



Response strategies of slash pine (*Pinus elliottii*) to cadmium stress and the gain effects of inoculation with *Herbaspirillum* sp. YTG72 in alleviating phytotoxicity and enhancing accumulation of cadmium

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Abstract

Phytoremediation using fast-growing woody plants assisted by plant growth-promoting bacteria (PGPB) on cadmium (Cd)-contaminated sites is considered a promising technique; however, its remediation efficiency is still affected by multiple factors. In this study, the mining areas' soil conditions were simulated with different Cd addition levels (0, 3, 6, 9 mg kg⁻¹) in order to investigate the response strategy to Cd stress of fast-growing economic tree species, slash pine (*Pinus elliottii*), and the effects of inoculation with the PGPB strain *Herbaspirillum* sp. YTG72 on the physiological activity and Cd accumulation of plants. The main results showed that there were significant ($p < 0.05$) increases in contents of chlorophyll and nutrient elements (P, K, Ca, and Mg) at low Cd addition level (3 mg kg⁻¹) compared to non-Cd addition treatment. When the additive amount of Cd increased, the growth of plants was severely inhibited and the content of proline was increased, as well as Cd in plants. Besides, the ratios of K:P, Ca:P, and Mg:P in plants were negatively correlated with the contents of Cd in plants and soils. Inoculation of *P. elliottii* with the PGPB strain *Herbaspirillum* sp. YTG72 improved the physiological functions of the plants under Cd stress and activated the antioxidant system, reduced the accumulation of proline, and decreased the ratios of K:P, Ca:P, and Mg:P in plant. More importantly, planting *P. elliottii* in Cd-contaminated soil could significantly ($p < 0.05$) reduce the Cd content in the rhizosphere soil, and furthermore, inoculation treatment could promote the reduction of soil Cd content and increased the accumulation of Cd by root. The results of the present study emphasized the Cd response mechanism of *P. elliottii* based on multifaceted regulation, as well as the feasibility of strain *Herbaspirillum* sp. YTG72 assisted *P. elliottii* for the remediation on Cd-contaminated sites.

Keywords Cd contamination · Cd toxicity · Microbe-assisted phytoremediation · Plant growth-promoting bacterium · Physiological response · Stoichiometric ratios

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Introduction

Heavy metals (HMs) contamination of soil in mining areas is one of the common environmental crises facing the world. In the process of mining production, the arbitrary accumulation of waste rock, tailings, and smelting slag, as well as the discharge of acidic waste, all cause HMs to be transferred to the environment and to accumulate continuously (Kan et al. 2021). Such adverse activities have led to a decline in soil quality and groundwater contamination, posing a serious threat to the ecological balance and human health (Fu et al. 2016). It was reported that 34.9% of industrial waste land and 33.4% of mining areas in China alone have HMs contamination, of which cadmium (Cd) contamination is particularly severe (Wang et al. 2014). The Cd content in Chinese soil is increasing at an average rate of

0.004 mg kg⁻¹ per year, and the over standard rate of Cd has reached 7.0% (Huang et al. 2019). How to effectively purify HMs contaminated soil has become an urgent problem to be solved in today's world.

Bioremediation, which has the advantages of being highly effective, economical, and ecologically sustainable, is recognized as a new method for soil HMs contamination treatment (Xin et al. 2020). Since HMs are difficult to biodegrade and are highly mobile and bioavailable in soil, the only way to reduce their levels in the soil is to accumulate them in plants and harvest them (Ismael et al. 2019). Therefore, phytoremediation is the only and effective bioremediation method to reduce HMs content of soil, in which the use of woody plants for remediation has received particular attention from scholars. Compared with herbaceous hyperaccumulators, fast-growing woody plants have a larger biomass, and are likely to accumulate more HMs than herbaceous hyperaccumulators. More critically, woody plants are often used as industrial timber forests and do not generally enter the food chain where they can cause secondary hazards to humans and animals (Marmioli et al. 2011). Woody plants not only produce a large amount of wood and biomass energy during the remediation process, but their well-developed root systems can also effectively prevent the expansion of HMs contamination caused by soil erosion (Laureysens et al. 2004; Wang et al. 2021a). Phytoremediation using high biomass, fast-growing tree species such as poplar, willow, eucalyptus, birch, and paulownia has been recognized as an effective method for the treatment of HMs contamination in mining areas (Pajevi et al. 2016). However, excess heavy metals in soil tend to cause oxidative stress in plants and interfered with normal metabolic processes, as evidenced by the reduction of chlorophyll, soluble sugar and protein contents, leakage of extracellular electrolytes, accumulation of proline, destruction of the antioxidant system, and nutritional imbalance (Zhang et al. 2020; Sardar et al. 2022). These adverse effects lead to inhibition of root growth, leaf yellowing, and biomass reduction, severely affecting the efficiency of phytoremediation (Hassan and Aarts 2011). Therefore, there is an urgent need to take effective measures to mitigate the toxicity of HMs to plants.

Inoculation of plants in unfavorable conditions with plant growth-promoting bacteria (PGPB) is an effective means of increasing the efficiency of phytoremediation. PGPB can regulate plant growth and development through a series of plant growth-promoting (PGP) properties they possess, such as phosphorus solubilization, nitrogen fixation, secretion of siderophore, indole acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, to improve the utilization of soil nutrients by plants, thus promoting plant growth (Begum et al. 2018). It has been proved that microorganisms can promote the conversion of HMs to non-toxic forms or directly absorb HM ions through ion exchange,

chelation, complexation, and physical adsorption on the cell surface, and promote/inhibit the accumulation of soil HMs by plants (Zhou et al. 2020). In addition, lipopolysaccharides of the bacterial outer membrane, antibiotics produced by bacteria, biosurfactants, and volatile organic compounds are capable of inducing and activating the antioxidant system of the plant, thereby reducing oxidative stress and increasing the tolerance of the plant to HMs toxicity (Etesami 2018). Previous studies have shown that *Burkholderia* sp., *Serratia* sp., *Micrococcus* sp., and *Arthroactor* sp. can effectively promote plant growth and increase Cd accumulation, which can serve as bioremediation agents for Cd-contaminated soils (Guo et al. 2011; El Aafi et al. 2012; Sangsuwan and Prapagdee 2021).

Slash pine (*Pinus elliottii*) is a fast-growing oil and timber tree species of Pinaceae, which is highly adaptable to adversity stresses (e.g., drought, high temperature, saline-alkali, HMs) (Zhang et al. 2022). Our previous studies showed that *P. elliottii* can be used as a pioneer tree species for phytoremediation of Cd-contaminated tailing areas; however, mechanistic insight of Cd action on *P. elliottii* remains unclear (Li et al. 2023a). *Herbaspirillum* sp. YTG72 is a Cd-tolerant PGPB isolated from the roots of woody plants in the mining area, which possesses a number of PGP properties such as IAA production, phosphorus solubilization, and nitrogen fixation (Li et al. 2023b). It was hypothesized that this strain has significant implications for the remediation of Cd-contaminated soil in *P. elliottii*. Consequently, the objectives of this study were (i) to investigate the effects of Cd stress on the growth, physiology, and antioxidant activity of *P. elliottii* seedlings, and to analyze the key factors affecting the response of plant to Cd stress; (ii) to investigate the effects of the addition with the strain YTG72 on the Cd tolerance of *P. elliottii* as well as on the Cd accumulation; and (iii) to assess the feasibility of microbe-assisted *P. elliottii* phytoremediation for Cd-contaminated soil. The results of this study are expected to provide more effective options for bioremediation of soil Cd contamination.

Materials and methods

Experimental materials

The *P. elliottii* annual seedlings used in the pot experiment were provided by the Experimental Center of Subtropical Forestry, Chinese Academy of Forestry, Jiangxi Province, China, with a plant height of 15.2 cm and a stem diameter of 1.60 mm.

The strain *Herbaspirillum* sp. YTG72 (GenBank accession number: OQ103356) was isolated from the roots of Indian azalea (*Rhododendron simsii*) and it possessed a wide range of PGP properties in terms of phosphate solubilization (phosphate solubilization index of 1.65) and the production

of IAA ($8.26 \mu\text{g mL}^{-1}$), nitrogenase (11.08 U L^{-1}), ACC deaminase ($115.37 \text{ pg mL}^{-1}$), and siderophore (77.24%) (Li et al. 2023b). Before the inoculation treatment of plants, the strain was inoculated in LB liquid medium and incubated at 28°C and 180 r min^{-1} for 24 h, and then the culture solution was diluted with sterile deionized water to $\text{OD}_{600}=0.5$ (bacterial density was about $5 \times 10^8 \text{ CFU mL}^{-1}$) to be used.

The soil used in this experiment was collected from the surface layer of 0–20 cm in the uncontaminated area around the mine ($27^\circ 37' \text{ N}$, $114^\circ 52' \text{ E}$) in Yushui District, Xinyu City, Jiangxi Province, and its basic properties are shown in Table S1. According to the risk screening value of Cd in the national standard GB 15618–2018, 10, 20 and 30 times of the value were used as the values of low (3 mg kg^{-1}), medium (6 mg kg^{-1}), and high (9 mg kg^{-1}) Cd addition level, and the Cd-contaminated soil was prepared as described by Li et al. (2023b). The actual Cd contents of the soils after anthropogenic contamination were 3.673 mg kg^{-1} , 6.455 mg kg^{-1} , and 9.511 mg kg^{-1} , respectively. The soil was sterilized at 121°C for 2 h before being used for pot experiment.

Pot experimental design

The pot experiment was conducted from July 2022 to November 2022 in an open-sided greenhouse in Experimental Center of Subtropical Forestry ($27^\circ 82' \text{ N}$, $117^\circ 67' \text{ E}$). Each pot ($15 \text{ cm} \times 12 \text{ cm}$) contained 2 kg of soil and was planted with one seedling, for a total of 36 seedlings per treatment. A separate set of treatment was set up for each Cd addition level of soil, without planting seedlings and without inoculation, to explore the Cd content lost due to watering in the experiment, with 5 pots set up for each level. *P. elliotii* seedlings were transplanted to Cd-contaminated soil for 1 week before inoculation treatment. A total of 50 mL of *Herbaspirillum* sp. YTG72 culture solution ($5 \times 10^8 \text{ CFU mL}^{-1}$) was inoculated on the roots of each plant by using a sterile syringe on 0 days, 30 days, 60 days, and 90 days of the experiment, and inoculated with sterile cultures as a control treatment (CK), for a total of four inoculations in the experiment. The exudate from the watering process was poured back into the soil, and plant growth conditions of each treatment such as light and humidity were kept consistent, and plants on soil remained unfertilized. After 120 days, plants were harvested and soil attached to the roots within 2 mm was collected.

Measurement method of each indicator

Plant growth indicators

Plant height and stem diameter of each seedling were measured before inoculation treatment and after harvest. At the end of the experiment, six seedlings were randomly

selected from each treatment and sampled in triplicate, for a total of 18 plants. Plant samples were rinsed with running water, desorbed in 20 mM $\text{Na}_2\text{-EDTA}$ for 20 min (Wang et al. 2021b), and then rinsed repeatedly with deionized water. The plant samples were placed in the oven by root, stem, and leaf, respectively, at 105°C for 30 min and then dried at 65°C to constant weight, and the dry biomass of each part was recorded. Six seedlings with average growth randomly selected from each treatment, and their roots were cut and cleaned. The root system was scanned and analyzed using a root scanner (Microtek ScanMaker i800 Plus, China) and a root analysis system (Wseen LA-S, China).

Element content

The dried plant samples in the “Plant growth indicators” section were crushed with a grinder and passed through a 2-mm nylon sieve. Each plant sample (0.2 g) was digested and prepared according to the Chinese standard GB 5009.268–2016. Soil samples were air-dried, ground, and passed through a 0.15-mm nylon sieve. Each soil sample (0.1 g) was digested and prepared according to the Chinese standard HJ 803–2016. The contents of total Cd, total phosphorus (P), total potassium (K), total calcium (Ca), and total magnesium (Mg) in the samples were measured by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer ELAN DRC-e, USA).

Plant physiological indicators

The remaining 18 plants in the “Plant growth indicators” section were rinsed with running water, and the root, stem, and leaf samples of each treatment were wrapped in tinfoil and immersed in liquid nitrogen for 2 min. Then, transferred to an ultra-low temperature refrigerator at -80°C for storage until use. Chlorophyll content was determined by acetone (80%) extraction method (Wellburn 1994). Soluble sugar content was determined by anthrone colorimetric method (Buisse and Merckx 1993). Soluble protein content was determined by BCA method (Smith et al. 1985). Proline (Pro) content was determined by acid ninhydrin colorimetric method (Bates et al. 1973). Peroxidase (POD) activity was determined by guaiacol method (Doerge et al. 1997). Catalase (CAT) activity was determined by H_2O_2 -UV colorimetric method (Aebi 1984).

Assessment of the enrichment and transport capacity of *P. elliotii* for Cd

The content of whole plant was used to estimate the overall accumulation of Cd by the plant and was calculated as:

Table 1 The growth indicators of *P. Elliottii* seedling under different Cd addition levels and inoculation treatment

Treatment	Cd levels (mg kg ⁻¹)	Plant height increment (cm)	Stem diameter increment (mm)	Root length (cm)	Root surface area (cm ²)	Root volume (cm ³)
Inoculation (YTG72)	0	8.9 a	1.85 ab	278.70 a	37.01 ab	0.73 a
	3	8.2 b	1.87 ab	272.62 a	35.26 abc	0.63 b
	6	5.6 d	2.01 a	265.68 ab	38.67 a	0.81 a
	9	4.7 e	1.85 ab	240.72 bc	32.09 bcd	0.58 b
Non-inoculation (CK)	0	5.2 d	1.55 d	213.81 cd	28.69 d	0.54 b
	3	7.0 c	1.65 cd	218.38 cd	26.36 d	0.40 c
	6	3.6 f	1.81 bc	195.56 d	29.50 d	0.59 b
	9	3.8 f	1.73 bc	219.66 cd	30.94 cd	0.58 b
Significances	Cd	****	**	ns	ns	****
	Inoculation	****	****	****	****	****
	Cd×Inoculation	****	ns	ns	ns	**

Different letters denote significant differences between the treatments ($p < 0.05$). The p values of ANOVAs of Cd levels, inoculation treatments, and their interactions were also shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, ns, not significant)

$$C_{\text{wholeplant}} = \frac{C_{\text{root}} \times B_{\text{root}} + C_{\text{stem}} \times B_{\text{stem}} + C_{\text{leaf}} \times B_{\text{leaf}}}{B_{\text{root}} + B_{\text{stem}} + B_{\text{leaf}}}$$

where C is the content of Cd in each plant organ, and B is the dry biomass of each plant organ.

The bioconcentration factor (BCF) and the translocation factor (TF) were used to assess the enrichment and translocation capacity of plants for soil Cd and were calculated as follows (Ali et al. 2013):

$$BCF_{\text{root/stem/leaf}} = \frac{C_{\text{root/stem/leaf}}}{C_{\text{soil}}}, TF_{\text{stem/leaf}} = \frac{C_{\text{stem/leaf}}}{C_{\text{root}}}$$

where C is the content of Cd in each plant organ or soil.

Statistical analysis

The experimental data were processed using Microsoft Excel 2019 and IBM SPSS 26. One-way analysis of variance (ANOVA) was conducted to compare the means of the parameters. Two-way ANOVA was conducted to analyze the differences of different treatments on the parameters. The Pearson correlation analysis and linear regression analysis were performed to compare the correlation between the indicators.

Results

Plant growth

Both different Cd addition levels and inoculation treatments significantly affected all growth indicators of *P. Elliottii* (Table 1 and Fig. 1). There was a trend of first increasing

and then decreasing in plant height increment and dry biomass of root, stem, and leaf, with increasing levels of Cd addition. The stem diameter increment increased under Cd stress, while the root length and root surface area were less affected by Cd stress ($p > 0.05$). Inoculation of plant with strain YTG72 effectively promoted the increase of

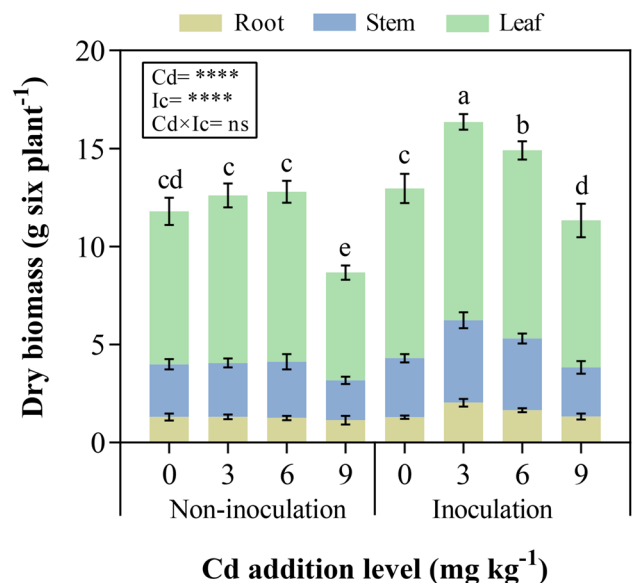


Fig. 1 Dry biomass of *P. Elliottii* under different Cd addition levels and inoculation treatment. Different letters denote significant differences in total plant dry biomass between the treatments ($p < 0.05$). The error bars denote the standard deviations from the mean. The p values of ANOVAs of Cd addition levels, inoculation treatments, and their interactions were shown in a square frame (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, ns, not significant), where “Cd” denotes Cd addition level, “Ic” denotes inoculation treatment, and “Cd×Ic” denotes their interactions. The same below

aboveground parts and below-ground part indicators of seedlings under Cd stress. In particular, the dry biomass of root, stem, and leaf of seedlings grown under Cd stress increased by 16.6–54.8%, 23.4–53.0%, and 10.8–36.2%, respectively, after inoculation with YTG72 compared to the control. It is noteworthy that this growth-promoting effect of the strain on plants gradually decreased with increasing soil Cd content.

Physiological characteristics

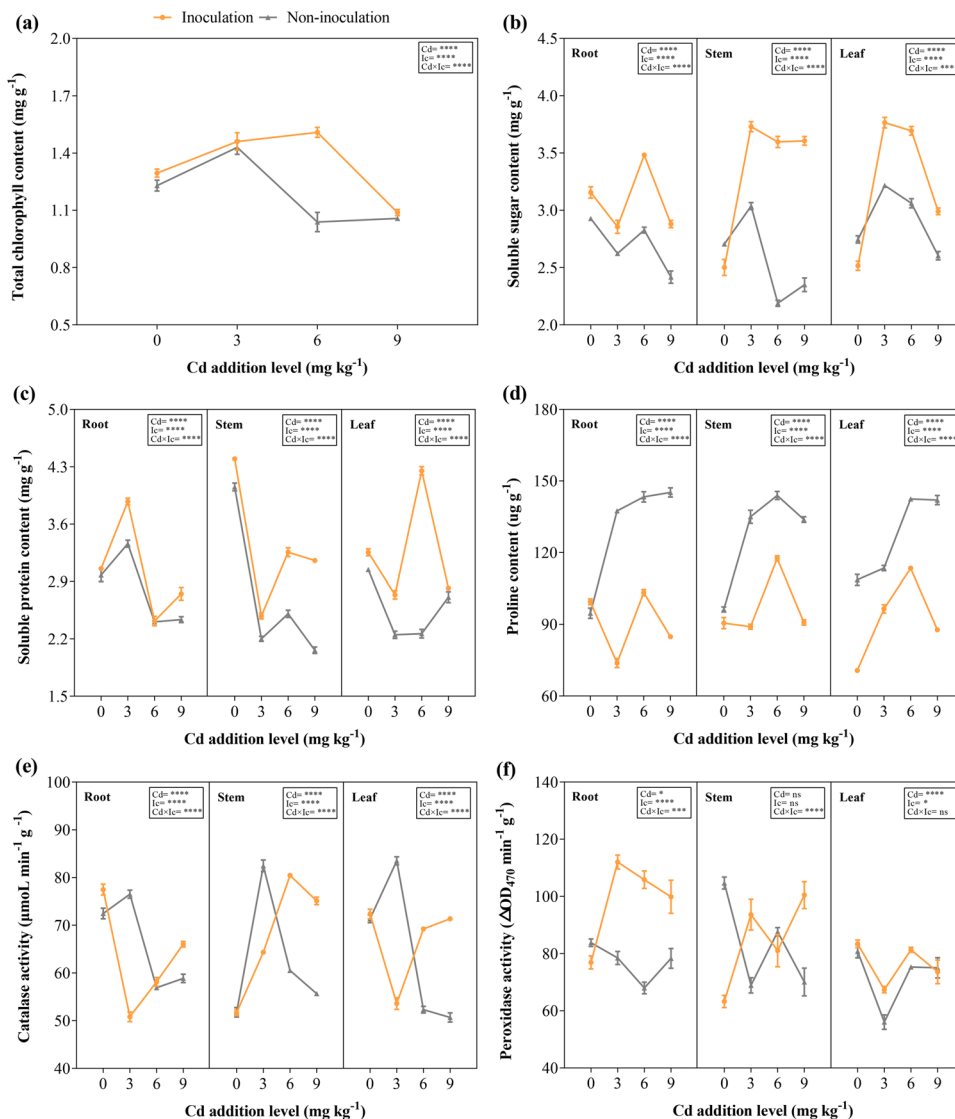
The effects of different Cd addition levels on chlorophyll, soluble sugars, and soluble proteins in plants generally showed a trend of low-promoted and high-suppressed condition (Fig. 2), and this effect was more pronounced for soluble sugar content in the aboveground parts (stem and leaf) and for soluble protein content in the below-ground part (root). The inoculation treatment increased the contents of chlorophyll, soluble sugar, and soluble protein in *P. elliotii*

under Cd stress to some extent. In particular, the inoculation treatment showed the most significant ($p < 0.05$) increase in chlorophyll and soluble sugar contents of *P. elliotii* under medium Cd stress (6 mg kg⁻¹), which increased chlorophyll content by 45.7% and increased root, stem, and leaf soluble sugar content by 23.2%, 64.3%, and 20.6%. The effects of inoculation on the increase of root, stem, and leaf soluble protein content varied with the soil Cd content.

Antioxidants

The changes in Pro, CAT, and POD under different treatments are shown in Fig. 2. The Pro content in root, stem, and leaf of seedlings was significantly ($p < 0.05$) increased under Cd stress. Inoculation of plant with strain YTG72 reduced the Pro content in root, stem, and leaf of Cd-stressed seedlings by 27.9–46.3%, 18.3–34.0%, and 15.0–38.2%, respectively. With the non-inoculation treatment, the CAT activity tended to increase with

Fig. 2 Physiological and stress tolerance indicators of *P. elliotii* root, stem, and leaf under different Cd addition levels and inoculation treatment. (a) Total chlorophyll content, (b) soluble sugar content, (c) soluble protein content, (d) proline content, (e) catalase (CAT) activity, and (f) peroxidase (POD) activity



increasing Cd addition levels, followed by a decreasing trend. The effects of inoculation on the CAT activity of plants varied depending on the Cd addition levels. Inoculation of YTG72 reduced CAT activity of plant at low Cd stress (3 mg kg⁻¹) and increased CAT activity at medium (6 mg kg⁻¹) and high (9 mg kg⁻¹) Cd stress, with the most significant increase in CAT activity of aboveground parts (32.9–34.9% for stem and 32.3–40.8% for leaf). The POD activity decreased significantly ($p < 0.05$) with the increase of soil Cd content, and inoculation of YTG72 effectively increased the POD activity of organ part under Cd stress conditions, with the most significant increase on root POD activity (27.5–55.6%).

Nutritional element contents and their stoichiometric ratios

The contents of total P, total K, total Ca, and total Mg in the root, stem, and leaf of seedlings under different treatments are shown in Fig. 3. The presence of Cd in the soil increased the P, K, Ca, and Mg contents in seedlings to some extent. The P content in root, stem, and leaf of seedlings increased

under Cd treatment, while the K, Ca, and Mg content showed a trend of increasing and then decreasing with increasing soil Cd content, among which P content in root, stem, and leaf was highest under medium Cd stress, while the contents of K, Ca, and Mg was highest under low Cd stress. Inoculation with YTG72 increased the contents of nutrient elements in root, stem, and leaf under no/low Cd stress condition, while there was no significant difference overall under medium and high Cd stress conditions.

Both the Cd addition levels and the inoculation treatments significantly ($p < 0.0001$) affected the stoichiometric ratios between P, K, Ca, and Mg in *P. elliptica* seedlings. The K:P, Ca:P, and Mg:P in root, stem, and leaf decreased significantly ($p < 0.05$) with increasing soil Cd content, and these ratios were highest when plants were in non-Cd addition conditions (Table 2). In contrast, there was no significant regularity of K:Ca, K:Mg, Ca:Mg with the increase of soil Cd content (Table S2). Compared with the control, inoculation of YTG72 significantly ($p < 0.05$) reduced K:P, Ca:P, and Mg:P in root, stem, and leaf, while it had less effect on K:Ca, K:Mg, and Ca:Mg.

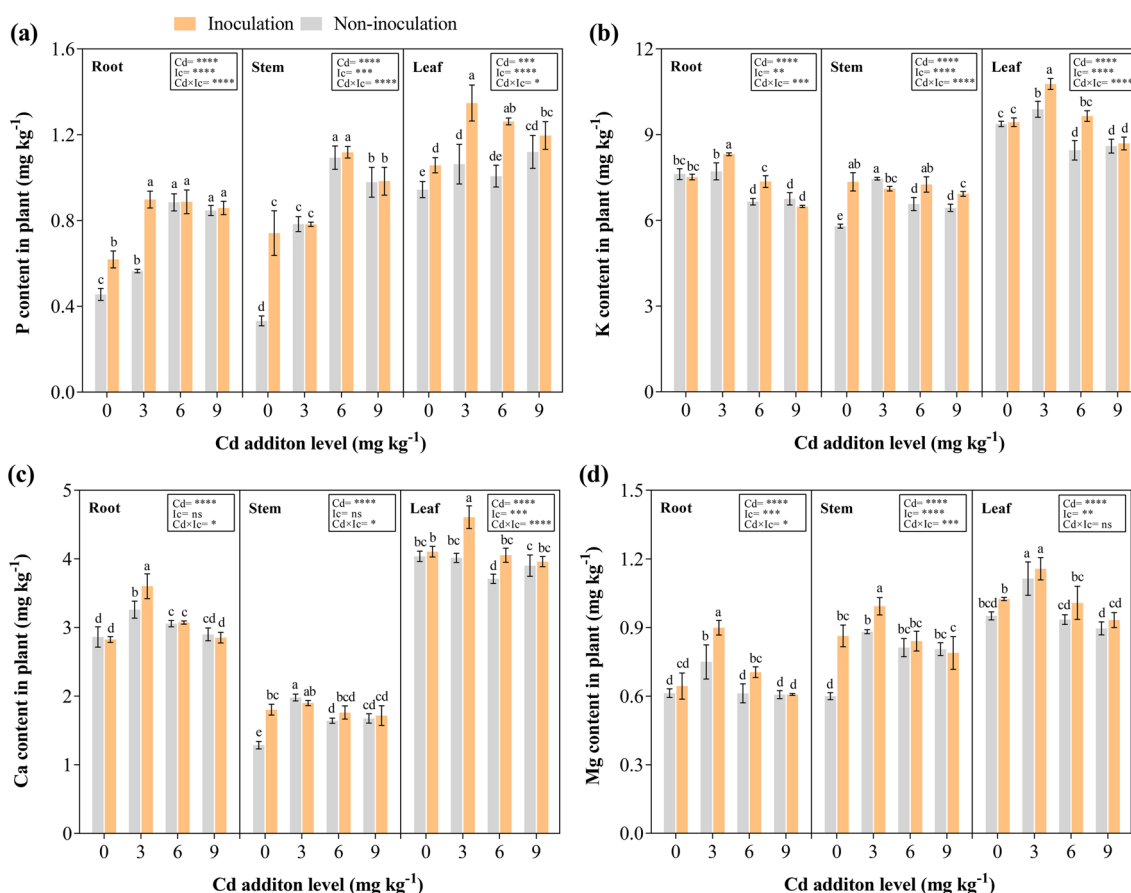


Fig. 3 Nutrient content of *P. elliptica* root, stem, and leaf under different Cd addition levels and inoculation treatment. (a) P content, (b) K content, (c) Ca content, and (d) Mg content. Different letters denote

significant differences between the treatments ($p < 0.05$), and the error bars denote the standard deviations from the mean

Cd content and accumulation characteristics

The total Cd content in soil after different treatments is shown in Table 3. As affected by Cd loss due to watering during the experiment, the final Cd content in the contaminated soil was reduced by 11.2–19.6% from the initial contaminated soil. Planting of *P. elliptii* seedlings was effective in reducing the Cd content in the final test soil, which resulted in a reduction of 24.8–30.1%, 12.2–23.4%, and 15.8–20.8% in low, medium, and high Cd-contaminated soils, respectively. There was a significant promoting effect ($p < 0.05$) to the reduction of Cd content in contaminated soils due to inoculation treatment, as evidenced by the fact that the inoculation treatment promoted a reduction of 7.0%, 12.7%, and 6.0% in low, medium, and high Cd-contaminated soils, respectively.

The Cd content in plants under different treatments is shown in Fig. 4. The distribution of Cd content in *P. elliptii* was root > stem > leaf. In the Cd treatment group, the Cd content in root, stem, leaf, and whole plant were 84.23–137.65 mg kg⁻¹, 17.75–26.59 mg kg⁻¹,

11.35–16.19 mg kg⁻¹, and 21.51–32.55 mg kg⁻¹, respectively. In addition, the Cd content in root and stem of each treatment tended to increase with increasing Cd addition levels, while the Cd content in leaf was highest under low Cd addition level and did not show significant difference ($p > 0.05$) with increasing Cd addition levels. Inoculation with YTG72 promoted the accumulation of Cd in root, leaf, and whole plants, with increases in Cd content of 21.3–55.6%, 12.9–33.0%, and 17.4–43.6%, respectively.

P. elliptii had good enrichment capacity for Cd, as shown by BCFs > 1 in root, stem, and leaf (Table 4). Among them, the root had the strongest enrichment capacity for Cd, with all BCFs greater than 10. The Cd translocation of plant aboveground parts was limited, and the TFs of stem and leaf were < 1. All inoculation treatments effectively improved the Cd enrichment capacity of root, and their BCFs were 1.56, 1.21, and 1.23 times higher than that of non-inoculation treatments, respectively, while the Cd enrichment and transport capacity of stem and leaf were less affected.

Table 2 The ratios of K:P, Ca:P, and Mg:P of *P. elliptii* root, stem, and leaf under different Cd addition levels and inoculation treatment

Treatment	Cd levels (mg kg ⁻¹)	K:P			Ca:P			Mg:P		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Inoculation (YTG72)	0	12.18 c	10.02 b	8.92 bc	4.58 c	2.46 b	3.88 b	1.04 b	1.17 bc	0.97 bc
	3	9.27 d	9.09 b	8.01 de	4.01 d	2.43 b	3.42 de	1.00 b	1.27 b	0.86 d
	6	8.31 e	6.49 c	7.65 ef	3.47 e	1.57 cd	3.21 e	0.79 c	0.75 d	0.80 de
	9	7.56 f	7.06 c	7.27 f	3.32 e	1.75 c	3.32 de	0.71 cd	0.80 d	0.78 e
Non-inoculation (CK)	0	16.76 a	17.5 a	9.93 a	6.29 a	3.88 a	4.28 a	1.35 a	1.81 a	1.01 ab
	3	13.66 b	9.54 b	9.34 b	5.77 b	2.53 b	3.79 b	1.33 a	1.13 c	1.05 a
	6	7.53 f	6.01 c	8.39 cd	3.46 e	1.50 d	3.69 bc	0.69 d	0.74 d	0.93 c
	9	7.97 ef	6.60 c	7.69 ef	3.42 e	1.72 e	3.49 cd	0.72 cd	0.82 d	0.80 de
Significances	Cd	****	****	****	****	****	****	****	****	****
	Inoculation	****	****	****	****	****	****	****	****	****
	Cd × Inoculation	****	****	ns	****	****	ns	****	****	**

Different letters denote significant differences between the treatments ($p < 0.05$). The p values of ANOVAs of Cd levels, inoculation treatments, and their interactions were also shown. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, ns, not significant)

Table 3 Total Cd contents of contaminated soil under different treatments

Cd levels (mg kg ⁻¹)	Treatment	Rhizosphere soil (mg kg ⁻¹)	Initial test soil (mg kg ⁻¹)	Final test soil (mg kg ⁻¹)
0	Inoculation	0.004 g	0.244 ± 0.015	0.129 ± 0.027
	Non-inoculation	0.065 g		
3	Inoculation	2.064 f	3.673 ± 0.012	2.952 ± 0.043
	Non-inoculation	2.219 e		
6	Inoculation	4.393 d	6.455 ± 0.030	5.733 ± 0.116
	Non-inoculation	5.032 c		
9	Inoculation	6.478 b	9.511 ± 0.108	8.184 ± 0.049
	Non-inoculation	6.888 a		

Different letters denote significant differences between the treatments ($p < 0.05$)

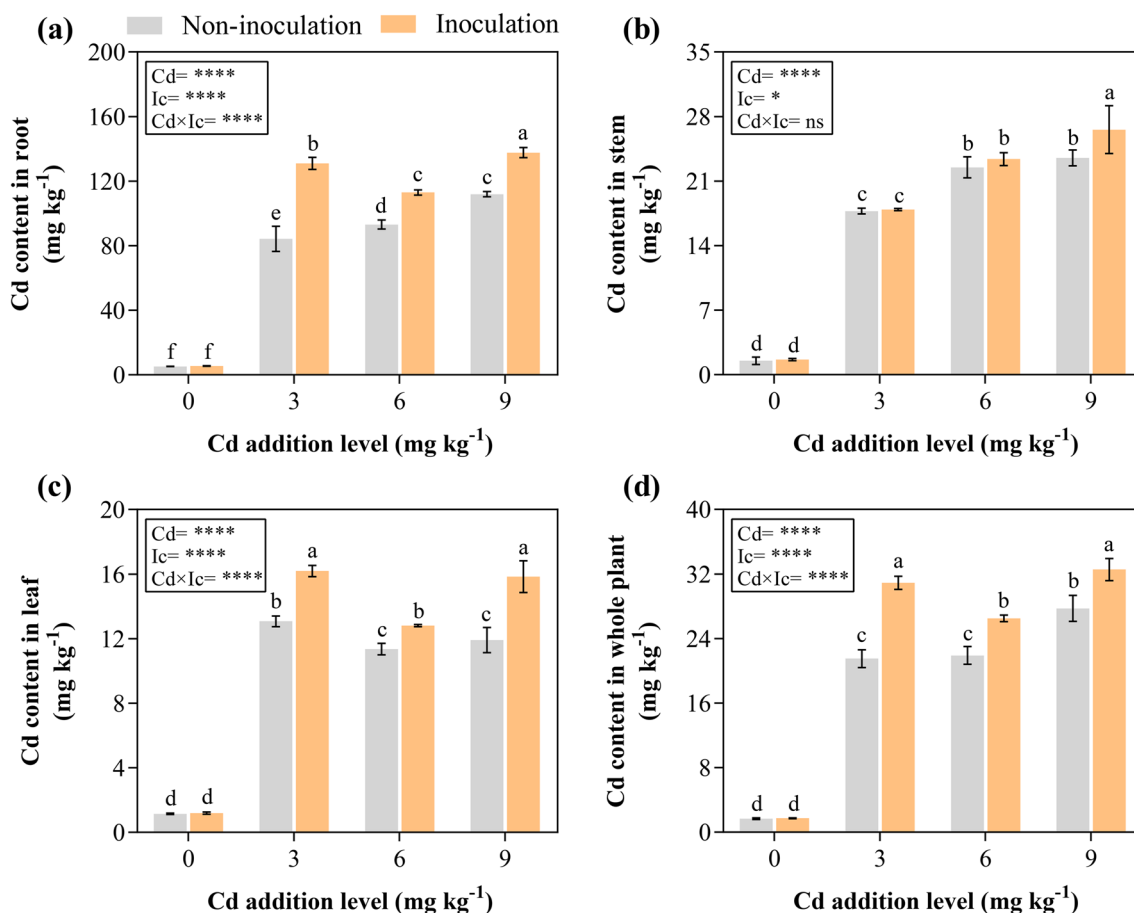


Fig. 4 Cd content of *P. Elliottii* under different Cd addition levels and inoculation treatment. (a) Root, (b) stem, (c) leaf, and (d) whole plant. Different letters denote significant differences between the treatments ($p < 0.05$), and the error bars denote the standard deviations from the mean

The Pearson correlation analysis and linear regression analysis

The Pearson correlation coefficients between the indicators of *P. Elliottii* are shown in Fig. 5. Whether inoculated or not,

plant biomass was negatively correlated with Cd content in plant ($p < 0.01$), while biomass was positively correlated with P, K, Ca, and Mg contents in plant ($p < 0.01$). The contents of P, K, Ca, and Mg were positively correlated with each other ($p < 0.05$). There were some significant

Table 4 The BCFs and TFs of Cd in the root, stem, and leaf of *P. Elliottii* under different Cd addition levels and inoculation treatment

Treatment	Cd levels (mg kg ⁻¹)	Root	Stem		Leaf	
		BCF	BCF	TF	BCF	TF
Inoculation (YTG72)	3	44.41 a	6.07 a	0.14 c	5.48 a	0.12 b
	6	19.71 c	4.08 b	0.21 b	2.24 c	0.11 b
	9	16.82 d	3.25 c	0.19 b	1.94 d	0.12 b
Non-inoculation (CK)	3	28.52 b	6.01 a	0.21 b	4.43 b	0.16 a
	6	16.25 d	3.92 b	0.24 a	1.98 d	0.12 b
	9	13.68 e	2.88 d	0.21 b	1.46 e	0.11 b
Significances	Cd	****	****	****	****	***
	Inoculation	****	*	****	****	*
	Cd × Inoculation	****	ns	**	****	*

Different letters denote significant differences between the treatments ($p < 0.05$). The p values of ANOVAs of Cd levels, inoculation treatments, and their interactions were also shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, ns, not significant)

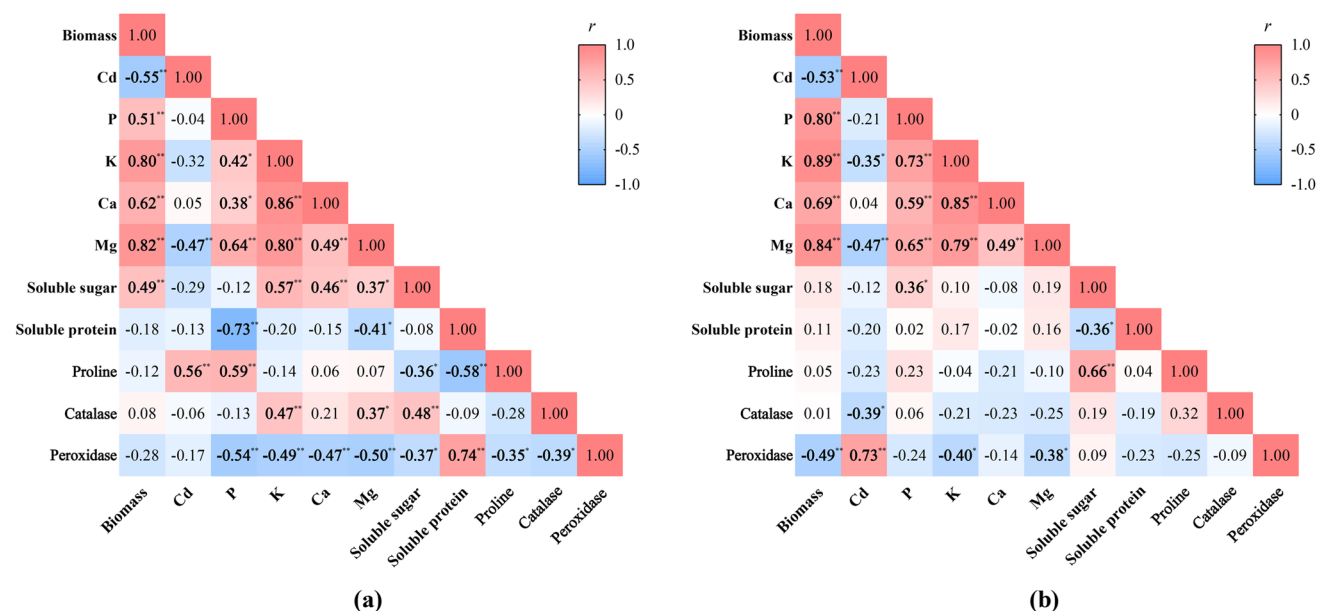


Fig. 5 The Pearson correlation coefficients between each indicator of *P. Elliottii* under (a) non-inoculation and (b) inoculation condition. Bold numbers indicate significant correlations (* $p < 0.05$; ** $p < 0.01$, two-tailed)

positive/negative correlations between soluble sugar, soluble protein, Pro, CAT, POD, and most of the indicators in non-inoculated plants, but the correlations between these indicators weakened under the inoculation treatment, and most of them shifted to non-significant ($p > 0.05$) or even opposite correlations.

Linear regression analysis was performed to further investigate the relationship between Cd and P, K, Ca, and Mg stoichiometric ratios in plants (Fig. 6 and Fig. S1). The results of the analysis showed that there were significant ($p < 0.05$) negative correlations between the Cd content in plant root, stem, and leaf and the ratios of their K:P, Ca:P, and Mg:P, with regression coefficients < 0 . Among them, the regression coefficients became larger after inoculation with YTG72 treatment, indicating a weakened negative correlation between indicators. In addition, the Cd content in root and stem of the non-inoculated treatment showed negative correlations with K:Ca and K:Mg, while there was no correlation between Cd content in root, stem, and leaf under inoculation treatment and K:Ca, K:Mg, and Ca:Mg ($p > 0.05$).

Discussion

Morphological and physiological response of *P. Elliottii* to Cd stress

Cd stress usually inhibits cell division in plant meristematic tissues and hinders water and nutrient uptake by the root system, thus adversely affecting dry biomass accumulation

in plants (Bali et al. 2020). Interestingly, there was a significant increase in dry biomass, plant height increment, and stem diameter increment of seedlings by low Cd stress treatments, and similar phenomena have been reported in previous studies on peanut (*Arachis hypogaea*) and pakchoi (*Brassica campestris*) (Wu 2008; Zhang et al. 2011). It has been demonstrated that low Cd stress can stimulate the root system to release enzymes, organic acids, amino acids, phenolics, and other root secretions as potential chelate ligands for Cd^{2+} , which can reduce Cd toxicity and stimulate the growth of the plant by decreasing the bioavailability of Cd in the soil (Patra et al. 1994; Bali et al. 2020). Under the presence or absence of Cd stress, inoculation with strain YTG72 was effective in promoting seedling growth, which was attributed to the elemental dilution effect due to the PGP properties of strain YTG72 that promoted an increase in plant biomass, thereby reducing the toxic effect of Cd on plants (Huang et al. 2017).

Cd stress interferes with plant photosynthesis and ultimately inhibits seedling growth by destroying the structure of chloroplasts, inhibiting light energy uptake, disrupting ion exchange, and decreasing the activity of enzymes essential for the Calvin cycle (Handa et al. 2018). The chlorophyll content of *P. Elliottii* seedlings under high Cd stress was reduced, which could be attributed to an increase in the activity of the chlorophyll-degraded enzyme chlorophyllase under HMs stress conditions (Eltahawy et al. 2022). Inoculation with strain YTG72 increased the chlorophyll content of *P. Elliottii* to some extent, as did Shahid's study (2019) of flax (*Linum usitatissimum*) on inoculation with *Serratia*

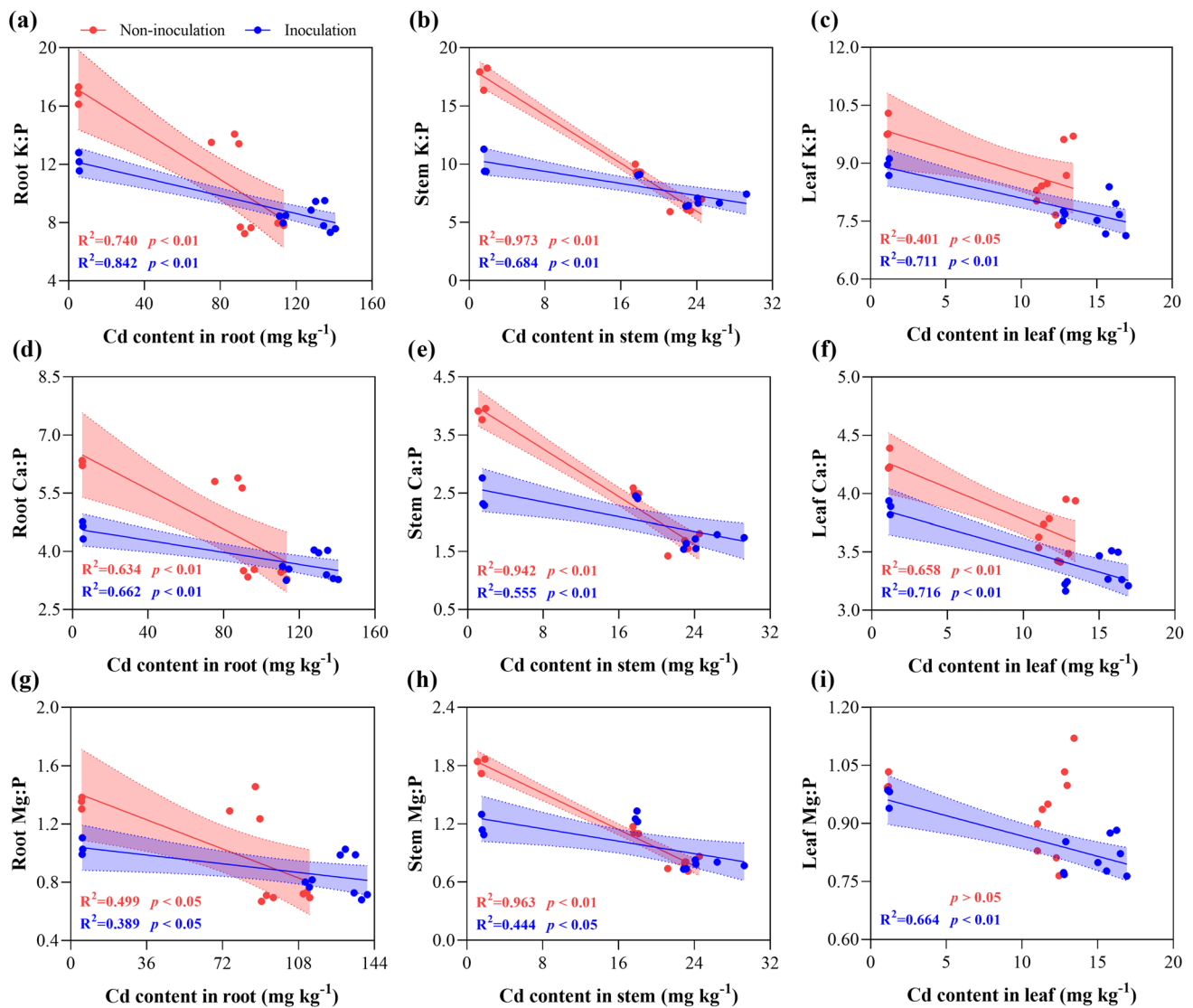


Fig. 6 Correlations between plant Cd contents and the ratios of plant nutrient element (K:P, Ca:P, and Mg:P) under inoculation/non-inoculation condition

sp. CP-13. Bacteria could prevent HMs from moving to the plant by attaching them to the cell wall, which may serve the purpose of reducing the effects of HMs on the plant and counteracting premature leaf senescence (Eltahawy et al. 2022). In addition, the application of strain YTG72 may have also activated the enzyme pathways of chlorophyll biosynthesis and increased the bioavailability of minerals (e.g., P, K, Ca, and Mg), which resulted in photosynthetic efficiency (Shahid et al. 2020), as confirmed by the increase in dry biomass of the inoculated *P. elliptii* seedlings.

Soluble sugars and soluble proteins, as important metabolic substrates during plant growth, have dynamic roles in controlling many processes in the developmental stages of plants (Sardar et al. 2022). Low Cd stress increased the content of aboveground soluble sugars and root soluble

proteins, which can be explained by the release of different types of proteins and carbohydrates from different tissues of the plant to regulate cellular osmotic pressure to maintain normal metabolism (Zeng et al. 2020). Increased soluble protein content also helps plants to enhance the synthesis of Cd-binding proteins/polypeptides to resist Cd toxicity (Yang et al. 2018). Previously, it was reported that the contents of soluble sugars and soluble proteins in mulberry (*Morus alba*) and camphor tree (*Cinnamomum camphora*) also increased under Cd stress (Zhou et al. 2019; Zeng et al. 2020). The significant decrease in soluble sugar content under high Cd stress may be attributed to the destruction of chloroplasts and subsequent decrease in photosynthetic activity with increasing Cd toxicity. Plants perhaps consumed some soluble sugars in order to mitigate the toxicity

of Cd and enhance their physicochemical activities (Sardar et al. 2022). Similarly, the significant decrease in soluble protein content under Cd stress may be due to protein degradation, or inactivation of biosynthetic enzymes involved in protein synthesis (Hakeem et al. 2022). The decrease in soluble protein content may also modulate the activity of antioxidant enzymes in response to Cd-induced reactive oxygen species (ROS) generation and phytochelatin biosynthesis, leading to intracellular Cd compartmentalization (Hossain et al. 2012). The inoculation with strain YTG72 increased the soluble sugar and soluble protein contents of seedlings under Cd stress, indicating that strain YTG72 can regulate the physicochemical activities of the plant to resist Cd stress, which is similar to the findings of Shah et al. (2020) and Tanveer et al. (2023). The increased levels of these substances may be due to the production of PGP metabolites by the bacteria, which increases plant metabolism, nutrient assimilation, and protein synthesis (Bruno et al. 2021).

Accumulation of Pro in plants under HMs stress is considered one of the primary signals of stress perception (Abbas et al. 2021). As an important osmolyte, increased levels of Pro under abiotic stress conditions protect cells from stress injury through osmotic regulation and maintain the integrity of the plasma membrane (Ou et al. 2023). Cd stress induced an increase in Pro accumulation in seedlings, suggesting that Cd toxicity prompted *P. elliotii* to enhance Pro synthesis required to mitigate osmotic stress. It is believed that the increase of Pro content helps Pro develop bonds with metal contaminants and reduce metal toxicity in plants (Nawaz et al. 2015). After inoculation with YTG72, the Pro content of seedlings was significantly decreased, which indicated the improvement of physiological function of plants along with the alleviation of oxidative stress. This is similar to the findings of Ali et al. (2021) and Sabir et al. (2020), reflecting the ability of strain YTG72 to alleviate Cd stress. It is also important that the reduction in Pro helps to save energy used in life activities, making this conserved energy available for other metabolic processes to upscale plant growth and yield (Sabir et al. 2020).

In order to repair the damage caused by ROS, plants can utilize antioxidant defense mechanisms based on the function of various antioxidants such as CAT, POD, superoxide dismutase (SOD), ascorbate peroxidase (APX) (Kumar et al. 2023). In the absence of inoculation, there was a significant increase in CAT and POD in root, stem, and leaf at some level of Cd stress. The function of CAT and POD is to scavenge H_2O_2 accumulated in plant cells (Ou et al. 2023); thus, this suggests that *P. elliotii* has the ability to resist Cd stress by stimulating antioxidant defense mechanisms. However, the activities of CAT and POD were reduced under high Cd stress, which may be related to the increased level of lipid peroxidation or enzyme inactivation due to the overproduction of ROS in plants, as confirmed by earlier studies (Zeng

et al. 2020; Abbas et al. 2021). This may also be one of the reasons for the growth inhibition of *P. elliotii* under high Cd stress in this study. Inoculation with strain YTG72 reduced CAT activity at low Cd stress, which may be attributed to the dilution effect brought about by the rapid increase in plant biomass, or the bacterial inoculation effectively alleviated the oxidative stress of the plant at low Cd stress (Shahid et al. 2020). When Cd stress was intensified, both CAT and POD activities were significantly increased by inoculation with strain YTG72, which indicated that the strain was able to activate the antioxidant system of the plant, resulting in the amelioration of oxidative stress induced by Cd toxicity. It has been reported that some bacteria have the ability to activate genes involved in antioxidant production to encode antioxidant enzymes, such as the application of *Serratia marcescens* to soybean (*Glycine max*), which increased the expression of its antioxidant genes (CAT, APX, POD, and Cd-SOD) (El-Esawi et al. 2020). Therefore, improving the antioxidant system of *P. elliotii* by inoculation with YTG72 may be considered one of the mitigation strategies to alleviate intracellular Cd stress.

Regulation of Cd tolerance in *P. elliotii* by dynamic allocation of nutrient elements

The changes in the content of nutrient elements occurring in the plant reflect the growth status of the plant, as shown by the significant positive correlation between seedlings biomass and P, K, Ca, and Mg in this study. There are some similar chemical properties between Cd and P, K, Ca, and Mg, so Cd^{2+} in the soil will displace these ionic nutrients through specific binding sites on the cell membrane, thus interfering with the uptake of these nutrients by the plant root system, which will result in nutrient deficiencies and imbalances, leading to stunted plant growth (Rochayati et al. 2011; Etesami 2018). In contrast, in this study, the low Cd treatment induced an increase in nutrient content in *P. elliotii*, which is most likely the result of changes in the rate of nutrient absorption and distribution in the plant. This may also be a stress strategy employed by the plant to counteract the competition of Cd^{2+} for P, K^+ , Ca^{2+} , and Mg^{2+} binding sites in the cytoplasm, which is believed to contribute to the plant tolerance to HMs (Liu et al. 2011; Rengel et al. 2015). This is also similar to the results of previous studies on pakchoi (*Brassica campestris*) as well as black nightshade (*Solanum nigrum*) (Guo et al. 2009; Zhang et al. 2011), which showed a synergistic relationship between the accumulation of Cd and P, K, Ca, and Mg in plants under low Cd stress. More importantly, among these elements, the variation of P content in *P. elliotii* seems to be the key factor in its tolerance to Cd stress. Under different levels of Cd stress, the P content in root, stem, and leaf of seedling was higher than those of the control without

Cd stress. Higher P levels in plants have been suggested to promote ATP synthesis in plants, which maintains cellular energy flow, promotes biomass, and improves stress tolerance (Li et al. 2016; Sardar et al. 2022). Furthermore, the ratios related to P in *P. elliotii*, such as K:P, Ca:P, and Mg:P, showed a regular decrease under Cd stress. It has been demonstrated that plants alleviate Cd toxicity by regulating the distribution of nutrients such as P, K, Ca, and Mg in the organ. For example, the maintenance and reduction of Mg:K ratio in arabis (*Arabidopsis thaliana*) roots under Cd stress were beneficial to root growth (Carvalho et al. 2020), and the reduction of K:P in plants helped chili pepper (*Capsicum annuum*) adapt to thallium stress (de la Luz Buendia-Valverde et al. 2022). In this study, the correlation analysis also confirmed the role of P distribution on the response of *P. elliotii* to Cd stress, just as the Cd content in both soil and plant showed a negative correlation with plant K:P, Ca:P, and Mg:P. In other words, the decrease of these ratios in *P. elliotii* may have increased plant resistance to Cd stress. This imbalance between nutrients may influence the detoxification process of Cd in plants (Liu et al. 2011).

Inoculation of seedlings with strain YTG72 increased the contents of P, K, Ca, and Mg to a certain extent, which can be attributed to the fact that the IAA generated by strain YTG72 modifies membrane permeability, thus promoting the accumulation of nutrients (Mir et al. 2022). In addition, due to the increase in root surface area after inoculation with YTG72, more binding sites for nutrients were provided, which improved the nutrient uptake in *P. elliotii* under Cd stress (Shahid et al. 2020). The further increase of P content and the further decrease of K:P, Ca:P, and Mg:P under inoculation treatment also indicated that the strain could assist Cd-stressed plants to maintain the optimal level of nutrient elements in vivo by mobilizing P allocation in the plant, thus alleviating the Cd stress injury of plants (Shahid et al. 2019).

Application potential of *Herbaspirillum* sp. YTG72 assisted *P. elliotii* for the remediation on Cd-contaminated site

Due to its high mobility and bioavailability in soil, Cd can enter the plant root system through the apoplast pathway and the symplast pathway, and then be transported to the aboveground part of the plant through the xylem (Ismael et al. 2019). Numerous studies have shown that roots are the main organ of woody plants responding to Cd stress in soil (Zeng et al. 2018, 2020; Zhou et al. 2019). Plants are capable of storing or chelating metal ions in the root cell vacuole for detoxification (Haider et al. 2021); thus, Cd is often accumulated in the roots of plants as shown in the results of this study.

It is noteworthy that with the increase of soil Cd content, the root and stem Cd content increased continuously, while there was no significant difference in leaf Cd content. This similar phenomenon also appeared in previous studies on Cd uptake by willow species (*Salix* spp.) (Wang et al. 2021b). The Cd deposition in the root cell apoplast increases with soil Cd content, which in turn leads to increased plant Cd retention (Sterckeman et al. 2011). However, excessively high levels of Cd in the soil may limit the translocation of Cd to the leaves, so that most of the Cd is sequestered in the root system to reduce its toxic effects on the aboveground (Zeng et al. 2020).

Inoculation of seedling with strain YTG72 effectively promoted soil Cd uptake and enrichment by roots. Many studies have confirmed that inoculation of plants with PGPB favors the uptake of soil Cd by plants (Guo et al. 2011; Sangsuwan and Prapagdee 2021). In this study, it is hypothesized that the ability of YTG72 to solubilize phosphorus and produce IAA can acidify the soil environment, and increase the bioavailability of Cd in soil, thus promoting the uptake of Cd by plants. Inoculation treatments had less effect on Cd enrichment and translocation capacity of stem and leaf, which can be attributed to the limiting effect of microorganisms on aboveground Cd accumulation in plants (Shahid et al. 2020). Previous studies have confirmed that PGPB can enhance phosphate retention through siderophores formation, hormone stimulation, and root hair growth, thereby reducing HMs translocation (Shahid et al. 2020). This also suggests that the strain YTG72 could promote the sequestration of Cd in plant roots. In addition, inoculation with the strain increased the Cd content accumulated per unit biomass of *P. elliotii*, and Konkolewska et al. (2020) concluded that the plant HMs extraction efficiency increased with the increase of plant biomass production. This also means that inoculation with YTG72 helps to increase the phytoremediation efficiency of Cd-contaminated soil per unit area.

The BCF and TF values can be used to explore the suitability of plants for phytoextraction or phytostabilization of metal-contaminated soils. Plants with BCF and TF values greater than 1 are suitable for phytoextraction; plant species with TF values less than 1 are suitable for phytostabilization (Chojnacka et al. 2005). The BCF of *P. elliotii* for Cd was much larger than 1, while all TFs were less than 1, indicating the potential as a phytostabilization tree species. According to Baker and Brooks (1989), *P. elliotii* is not enough to be a hyperaccumulator for Cd (Cd content > 100 mg kg⁻¹), and in fact, woody plants can seldom reach this criterion. Nevertheless, as a fast-growing tree, *P. elliotii* can be used as a pioneer species for tailings phytoremediation because of its large aboveground biomass and the ability of its roots to effectively enrich Cd and reduce soil Cd content in a relatively short period of time.

Conclusion

Low Cd addition level (3 mg kg⁻¹) stimulated the growth of *P. elliotii*, while higher Cd addition level had a significant inhibitory effect on plant growth. Under Cd stress, *P. elliotii* can alleviate the Cd phytotoxicity through a series of mechanisms including plant biomass accumulation, nutrient uptake, osmotic system regulation, and antioxidant system activation. It is also found that the increase of P content as well as its proportion in nutrients of *P. elliotii* is a potential key mechanism for Cd tolerance. The present study provides an environmentally friendly solution for the remediation of Cd-contaminated soil. *P. elliotii* showed good enrichment ability for soil Cd and can be used as a phytostabilization tree species for Cd-contaminated site. Inoculation of *P. elliotii* with the PGPB *Herbaspirillum* sp. YTG72 improved the physiological activity and antioxidant levels of plants, thereby reducing the phytotoxicity of Cd, and that the inoculation treatment also promoted Cd accumulation in plants and reduced soil Cd content. Therefore, it is necessary for future implementation of phytoremediation to manage soil nutrients and apply microbial agents, which is of great significance for improving the efficiency of phytoremediation on contaminated sites.

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Author contribution Yanglong Li: investigation, data curation, formal analysis, methodology, writing—original draft. Xiangteng Chen: investigation, formal analysis. Yuhong Dong: methodology, writing—review and editing. Shumeng Wei: formal analysis. Mansheng Zeng: resources. Ruzhen Jiao: conceptualization, supervision, writing—review and editing.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors read and approved the final manuscript. The work described has not been published. It is not under consideration for publication elsewhere. Its publication has been approved by all coauthors. If the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Competing interests The authors declare no competing interests.

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