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Cadmium Uptake and Growth Responses of Potted Vegetables to the Cd-Contaminated Soil Inoculated with Cd-Tolerant *Purpureocillium lilacinum* N1

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Abstract: Bioremediation of Cd- (cadmium) contaminated soil using Cd-tolerant fungus is considered an eco-friendly and cost-effective technique. In this study, we isolated one fungal strain that was hyper-tolerant to Cd from a highly polluted river and conducted pot experiments to evaluate its effects on bioremediation. We found that the fungal strain belonging to the genus, *Purpureocillium lilacinum*, tolerated 12,000 mg/L Cd. SEM manifested that Cd can be bioaccumulated on the crumpled mycelial surface, generating plenty of metal precipitation particles. In addition, pot experiments showed that the inoculation of *P. lilacinum* N1 could reduce the total Cd content in soil (2.09% in low contaminated soil and 12.56% in high contaminated soil) and greatly promote plant growth (2.16~3.13 times). Although the Cd concentration of plants was increased by 112.8% in low contaminated soil and decreased by 9.5% in highly contaminated soil with the inoculation of *P. lilacinum* N1, the total uptake of Cd by plants was greatly improved—1.84~3.6 times higher than that in CK groups. All our results suggest that *P. lilacinum* N1 is a valuable candidate for the bioremediation of Cd-contaminated soils because of its dual effects on the total Cd content in soil and Cd uptake in plants.

Keywords: cadmium; *Purpureocillium lilacinum*; Cd-resistant; bioremediation; growth-promoting

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1. Introduction

Cadmium (Cd) is a highly toxic heavy metal element ubiquitous in soil and is threatening human health more and more seriously. In the natural environment, Cd pollution is mainly derived from anthropogenic inputs, including industrial and agricultural activities, such as metal smelting and indiscriminate fertilizer and pesticide application [1,2]. According to the China Soil Environmental Quality Standard (GB15618-1995) and the National Food Safety Standard (GB 2762-2017), the recommended limits for Cd concentration in the environment and food are 0.3 mg/kg in acid paddy soil (pH < 6.5), and 0.2 mg/kg in brown rice and leafy vegetables, respectively [3,4]. These are the standardized limit values that must be adhered to, to ensure safe agricultural production and human health. However, with the rapid industrial development, the cadmium content in paddy fields is up to 60 mg/kg in some areas of Hunan Province [5]. In addition, according to a comprehensive investigation of 15 towns in a county of Hunan Province, the Cd concentration in 57.5% of the soil samples and 59.6% of rice grains exceeded the national standard limit [6]. Long-term intake of Cd can cause severe bone metabolism disease, cardiovascular disease, and kidney damage [7–9]. Therefore, it is urgent to develop efficient strategies to remediate Cd-contaminated soils to safeguard people's health.

Bioremediation has received more and more concerns in recent years, and the application of microorganisms is considered an eco-friendly and cost-effective technology [10–14]. Fungi have developed metal resistance mechanisms and can survive even under high Cd stress. The organic acids and metal-binding proteins produced by fungi or functional groups like amino, carboxyl, hydroxyl, and sulfhydryl groups existing on fungi cells, usually involves in biosorption, bioaccumulation, biotransformation, or biomineralization of Cd in the process of bioremediation [15,16]. These physical-chemical processes could alter the fractions of Cd in soil, thus changing its bioavailability. In addition, some fungi boost a plant's antioxidant system, which aids in the reduction of Cd's damaging effects [17,18]. Many fungi species have been isolated from Cd-contaminated sites and used for soil remediation, such as *Aspergillus niger*, *Mucor circinelloides*, *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma virens*, *Fusarium oxysporum*, *Leptobacillium chinense*, and arbuscular mycorrhizal fungi [18–24]. Given the difference in the removal efficiency of Cd and protective effects on plants by various fungi, it is essential to explore more valuable fungi resources.

We summarized the effects of microbial remediation on soil Cd pollution into two aspects. Firstly, from the perspective of the changes of soil's total Cd content and Cd fractions, the application of microorganisms to the soil can alter Cd fractions, resulting in the inhibition or improvement of Cd bioavailability, thus reducing or promoting the uptake of Cd in plants [25–27]. Microorganisms can potentially reduce the total Cd content in the soil [28]. Secondly, from the perspective of plants, the application of microorganisms can promote the growth of plants, increase the total uptake of Cd [20], or reduce the concentration of Cd in plants [29]. For non-edible plants like hyperaccumulators, promoting plant biomass and increasing total Cd uptake can improve the remediation efficiency of Cd pollution [30]. For edible plants, reducing Cd content in edible parts below the standard limit is the primary objective of remediation [10]. Therefore, screening some strains which have dual effects on the total Cd content in soil and Cd uptake in plants has a tremendous application value.

In the present study, one Cd-tolerant fungus strain was isolated from highly Cd-contaminated soils. The purpose of this study was to evaluate its ability for bioremediation of Cd-contaminated soils. Pot experiments were designed to determine the effects of inoculation of the fungal isolate on total Cd content and Cd fractions in soils, and plant biomass and Cd concentration in plants. The results obtained showed progress in applying *Purpureocillium lilacinum* for the bioremediation of Cd-contaminated soil.

2. Materials and Methods

2.1. Fungal Isolation, Identification and Characterization

Three topsoil samples (0–30 cm) were collected from river sediment in Zhongwan village (N 27°46′, E 112°52′), Xiangtan City, Hunan Province, China. This river sediment has been contaminated with untreated industrial wastewater over ten years ago, and the Cd concentration in sediment was up to 4224 mg/kg according to our previous investigation [5]. Three sampling sites were selected at intervals of about 20 m along the river. After that, the samples were kept on dry ice and sent to the laboratory within four hours.

For the isolation of Cd-tolerant fungi, 10 g sediment samples were fully suspended in 100 mL sterilized water, serially diluted (up to 10^{-7}), and then spread on nutrient broth (NB) agar plates (10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride, 20 g/L agar, pH = 7) in the presence of Cd (II) (supplemented with 500 mg/L $CdCl_2$) at 30 °C for 48–96 h. The colonies were selected and inoculated on new plates containing a gradual increase concentration of Cd (1000, 2000, 4000, 6000, 9000, 12,000 mg/L). Finally, one Cd-tolerant fungal strain N1 was found to be tolerant to 12,000 mg/L Cd, and this fungal strain was selected for further studies.

The spore suspension (10^7 spores/mL) of the fungal isolate N1 was first prepared before inoculating into NB broth. For fungal molecular identification, 1 mL spore suspension was inoculated into 100 mL NB broth for 3 days at 30 °C, 170 rpm. The culture was

centrifuged at 4000 rpm for 8 min to collect mycelial pellets and then ground with liquid nitrogen. Extracted genomic DNA and amplification of the internal transcribed spacers (ITS) DNA was done using the primer set ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). Sequence data were analyzed using NCBI BLASTn search (http://www.ncbi.nlm.nih.gov, accessed on 10 December 2020) to identify its homology. These sequences with high similarity from the database were downloaded and a phylogenetic tree was constructed using the neighbor-joining method in MEGA X (Pennsylvania State University, State College, PA, USA) to analyze the evolutionary relationship. The sequence was deposited in the GenBank NCBI database (accession no. SAMN19304788, accessed on 21 May 2021).

2.2. Temperature and pH Tolerance

For determining temperature tolerance, 2 mL spore suspension were inoculated into 98 mL NB liquid medium at different temperatures of 25, 30, 35 and 40 °C for 4 days. For examining pH tolerance, 2 mL spore suspension were inoculated into 98 mL NB liquid medium at different initial pH of 2, 4, 6, 8 and 10 at 30 °C for 4 days. The pH of the media was adjusted using 1 M H_2SO_4 or NaOH; 2 mL samples were taken every day and centrifuged at 10,000 rpm for 10 min. The pH of the supernatant was determined using a pH meter (BPH-220, Bell Instrument Equipment Co., Ltd., Dalian, China). The wet weight of mycelial pellets was determined to evaluate the growth of strain. Three replicates were set for each temperature and pH gradient.

2.3. Biosorption Experiment of Cd (II) in Aqueous Solution

Biosorption experiments were carried out by inoculating 2 mL spore suspension into 98 mL NB liquid medium at different initial concentrations of Cd (II) ranging from 10 to 500 mg/L for 10 days at 30 °C, 170 rpm; 2 mL samples were taken every 2 days and centrifuged at 10,000 rpm for 3 min. The supernatant was stored at 4 °C and used to determine Cd concentration. All experiments were carried out in triplicate.

The fungal strain N1 was cultivated in NB broth containing 9000 mg/L Cd (II) to observe its morphology before and after the biosorption of Cd (II). The culture was centrifuged at 10,000 rpm for 5 min and the mycelial pellets were fixed in 2.5% glutaraldehyde solution and placed at 4 °C overnight. The fixed samples were observed using a scanning electron microscope (SEM, EM-30C, COXEM, Daejeon. Korea).

2.4. Pot Experimental Design

Cadmium-contaminated mine soil samples were collected from farmland soil around several zinc smelters that have been contaminated for twenty years in Hengyang City (N 26°59′, E 112°48′), Hunan Province, China. The samples were collected from a relatively higher contaminated region (High-Cd) and a less contaminated region (Low-Cd). After removing the stones, plant debris and other coarse materials, the soil samples were air-dried at room temperature and then ground to pass through a 10-mesh sieve for pot experiments. Soil pH, organic matter, total Cd content and the Cd fractions were determined.

Both High-Cd and Low-Cd soil samples were applied in pot experiments, with each pot (length: 23 cm, weight: 17 cm, height: 13 cm) containing 1 kg soil. The pots had holes at the bottom to ensure good drainage. Before pot experiments, the fungal spore suspension was cultivated in the sterile NB liquid medium for 3 days. A proportion of the fungal inoculum was filtered using a 0.45 μm cellulose–acetate membrane; the supernatant and the fungal pellets were collected, respectively. Fungal pellets were re-suspended in sterile distilled water with the same volume as supernatant to obtain the mycelia pellets without metabolites. Therefore, 4 treatments with 3 replicates were set up, yielding a total of 24 pots (2 Cd level, 4 treatments, and 3 replicates). Four treatments were as follows: (1) soil + distilled water (CK); (2) soil + inoculum (SI); (3) soil + supernatant (SS); (4) soil + re-suspended mycelia pellets (SR). Ten vegetable seeds (*Brassica Chinensis* L.) were sown in each pot, covered with a thin layer of soil, and watered thoroughly with a volume of

400 mL of the prepared liquid. Each pot was watered with 100 mL corresponding liquid 4 times in the first 10 days and watered with 100 mL distilled water 10 days later. After 30 days, the plants were carefully removed from the pots and rinsed with distilled water. All the pots were placed in natural light and kept at around 25 °C for room temperature.

A phenomenon in the growth process was that seeds sprayed with inoculum and supernatant (SI and SS) did not germinate or survive when emerged. After 30 days of growth, the rest of the grown vegetables were harvested, washed with distilled water, and dried with filter paper. After measuring the wet weight, the plants were dried to a constant weight at 80 °C for Cd concentration determination. Soil samples were collected after harvesting for measurement of total Cd concentration and Cd fractions.

2.5. Total Cd Content and Fraction Analysis in Soil and Plant Samples

According to reported methods, soil samples were air-dried at room temperature and then powdered to pass through a 100-mesh sieve for total Cd determination [31]; 0.200–0.500 g samples were digested in a mixture of HNO_3/HF/$HClO_4$ (10/5/2, *v/v/v*) solution following a digestion procedure: 110 °C for 30 min, 140 °C for 30 min, and 180 °C until the samples were completely digested, and clarified digestion fluid obtained. Similarly, 0.200–0.500 g plant samples were digested in a mixture solution of 8 mL HNO_3 and 4 mL $HClO_4$ following the same digestion procedure as soil digestion. Reagent blanks and standard reference materials (GBW07401) were included in each digestion batch to ensure the analytical quality. The relative standard deviations were less than 5%. The concentration of Cd in the digested fluid obtained was measured using inductively coupled plasma atomic emission spectrometry (ICP-AES, Optima 5300DV, PerkinElmer, Shelton, CT, USA) by measuring the absorbance values under the emission wavelength of Cd at 228.8 nm.

A three-step sequential extraction procedure was carried out to determine the fractions of Cd in soil samples according to the modified BCR (European Community Bureau of Reference) [32,33], in which the Cd was divided into four fractions: weak acid soluble Cd (F1), reducible Cd (F2), oxidizable Cd (F3), and residual Cd (F4). Generally, acid-soluble Cd in the soil can be more easily absorbed by plants, and oxidation and residual Cd are not likely to be utilized [34]. For each liquid extract and digested solution, ICP-AES was used to determine the fractions of heavy metals.

2.6. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance and the Tukey's test ($p < 0.05$) in the Minitab 17 (Minitab Inc, State College, PA, USA) and Figures were drawn using Origin 9.0 (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Identification and Characterization of Cd-Tolerant Fungal Strain

We isolated fungi from heavily polluted river sediment with NB broth. Initially, few colonies were obtained at the concentration of 500 mg/L Cd (II), but only one Cd-tolerant fungal strain was obtained when Cd (II) concentration was increased to 6000 mg/L. The minimum inhibitory concentration (MIC) of Cd (II) for this isolate was up to 12,000 mg/L. According to the internal transcribed spacers (ITS) rDNA gene sequencing results, the isolated fungal strain showed 100% similarity with the sequences of *Purpureocillium lilacinum*.

In addition, we only observed few colonies, even when the initial Cd concentration was 500 mg/L. Our results showed that few microorganisms were existing in these extremely heavily contaminated sites (Cd content > 4224 mg/kg). Generally, soil fungal populations decreased with increasing concentration of heavy metals [16,35]. Several kinds of research have shown that fungi appeared to be more adaptable to the toxicity of heavy metals than bacteria and could survive in extremely harsh environments [36–38]. Furthermore, a wide range of fungi such as *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, etc., have been frequently isolated from contaminated soils, harboring a strong resistance to heavy

metals in many studies [16,35,39–41]. However, the maximum tolerance against Cd (II) of these reported fungi was usually in the range of 500~1600 mg/L [16,42]. Liaquat et al. has reported that the maximum tolerance against Cd (II) of *Komagataella phaffii*, *Aspergillus*, and *Trichoderma harzianum* were 6000 mg/L, 4000 mg/L, and 2500 mg/L, respectively [41]. The Cd tolerance limit of isolated *P. lilacinum* in this study was up to 12,000 mg/L, exhibiting an extraordinary acceptance for Cd, making it a potential candidate for bioremediation of Cd pollution.

The morphology of strain N1 (Figure 1A) showed a suede-like lilac colony with whitish mycelia. The phylogenetic tree (Figure 1B) constructed with MEGA X showed evolutionary relationships between the strain N1 and other reported species of *P. lilacinum*. The effects of temperature and pH on the growth of strain N1 were conducted, and the wet weight of mycelial pellets was used to evaluate the growth of strain (Figure 1C, D). We observed that strain N1 could grow under a broad range of temperature from 25 °C to 40 °C, and a broad range of pH from an acidic medium at pH 4 to alkaline at pH 8. However, the growth of strain N1 was inhibited entirely at pH 2 and pH 10. The optimal pH and temperature of strain N1 were 6 and 30 °C, respectively. During the four days of growth, the pH of supernatant decreased slightly at an initial pH of 4, 6 and 8, which indicated that some organic acids may be produced by *P. lilacinum* N1 to reduce the pH. The pH of supernatant remained unchanged at initial pH of 2 and 10, which was mainly due to the complete inhibition of fungal growth at pH 2 and pH 10 (Figure 1E).

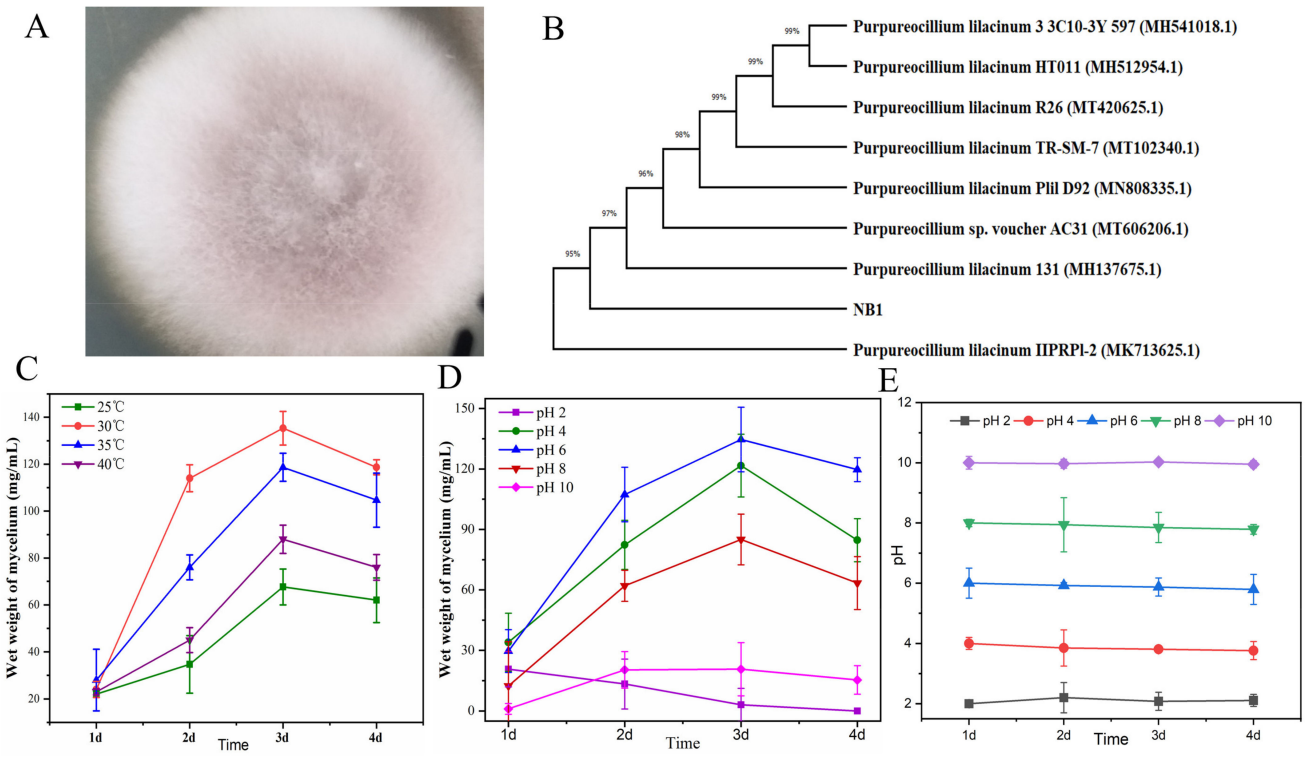

Figure 1. (**A**) The fungal morphology on the plate; (**B**) The phylogenetic tree based on the ITS DNA sequence data of *P. lilacinum* N1 using MEGA X; (**C**) The wet mycelial weight of *P. lilacinum* N1 at different temperatures and (**D**) pH; (**E**) The pH variation of supernatant at different initial pH during four days.

3.2. Biosorption of Cd (II) by P. lilacinum N1 in Aqueous Solution

The Cd biosorption efficiency of *P. lilacinum* N1 was assessed in an aqueous solution at different initial Cd concentrations ranging from 10 to 500 mg/L (Figure 2A). It was observed that the biosorption efficiency increased gradually in the first 4 days and tended towards stability from the 4th day. The maximum removal efficiency gradually decreased from 70.67% ± 4.47% to 9.05% ± 1.98% when Cd (II) concentration increased from 10 mg/L

to 500 mg/L Cd. The decrease of removal efficiency may be due to the limited binding sites on cell surface and the decrease of biomass. At lower concentration of Cd (II), the *P. lilacinum* N1 can grow rapidly and provide enough binding sites for Cd (II) adsorption. On the contrary, the higher concentration of Cd (II) inhibited the growth of *P. lilacinum* N1, and the binding sites were saturated only by a small part of Cd (II), leading to the decrease of biosorption efficiency of Cd (II).

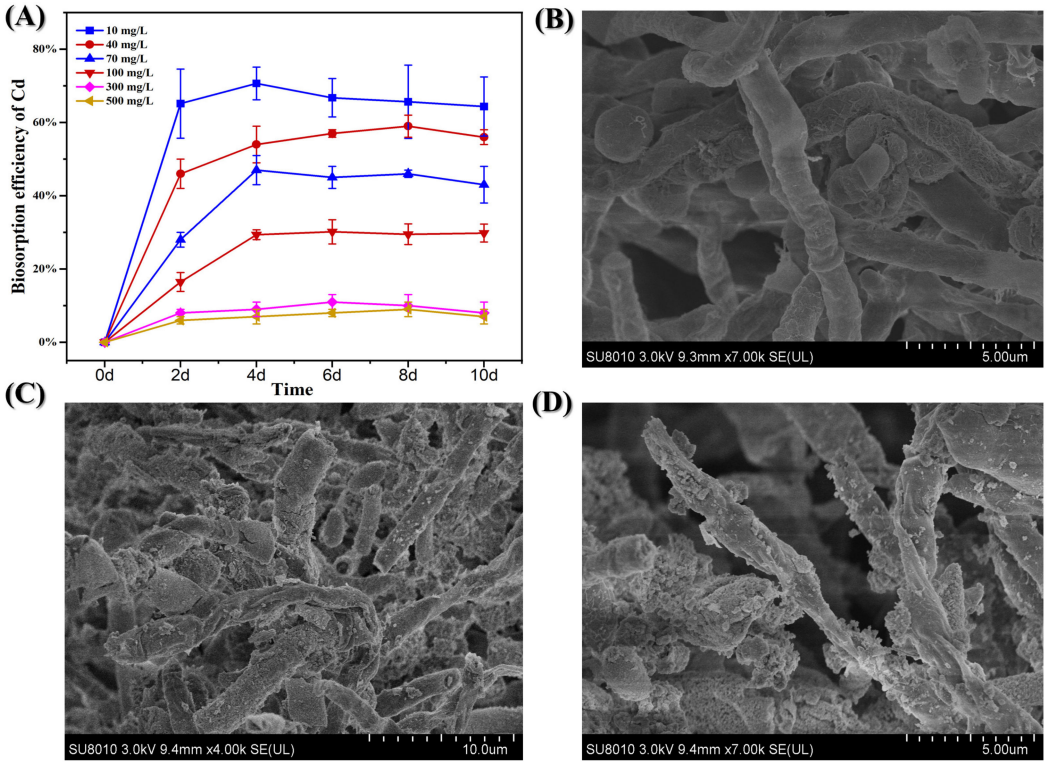

Figure 2. (**A**) The Cd biosorption efficiency of *P. lilacinum* N1 at initial Cd concentrations of 10 mg/L and 100 mg/L; (**B**) SEM image of normal mycelia. SEM image of mycelia grown in the NB liquid medium supplemented with 9000 mg/L of Cd (II) with the resolution of 10 μm (**C**) and 5 μm (**D**).

SEM was used to analyze the morphological changes of *P. lilacinum* N1 under Cd stress. The mycelia of strain N1 was plump and intact in the control group (Figure 2B). However, apparent irregular folds and disruption were observed in the mycelia of strain N1 growing in the NB broth with 9000 mg/L Cd (II) (Figure 2C, D). On the crumpled mycelial surface of *P. lilacinum* N1, many metal precipitations were formed with different particle sizes. Many findings have observed similar morphological changes in the mycelium of fungi exposed to heavy metals [41,43]. Many active groups like amino, phosphate, and carboxyl groups existing on fungi cells, combine with Cd to form the inorganic minerals and reduced Cd toxicity [43]. Previous studies have shown that the extracellular polymer substances of *P. lilacinum* are involved in the biomineralization of jarosite minerals, which significantly alleviated the damage of Fe^{3+} to the fungal cells [44,45]. Thus, we speculated that the biomineralization process on the surface of cells could also alleviate the damage of Cd^{2+} to the *P. lilacinum*. Biomineralization may also be the reason for forming an enormous number of mineral particles on the cell surface. Zeng et al. isolated one strain of *P. lilacinum*, which tolerated 80 mM Cd (i.e., 8960 mg/L Cd), and the adsorption capacity of Cd reached 24.23 mg/g [46]. Some other studies also showed that *P. lilacinum* is strongly resistant to various heavy metals such as chromium, arsenic, lead, copper, mercury, vanadium, etc. [47–50]. These results showed that *P. lilacinum* strongly resisted high Cd stress and could be a potential strain for Cd bioremediation.

3.3. Variation of Fractions of Cd in Soils after Pot Experiments

The results for the characterization of soil samples taken from a chunk of farmland around several abandoned zinc smelters were presented in Table 1. These farmlands have been subjected to Cd exposure for over twenty years. Soil pH values were 5.97 ± 0.15 and 5.22 ± 0.32 in low-contaminated and high contaminated regions, respectively. The soil samples were acidic, and pH values were lower in the samples which suffered more severe pollution. Similar results were obtained in the previous study [51] and this might be due to more extended periods of industrial acid wastewater irrigation in highly contaminated regions.

Table 1. The properties of soil samples from a high and low contaminated region.

Soil Properties (mg/kg)	Low-Cd	High-Cd
pH	5.97 ± 0.15 [a]	5.22 ± 0.32 [b]
Organic matters (%)	3.59 ± 0.149 [a]	1.72 ± 0.214 [b]
Total Cd	1.45 ± 0.263 [b]	20.18 ± 0.426 [a]
Weak acid soluble Cd (F1)	0.946 ± 0.108 [b]	14.09 ± 0.745 [a]
Reducible Cd (F2)	0.263 ± 0.056 [b]	3.837 ± 0.248 [a]
Oxidizable Cd (F3)	0.052 ± 0.011 [b]	1.254 ± 0.206 [a]
Residual Cd (F4)	0.189 ± 0.024 [b]	0.99 ± 0.103 [a]

Different letter within the same line means significant differences ($p < 0.05$), [a] means that the value was significantly higher than the value labeled with [b].

Soil total Cd content in highly contaminated soil was significantly higher than in lower contaminated soil, 20.18 ± 0.426 mg/kg and 1.45 ± 0.263 mg/kg, respectively. Soil organic matters and detailed fraction analysis, including weak acid-soluble, reducible, oxidizable and residual Cd, were much higher in the High-Cd group. The fractions of Cd in soils were critical for evaluating its bioavailability. Generally, acid-soluble Cd was much more available for plant uptake than oxidizable and residual Cd [5]. However, Cd in both low contaminated and high contaminated soil was mainly presented in the acid-soluble Cd (69.9% and 65.3%, respectively), indicating that the Cd in test soil can be easily transferred to pot vegetables.

The effects of *P. lilacinum* N1 on the Cd fractions in soils after pot experiments are shown in Figure 3. Compared to the CK group, total Cd content in low contaminated and high contaminated soil treated with re-suspended mycelia pellets reduced by 2.09% and 12.56%, respectively. In the low contaminated soil, the acid-soluble Cd decreased by 6.0%, whereas the residual Cd increased by 17.73%. The results indicated that *P. lilacinum* N1 could promote the immobilization of Cd in the low contaminated soil. The reduction of total Cd in highly contaminated soil was owed to the decrease of all the fractions, namely, 10.61% for F1, 18.02% for F2, 13.64% for F3, and 15.60% for F4, respectively. The results meant that *P. lilacinum* N1 could remove Cd in the soil through drainage.

The removal of heavy metals from the soil via fungus has been widely reported. For example, Xu et al. [52] and Ren et al. [24] used *Aspergillus niger* to remove the heavy metals in the sludge and contaminated soil, and found that the removal rate of Cd was up to 97.8% and 100% after bioleaching. *P. lilacinum* decreased the pH in culture media [53], which might be due to the excreted organic acid metabolites such as citric acid, succinic acid, oxalic acid, malic acid, and gluconic acid [54,55]. These organic acids can promote the activation of heavy metals in soil [56,57]. In addition, the release of dissolved organic carbon and the degradation of humic substances by fungal activity can increase the solubility and motility of heavy metals [58,59]. *P. lilacinum* can degrade humic substances, which may also contribute to the reduction of Cd in contaminated soil [60,61].

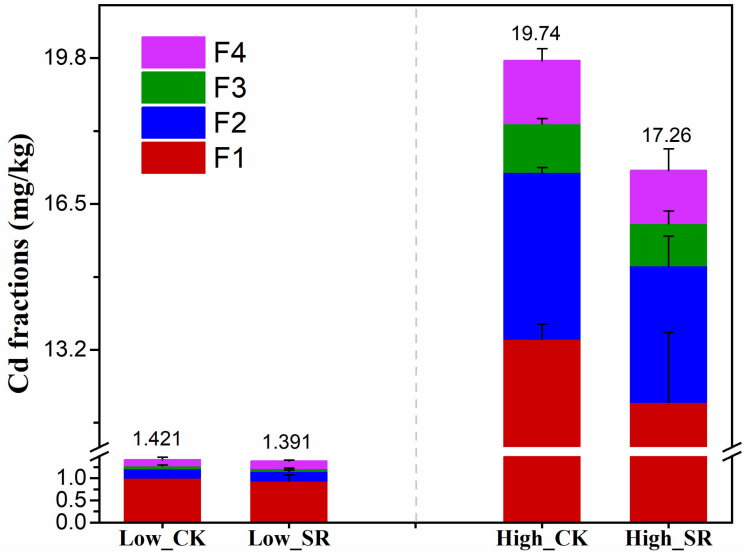

Figure 3. Cd fractions in soils after pot experiments. T signs presented on every bar mean error bars. Low_CK: low contaminated soil + distilled water; Low_SR: low contaminated soil + re-suspended mycelia pellets; High_CK: high contaminated soil + distilled water; High_CK: high contaminated soil + re-suspended mycelia pellets; F1: weak acid-soluble Cd; F2: reducible Cd; F3: oxidizable Cd; F4: residual Cd.

3.4. Effects of Fungal Inoculation on the Growth of Plant Samples

The results for plant growth and Cd accumulation were shown in Figure 4. In terms of wet weight and dry weight, it was axiomatic that the treatments with re-suspended mycelia pellets promoted plant growth. The wet weight of the SR groups was 2.76 and 2.56 times that of the CK groups in low contaminated and high contaminated soil, respectively. Likewise, the dry weight of the SR groups was 3.13 and 2.16 times that of the CK groups, respectively. High concentration of Cd in soil inhibited plant growth, which can be alleviated by applying *P. lilacinum* N1. In terms of Cd accumulation, it is evident that the application of strain N1 had different impacts on the Cd concentration in plants. In low contaminated soil, the Cd concentration of plants in SR groups was increased by 112.8% compared to the CK groups, whereas it decreased by 9.5% in highly contaminated soil. The total uptake of Cd by plants was significantly improved with the inoculation of *P. lilacinum* N1. The total uptake of Cd by plants in SR groups was 3.6 times and 1.84 times higher than that in CK groups, in low contaminated and high contaminated soil, respectively (Table 2).

Table 2. The total uptake of Cd content in plants.

Groups	CK (μg)	SR (μg)
Low contaminated soil	0.181 ± 0.044	0.833 ± 0.093
High contaminated soil	1.664 ± 0.333	4.718 ± 0.920

Studies have shown that some fungi can promote plant growth and reduce Cd content in plants. For example, Zhang et al. reported that the application of *Mucor circinelloides* and *Trichoderma asperellum* could increase 13.49–39.53% shoot fresh weight and decrease 19.7–38.92% Cd content in *Arabidopsis* [18]. However, the decrease of Cd content in *Arabidopsis* was mainly due to the reduction of bioavailable Cd rather than total Cd content in soil by *Mucor circinelloides* and *Trichoderma asperellum*. Indeed, the reduction in plant Cd concentration can also be influenced by increased plant biomass and subsequent "dilution effects" [62]. In this study, *P. lilacinum* N1 greatly promoted the growth of plants (2–3 times). Additionally, it reduced the total Cd content in soils (2.09% in Low-SR and 12.56% in High-SR), which can greatly reduce the Cd concentration in plants. However,

the Cd concentration of plants in SR groups was increased by 112.8% in low contaminated soil and decreased only by 9.5% in highly contaminated soil, which cannot be explained by "dilution effects". These results showed that *P. lilacinum* N1 not only promoted plant growth, but also enhanced the uptake of Cd by plants.

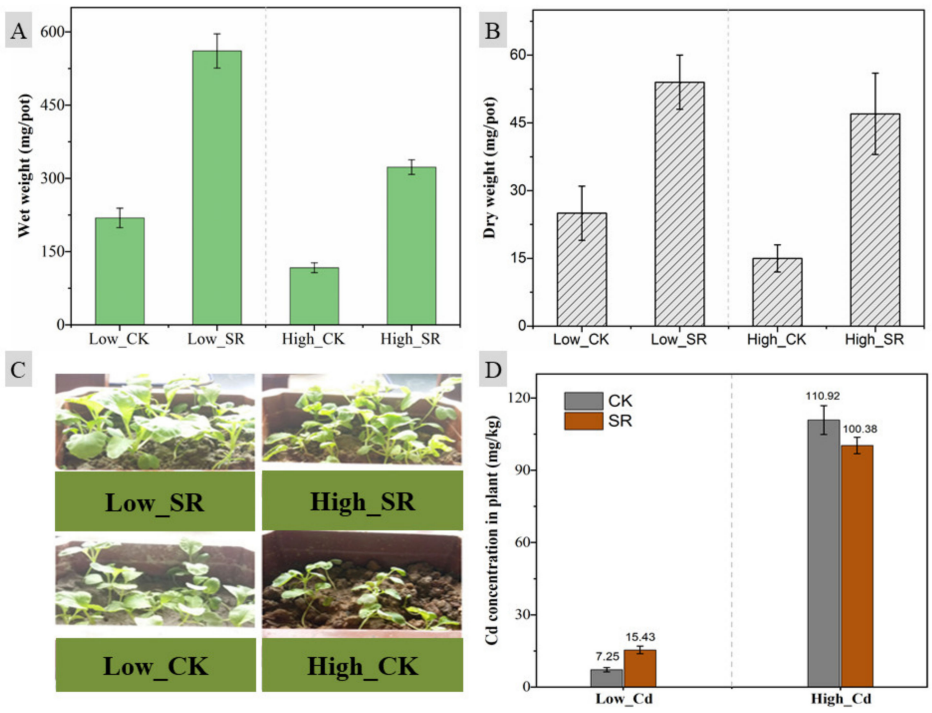

Figure 4. The effects of fungal inoculation on the growth of plant samples: (**A**) The wet weight and (**B**) the dry weight of all plant samples in a pot; (**C**) Plants in the pots before sampling; (**D**) The Cd concentration of the dry plants.

There are only a few studies on the bioremediation of heavy metal-contaminated soil by *P. lilacinum*, and the remediation efficiency is quite different. It was found that *P. lilacinum* could significantly promote plant biomass and reduce arsenic content in the plants [63]. Moreover, Gong et al. indicated that *Purpureocillium* sp. could protect the growth of plants under Cu stress in mangrove ecosystems and reduce the uptake of Cu, but the total Cu content in soil increased. *Purpureocillium* sp. increased the concentration of carbonate-bound Cu, Fe/Mn oxide-bound Cu, and organic-bound Cu in soil [64]. Furthermore, one study has shown that *P. lilacinum* enhanced 30~45% of plant biomass and improved 10~15% of Cd concentration in plants [65]. Another study also showed that the application of *P. lilacinum* has facilitation to biomass, but probably has no effects on Cd concentration in plants [66]. These conflicting results may be caused by the differences in metal characteristics and heavy metal accumulation ability of plants. Although the concentration of heavy metals in plants remained unchanged or even decreased, the total uptake may be greatly increased considering the biomass [66,67]. In this study, the total uptake of Cd by plants was greatly improved with the inoculation of *P. lilacinum* N1, 1.84~3.6 times higher than that in CK groups. These data indicated that *P. lilacinum* can be an outstanding candidate for Cd bioremediation because of its double effects on the total Cd content in soil and Cd uptake in plants.

It was noteworthy that the treatments, including SI and SS (soil sprayed with inoculum and supernatant), did not germinate or survive. The difference between them and the survival groups was the fermented culture medium. We hypothesized that some metabolites produced during fungal growth might inhibit seed germination. Many fungi have been reported to inhibit seed germination, such as certain strains of *Aspergillus*, *Cladosporium*, *Fusarium*, and *Macrophomina* [68]. However, contrary to previous studies, the cell-free su-

pernatant of *P. lilacinum* in PDA culture medium increased seed germination and promoted plant growth [69]. Thus, we speculated that some components of NB nutrient media or metabolites produced by *P. lilacinum* N1 in NB media might inhibit seed germination.

3.5. Implications for Cd Remediation

P. lilacinum was mainly known for its nematophagous capacity [70], and most studies were focused on its roles in biocontrol against etiological agents of the plant diseases, such as *Meloidogyne incognita* [63,71,72], *Sweet-potato whitefly* [73], *Penicillium digitatum* [74]. It also possesses multiple characteristics such as indoleacetic acid, siderophores, phosphate solubilization, and potash solubilization [53,75–77]. These functional traits can promote soil fertility and plant growth [78]. In addition, this study showed that *P. lilacinum* could increase the total uptake of Cd in plants and reduce total Cd content in the soil, indicating that this strain can be applied for the treatment of Cd-contaminated soil. Notably, the Cd content was 1.45 mg/kg in low contaminated soil and 20.18 mg/kg in highly contaminated soil, whereas the Cd concentration in plants was about 5~10 times higher than that in soil, which far exceeded the guidance limit of the China Food Safety National Standard (GB 2762-2017) of 0.2 mg/kg [79]. Therefore, crop variety and soil pollution should also be considered when applying microbial agents. To harvest high-quality vegetables while avoiding Cd harm to human health, low Cd-accumulating vegetables should be cultivated in low Cd-polluted soil as much as feasible, together with the Cd-reduction effect of microbial agents. In the highly polluted soil, microbial-assisted hyperaccumulators have more advantages than microbes or phytoextraction alone to remove Cd from soil gradually. It needs to be processed repeatedly for a long time before the effects can be seen. Further investigations need to analyze the complex interactions between microbes and hyperaccumulators towards the chemical and biological perspectives [80]. Hyperaccumulators were more valuable than vegetables (*Brassica Chinensis* L.) planted in this study, due to their high biomass and enrichment ability under high Cd stress. *P. lilacinum* isolated in this study further enriched the microbial resources of bioremediation for Cd-contaminated soil.

4. Conclusions

In this study, *P. lilacinum* N1 was isolated from a highly Cd-contaminated sediment sample, which showed hyper-tolerance to Cd. It was able to grow in the presence of Cd concentration up to 12,000 mg/L in NB medium. The maximum removal efficiency gradually decreased from 70.67% ± 4.47% to 9.05% ± 1.98% when Cd (II) concentration increased from 10 mg/L to 500 mg/L Cd. Compared to the CK groups, total Cd content in low contaminated and high contaminated soil with fungal inoculation was reduced by 2.09% and 12.56%, respectively. In the low contaminated soil, the acid-soluble Cd decreased by 6.0%, whereas the residual Cd increased by 17.73%. In high contaminated soil, four fractions of Cd decreased by 10.61~18.02%. *P. lilacinum* N1 demonstrated tremendous capacity to promote the growth of plants, with the wet weight and dry weight of plants 2.16~3.13 times that of the control groups. In addition, the Cd concentration in plants with fungal inoculation was increased by 112.8% in low contaminated soil, whereas it decreased by 9.5% in highly contaminated soil. The total uptake of Cd by plants with fungal inoculation was 1.84~3.6 times higher than that in control groups. To sum up, this study found that using a microbial-assisted plant technique to bioremediate Cd-contaminated soil is a promising method, and *P. lilacinum* isolated in this study further showed an enrichment in the microbial resources.

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