker than that in the CK group (BCF = 3.064). The S3 and S7 groups showed no enrichment of Cd. Also, the enrichment coefficient was significantly lower than that in the CK group. No enrichment of Pb, As, Hg, and Cr was observed (BCF < 1.2). However, the BCF of Pb in the S1-S7 groups was higher than that in the CK group. The enrichment coefficient of Hg in the S2 and S6 groups was slightly greater than that in the CK group. The BCF of As was slightly greater in the S1, S3, S5, and S7 groups compared with the CK group.

Correlation analysis: The correlation analysis results of 10 nutritional elements in rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis* are shown in Table 8. Na was extremely significantly positively correlated with Fe and Ca, Ca was significantly positively correlated with Fe and Cu, Mg was positively correlated with Fe and Cu, and Cu was positively correlated with Zn in the rhizomes. Fe and Mn and Zn were extremely significantly positively correlated, whereas Mn and Zn were significantly positively correlated in the rhizosphere soil. Certain correlation existed between the elements in rhizomes and rhizosphere soil. Cu in the rhizosphere soil was positively correlated with Mn in rhizomes, and a positive correlation existed between rhizomes and rhizosphere soil of Zn.

The correlation analysis results of 5 heavy metal elements in rhizomes and rhizosphere soil are shown in Table 9. A significant negative correlation between As and Hg in the rhizomes, a significant negative correlation between Pb and Hg in soil, and a positive correlation between Hg in rhizomes and Cr and Pb in soil were observed.

Table 7. Contents of five heavy metals in the rhizomes and rhizosphere soil ($n = 3$，$mg \cdot kg^{-1}$).

Group	Pb_Y	Cd_Y	As_Y	Hg_Y	Cr_Y	Pb_T	Cd_T	As_T	Hg_T	Cr_T
S1	5.410d	0.756b	1.765b	0.076f	1.926a	22.215g	0.344e	20.205e	1.182d	59.472d
S2	6.041c	0.932a	0.929e	0.146b	1.751b	25.632c	0.789a	21.670b	1.159f	59.243e
S3	4.012f	0.146h	1.772b	0.082e	1.067f	26.294b	0.279g	20.681d	1.231c	61.064c
S4	5.306e	0.676e	0.686g	0.085d	1.096e	24.268f	0.436b	23.190a	1.165e	51.727f
S5	3.374g	0.721d	1.646c	0.046h	1.016g	24.745d	0.315f	18.465g	1.115g	36.426h
S6	6.153a	0.525f	0.844f	0.158a	1.314d	29.184a	0.425d	19.493f	1.059h	66.421a
S7	6.117b	0.395g	1.781a	0.073g	0.830h	21.773h	0.429c	16.891h	1.716a	50.527g
CK	3.261h	0.737c	1.030d	0.139c	1.714c	24.549e	0.241h	21.055c	1.292b	62.125b
Screening value ($mg \cdot kg^{-1}$)	5	1	2	0.2	2	120	0.3	30	2.4	200

Note: "Y" indicates rhizomes and "T" indicates rhizosphere soil

Table 8. Correlation analysis of 10 nutrient elements in the rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis*.

Element	N_Y	P_Y	K_Y	Ca_Y	Mg_Y	Na_Y	Fe_Y	Cu_Y	Mn_Y	Zn_Y	N_T	P_T	K_T	Ca_T	Mg_T	Na_T	Fe_T	Cu_T	Mn_T	Zn_T
N_Y	-																			
P_Y	0.398	-																		
K_Y	0.614	0.584	-																	
Ca_Y	0.229	0.149	-0.299	-																
Mg_Y	0.094	0.486	0.197	0.624	-															
Na_Y	0.435	0.363	0.074	0.862^{**}	0.591	-														
Fe_Y	0.449	0.312	0.165	0.804^{*}	0.779^{*}	0.843^{**}	-													
Cu_Y	0.010	0.371	-0.140	0.725^{*}	0.715^{*}	0.609	0.456	-												
Mn_Y	0.185	0.496	0.391	0.370	0.408	0.657	0.517	0.233	-											
Zn_Y	0.378	0.327	-0.019	0.706	0.554	0.511	0.460	0.829^{*}	0.071	-										
N_T	0.108	0.243	0.198	-0.054	0.114	-0.071	0.280	-0.451	0.269	-0.280	-									
P_T	-0.373	0.548	0.088	0.108	0.648	0.062	0.273	0.277	0.354	0.016	0.495	-								
K_T	0.560	0.596	0.340	0.206	-0.072	0.542	0.150	0.191	0.466	0.173	-0.176	-0.200	-							
Ca_T	0.375	0.515	0.453	0.057	0.271	0.137	-0.040	0.589	0.015	0.653	-0.543	-0.067	0.378	-						
Mg_T	0.186	0.667	0.686	-0.354	0.356	-0.116	-0.027	0.135	0.013	0.003	-0.046	0.412	0.185	0.562	-					
Na_T	0.357	-0.267	0.059	0.441	0.345	0.404	0.411	0.469	-0.120	0.549	-0.581	-0.451	-0.079	0.404	-0.046	-				
Fe_T	-0.229	0.429	0.185	-0.021	0.338	-0.099	-0.192	0.554	0.184	0.474	-0.236	0.419	-0.032	0.688	0.366	-0.026	-			
Cu_T	0.570	0.571	0.517	0.342	0.187	0.607	0.400	0.211	0.831^{*}	0.324	0.176	0.010	0.689	0.310	-0.009	-0.019	0.226	-		
Mn_T	-0.371	0.349	0.158	-0.148	0.239	-0.190	-0.309	0.445	0.205	0.292	-0.245	0.427	-0.072	0.587	0.347	-0.121	0.977^{**}	0.175	-	
Zn_T	-0.161	0.210	-0.093	0.183	0.352	-0.123	-0.094	0.613	-0.150	0.709^{*}	-0.213	0.280	-0.255	0.613	0.129	0.164	$.855^{**}$	0.003	0.759^{*}	-

Note: "*" indicates a significant correlation ($p<0.05$); "**" indicates a very significant correlation ($p<0.01$)

Table 9. Correlation analysis of five heavy metals in the rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis*.

Element	Cr_Y	As_Y	Cd_Y	Pb_Y	Hg_Y	Cr_T	As_T	Cd_T	Pb_T
As_Y	-0.225	-							
Cd_Y	0.645	-0.451	-						
Pb_Y	0.04	-0.228	0.064	-					
Hg_Y	0.493	-0.734^{*}	0.257	0.305	-				
Cr_T	0.528	-0.329	-0.133	0.314	0.770^{*}	-			
As_T	0.422	-0.656	0.337	-0.072	0.338	0.345	-		
Cd_T	0.214	-0.41	0.466	0.676	0.401	0.096	0.222	-	
Pb_T	-0.015	-0.499	-0.148	0.019	0.614^{*}	0.422	0.224	0.118	-
Hg_T	-0.386	0.438	-0.366	0.178	-0.265	-0.142	-0.55	-0.075	-0.636*

Note: "*" indicates a significant correlation ($p<0.05$); "**" indicates a very significant correlation ($p<0.01$)

Discussion

Artificial cultivation is an effective method to address the rapid depletion of the wild resources of *P. polyphylla* var. *yunnanensis* and meet current medical demands. However, transitioning from planting to harvesting generally takes 5-6 years. The fertility of the soil environment decreases, the soil-borne pathogens accumulate, and the frequency of applying pesticides and fertilizers increases with the increase in the number of growth years, thereby increasing the management cost. Nutrient elements play a vital role in the growth of plants. Macroelements provide nutrients and influence the synthesis of coenzymes in plants; microelements participate in the physiological and biochemical processes of plants (Zhuang *et al*., 2022; Li *et al*., 2023). This study showed that the contents of 10 nutrient elements in the rhizosphere soil and rhizomes of *P. polyphylla* var. *yunnanensis* increased after applying the phosphate-solubilizing bacteria. The total content of nutrient elements in rhizomes and rhizosphere soil in the CK group was lower than that in the S1-S7 groups, among which the contents in the S2 and S7 groups were the highest. These results showed that different phosphate-solubilizing bacteria promoted the accumulation of nutrient elements in rhizomes and rhizosphere soil, thereby having a positive impact on nitrogen and phosphorus cycling and soil fertility. Adjustment of the soil microenvironment in which the medicinal plants grow by soil microorganisms can improve the nutritional status of the soil, which is beneficial to the production of medicinal materials and environmental protection.

The P element in the soil is mainly divided into organophosphorus and inorganic phosphorus; the inorganic phosphorus accounts for a relatively high proportion and hence is the main source of plant phosphorus nutrition (Wang *et al.*, 2023). K has a positive significance for the accumulation and transport of plant photosynthetic products (Dai *et al.*, 2023). Increasing the contents of N, P, and other nutrient elements in medicinal plants through fertilization is conducive to increasing the yield. In this study, the contents of N, P, Fe, Ca, Mg, Cu, and Zn in the rhizomes were higher in the S1-S7 groups compared with the CK group. The K, Ca, Mg, Na, Cu, and Zn contents in the rhizosphere soil displayed an increasing trend in the treatment groups compared with the CK group, indicating that inorganic phosphorus-solubilizing bacteria could promote the nutrient element cycling in rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis*. The increase in the nutrient content in rhizomes was the most significant in the S7 group compared with the CK group. The increase in nutrient content in rhizomes was the most significant in the S7 group. Meanwhile, the nutrient contents in both the rhizome and the rhizosphere soil were higher in the S2 group than in the CK group. This result indicated that *B. aryabhattai* Z6-1 could stably promote the accumulation of nutrients in rhizomes and soil to maintain soil fertility and the combined application of the three bacterial strains in this study was effective on rhizomes. N, P, K, Cu, Fe, Mg, Mn, Ca, and Zn were the characteristic nutrient elements in the rhizosphere soil of *P. polyphylla* var. *yunnanensis*. The contents of these elements were the highest in the rhizosphere soil in each treatment group. However, the contents of these elements in the rhizome in the S1 group did not increase significantly compared with that in the CK group. The action of this single bacterium may be influenced by time, which needs further elucidation. The enrichment coefficients of P and Cu in the rhizomes were significantly higher in the treatment groups compared with the CK group, showing a strong enrichment state. This indicated that the phosphate-solubilizing bacteria used in this study promoted the absorption of P and Cu elements in rhizomes but did not enrich the N, Na, Fe, and Mn absorption. Therefore, special attention should be paid to these elements which were not enriched during the cultivation of *P. polyphylla* var. *yunnanensis* and appropriate fertilizers should be applied to maintain soil nutrient levels. A universal positive correlation exists between various elements in the rhizome and rhizosphere soil. The elements in rhizomes are also positively correlated with those in the rhizosphere soil. Therefore, the nutrient elements in rhizomes and rhizosphere soil may have a synergistic effect. Improving the nutrient level in the soil can help in the nutrient utilization of *P. polyphylla* var. *yunnanensis*.

Soil is a medium for the material exchange between plants and the environment. The quality of soil also influences the growth of medicinal plants and the accumulation of effective components. Heavy metals not only pollute the soil but also accumulate in the plants through element circulation to impact the growth of the plants. Moreover, the heavy metals accumulated in plants may cause potential hazards to humans once they become medicinal herbs and enter the market. This study demonstrated a close correlation between the heavy metal content in rhizomes and rhizosphere soil in each treatment group with the phosphate-solubilizing bacteria applied. The contents of Hg, As, and Cr in the rhizomes and rhizosphere soil did not exceed the limit standards, whereas the Cd content in the rhizosphere soil in most treatment groups, except for the S3 and CK groups, exceeded the limit standard. The enrichment of Cd in rhizomes was significantly reduced by phosphate-solubilizing bacteria application, but Pb in the rhizomes in some treatment groups still exceeded the limit standard in this study. The influence of inoculation concentration and amount of bacteria on the content of each element in rhizomes and rhizosphere soil of *P. polyphylla* var. *yunnanensis* is not yet known. Therefore, the most suitable concentration and dosage of phosphate-solubilizing bacteria need to be determined. A positive correlation was observed between the contents of heavy metals in the rhizosphere soil and rhizomes. For example, Hg in rhizomes showed a synergistic effect with Cr and Pb in the rhizosphere soil. Therefore, besides paying attention to the treatment of bacteria during cultivation to promote plant growth, we should also monitor the changes in the contents of heavy metals, especially Cd and Pb, in plants to prevent heavy metal pollution in soil and medicinal materials. These elements circulate and accumulate between the soil and the plants, resulting in an excess of heavy metals in the medicinal parts of plants, influencing the quality and yield of medicinal materials, and thus impacting the safety of traditional Chinese medicine production.

The quality and yield need special attention during the cultivation of *P. polyphylla* var. *yunnanensis*; also, environmental protection cannot be ignored. In this study, the content of nutrient elements in rhizomes and rhizosphere soil increased, the enrichment ability of nutrient elements was enhanced, whereas the enrichment of heavy metals by rhizomes was reduced after applying phosphorus-solubilizing bacteria. Overall, the effects of applying phosphorus-solubilizing bacteria in the S2 and S7 groups were the most significant. The contents of nutrient elements in rhizomes and rhizosphere soil were increased significantly in the S2 group compared with the CK group. The nutrient contents were increased most significantly in the rhizomes in the S7 group compared with the CK group, with no accumulation of Cd. However, the pot experiment still had many limitations. These strains can be further inoculated to *P. polyphylla* var. *yunnanensis* plants grown in the field to evaluate the effects of phosphate-solubilizing bacteria on the nutrient elements and heavy metals from various perspectives, and to explore the stability and effectiveness of the strains under field conditions.

Conclusions

The application of phosphate-solubilizing bacteria enhances the absorption and utilization of nutrient elements in *Paris polyphylla* var. *yunnanensis*. Notably, the S2 treatment group, inoculated with *Bacillus aryabhattai* Z6-1, exhibited a significant increase in the content of nutrient elements in both the rhizomes and rhizosphere soil. In the S7 treatment group, where a combination of three bacterial strains (*Bacillus cereus* 1-1, *Bacillus aryabhattai* Z6-1, and *Bacillus aryabhattai* Z3-4) was used, the enhancement in nutrient element content was the most pronounced.

The inoculation of inorganic phosphorus-solubilizing bacteria reduced the enrichment ability of some heavy metals in *P. polyphylla* var. *yunnanensis*. Also, the enrichment of Cd in the S3 and S7 groups was significantly reduced. The contents of Pb and Cd in the rhizosphere soil slightly exceeded the risk-based screening value, whereas the contents of Hg, As, and Cr were all in the limit standard range.

Acknowledgement

This research was funded by Chongqing Municipal Education Commission (KJZD-M202401201).

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