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Remediation mechanism of "double-resistant" bacteria—*Sedum alfredii* Hance on Pb- and Cd-contaminated soil

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Abstract

Background: Concentrations of heavy metals continue to increase in soil environments as a result of both anthropogenic activities and natural processes. Cadmium (Cd) and lead (Pb) is one of the most toxic heavy metals and pose health risks to both humans and the ecosystem. Therefore, effectively solving the problem of heavy metal pollution is the concern of soil workers. Among the existing remediation techniques, only the combined use of microorganisms and plants for remediation of heavy metal-contaminated soil is the greenest and most developed one. Consequently, based on this background, this study investigates the remediation mechanism of Pb and Cd heavy metals using the combined action of bacteria and *Sedum alfredii* Hance.

Methods: In order to enrich the research theory of combined plant and microorganism remediation of heavy metal-contaminated soil, we constructed a heavy metal composite pollution remediation system by combining Pb and Cd-tolerant bacteria with the Pb and Cd hyperaccumulator plant—*Sedum alfredii* Hance to investigate its combined remediation effect on Pb and Cd composite contaminated soil.

Results: The results showed that resistant bacteria were able to promote enrichment of Pb and Cd in *Sedum alfredii* Hance and J2 (200 ml of bacterial solution) was significantly ($P < 0.05$) more effective than J1 (100 ml of bacterial solution). The resistant bacteria were able to alleviate the toxic effects of Pb and Cd heavy metals on *Sedum alfredii* Hance and promote growth while reducing rhizosphere soil pH. The resistant bacteria were able to significantly reduce the effective state of Pb and Cd in the rhizosphere soil ($P < 0.05$), with the greatest reduction in the effective state of Pb in treatment A (Cd7Pb100 mg/kg), where J2 was reduced by 9.98% compared to J0, and the greatest reduction in the effective state of Cd in treatment C (Cd28Pb400 mg/kg), where J2 was 43.53% lower than J0. In addition, the resistant bacteria were able to increase the exchangeable state Cd content by 0.97 to 9.85%. The resistant bacteria had a weakly promoting effect and a highly inhibitory effect on the absorption of Pb by *Sedum alfredii* Hance.

Conclusions: The resistant bacteria can change the rhizosphere environment and significantly improve the remediation effect of *Sedum alfredii* Hance on heavy metal cadmium. The role of "double-resistant" bacteria in promoting the accumulation of Cd was greater than that of Pb.

Keywords: "Double-resistant" bacteria, *Sedum alfredii* Hance, Lead and cadmium contamination, Combined remediation

Introduction

Human activities are one of the main causes of heavy metal pollution in soil. The rapid growth of human demand for resources and the excessive waste of energy have led to the release of more and more heavy metals

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into the natural environment. Soil contamination by heavy metals has become increasingly serious, resulting in environmental problems that urgently need solution. Contaminated soil is difficult to repair quickly by self-purification, and heavy metals can enter the human body through the food chain and damage human health (Islam et al. 2018; Martínez-Sánchez et al. 2013; Damodaran et al. 2013). There is still no very effective remediation technology for this difficult problem. The common remediation means are chemical remediation, physical remediation and bioremediation, and the combined phyto-microbial remediation technology in bioremediation is one of the most researched remediation techniques. Soil microorganisms, the most soil active components, play a crucial role in the remediation of soils contaminated by heavy metals. Microorganisms affect the absorption of heavy metals by plants by changing the morphology of heavy metals and the bioavailability of heavy-metal ions through extracellular immobilization, intracellular polymerization, and redox processes (Njagi et al. 2011; Pablo et al. 2018). Sheng et al. (2008) noted that some endophytic bacteria can activate heavy metals and improve their bioavailability by producing organic acids, iron carriers, and other substances. Luo et al. (2012) found that the endophyte *Bacillus* SLS18 can enhance the heavy-metal carrying capacity of sorghum. Also, Salt et al. (1999) discovered that the Cd content of *Brassica juncea* roots greatly increases after inoculation with rhizosphere bacteria. Not only that, Rajkumar and Freitas (2008) demonstrated that mycorrhizal fungi and growth-promoting bacteria can promote plant growth, while enhancing plant stress resistance and improving remediation of heavy metals. For the last few decades, there has been a growing consensus that environmentally sustainable and economically viable soil remediation methods should be developed. The use of plants to remediate soil heavy metal pollution is a hot research topic today and is currently the greenest option (Marques et al. 2009; Singh and Prasad 2015). However, plants that hyperaccumulate heavy metals tend to become stunted and present slow growth and remediation, low biomass, and difficult artificial cultivation (Reeves et al. 2018; Mehrdad et al. 2008). Furthermore, current phytoremediation technologies require weeks to years to exhibit signs of remediation. Considering the current global waste crisis, the remediation of soils with heavy-metal contamination by using a single technology is obviously impractical. Therefore, combining phytoremediation with other remediation technologies is the best choice to accelerate soil remediation.

The microorganisms that are commonly used to remediate heavy-metal-contaminated soil mainly include bacteria, yeast, and fungi (Pabst et al. 2010). Bacteria are considered as one of the most important bioremediation agents for heavy metals because they can adsorb heavy-metal ions on the surfaces of cell walls for detoxification purposes (Naik et al. 2012; Feng et al. 2012). *Bacillus* species not only adsorb soil heavy-metal ions, they also have good environmental compatibility, which is of great value in application (Oves et al. 2013; Long et al. 2017). *Sedum alfredii* Hance is a Zn/Cd/Pb hyperaccumulator plant in China that can grow normally in soils contaminated with high concentrations of heavy metals (Ye 2003; Yang et al. 2004). It exhibits a perennial growth habit, asexual reproduction, and large biomass. Moreover, it is suitable for mowing. At the same time, it has great potential for application in the remediation of heavy-metal-contaminated soil (Yang et al. 2002). Xiong et al. (2011) suggested that *Sedum alfredii* Hance has high patience and enrichment capacity for Pb and Cd. Li et al. (2011a, b) studied the remediation effects of two ecotypes of *Sedum alfredii* Hance on soils contaminated with Cd–BaP compounds and found that the remediation effect of the hyperaccumulator ecotype of *Sedum alfredii* Hance is better than that of the non-hyperaccumulator ecotype. The remediation of contaminated soil by microorganisms and plants can improve the soil environment and enhance the absorption capacity of plants for heavy metals. Moreover, microorganisms can change the combined forms of heavy metals and soil by producing metabolites that dissolve, complex, and redox heavy metals and heavy-metal-containing minerals (Francis and Dodge 1988; Kalinowski et al. 2000). Guo et al. (2011) revealed that inoculating the D54 strain, which can dissolve inorganic phosphorus and mineral metal elements, into heavy-metal-contaminated soil planted with *Sedum alfredii* Hance can improve the biomass and heavy-metal absorption of *Sedum alfredii* Hance. Zhang et al. (2018) showed that soil inoculation with *Mucor* or *Trichoderma* can increase the Pb and Cd content in *Arabidopsis thaliana*. Specifically, they found that the concentrations of Pb and Cd in *A. thaliana* have increased by 41.46% and 19.7%, respectively, after inoculation with *Mucor*, and by 20.33% and 38.92%, respectively, after inoculation with *Trichoderma*. Li et al. (2018) also found that inoculation of Cd-resistant *Enterobacter* strain into ore soil not only boosts the growth of *Centella asiatica* in mining areas, but also reduces the toxicity of Cd to plants. *Bacillus cereus* can increase the enrichment efficiency of heavy metals in plants by producing indole acetic acid and dissolving P (Dawwam et al. 2013). Nayak et al. (2018) demonstrated that under promotion by *B. cereus*, the removal rates of Zn and Cd in soil can reach 36% and 31%, respectively. This proves that *Bacillus* is capable of promoting the uptake and accumulation of heavy metals by plants. However, studies on the combined microbial and plant remediation of soils contaminated with the heavy metals Pb and Cd are rare, making information on the phytoremediation of Pb and Cd composite contaminated soil by *Bacillus* highly valuable. To this end, we used one of the main types of soil (laterite) in southern China as the substrate, and selected Pb and Cd-resistant strains combined with *Sedum alfredii* Hance to construct a combined enhanced microbe–plant system for the remediation of Pb- and Cd-contaminated soil. This work aimed to explore the remediation mechanism and effect of this system and to explore a new technical approach for the remediation of soil with heavy-metal contamination.

Materials and methods

Test soils

Quaternary red soil free from Pb and Cd pollution was collected from the farm of the College of Agriculture, Guangxi University. The basic physicochemical properties of the tested soil are shown in Table 1.

Test strain and plants

The test strain was the Pb and Cd-resistant bacterium E-2, which was identified as *Bacillus* via 16S rDNA sequence determination. The 16S rDNA sequence of this strain showed 99% consistency with that of *Bacillus niacini*. This bacterium was obtained through preliminary screening and domestication by our research team. The tested plants were the mine ecotype of *Sedum alfredii* Hance and were taken from an ancient Pb and Zn mining area in Zhejiang Province.

Methodology

After collection, the soil sample was air-dried, hammered, sieved through a five-mesh sieve, mixed evenly, and then placed into a basin. Analytically pure $Pb(NO_3)_2$ and $3CdSO_4{\cdot}8H_2O$ were dissolved in deionized water and added to the soil uniformly in the form of a spray. Each pot was filled with 4 kg of soil. Soil moisture content was maintained at 60% field capacity over 3 months of aging. Moisture is determined by weighing during the aging process, and moisture is added from time to time to ensure a stable moisture content. Soil pH was measured after aging. $CaCO_3$ was selected for adjustment. The amount of alkali needed to adjust the soil pH to the final value was calculated in accordance with the soil acid–base curve. Before adjustment, the soil was filtered through an 18-mesh sieve and then fully mixed with $CaCO_3$. Soil moisture content was maintained at 60% field capacity over 2 months of aging. The indexes in Table 1 were determined after aging.

The experiment on the remediation mechanism of the bi-resistant bacteria inoculated at different supplemental levels into soils with composite pollution and different Pb and Cd concentrations was a two-factor completely randomized block test. Factor A was the amount of bacteria added (bacterial suspension 10^7 cfc/ml). Three levels were set, namely, no bacterial suspension (J0), 100 ml bacterial suspension (J1), and 200 ml bacterial suspension (J2). Factor B was the concentration of Pb and Cd pollution. Six levels were set, namely, Cd0Pb0 (CK), Cd7Pb100 (A), Cd14Pb200 (B), Cd28Pb400 (C), Cd49Pb700 (D), and Cd77Pb1100 (E) in units of mg/kg, with three replicates for each treatment. Aged soil was passed through a sieve with a 5-mm aperture, and each pot was filled with 4 kg of soil. Urea and potassium dihydrogen phosphate were used as the base fertilizer and applied at the rates of 0.2 and 0.4 g per kg of soil, respectively. Five to six young plants with the same growth status were selected and transplanted into potted soil. Unified management was carried out in the later stage of growth. Taking the second day of planting as the first day, the available concentrations of Pb and Cd in soil were measured on the 1st, 8th, 22nd, 43rd and 60th days. The biomass of *Sedum alfredii* Hance was measured at harvest time at the end of the experiment on day 60. At the same time, fresh rhizosphere soil was collected, and various indexes, such as rhizosphere pH and available Pb and Cd, were measured.

Preparation of bacterial suspension: After activation, resistant bacteria were added to sterilized beef extract

Table 1 The basic physical and chemical properties of the tested soil

Treatment	pH	OM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	A-Pb (mg/kg)	A-Cd (mg/kg)
Cd0Pb0	5.80	10.04	101.76	38.30	42.60	0.001	0.002
Cd7Pb100	5.64	10.08	101.81	38.20	42.30	75.00	5.10
Cd14Pb200	5.63	10.06	101.80	36.80	42.80	148.00	10.20
Cd28Pb400	5.63	10.10	101.77	37.60	42.50	307.00	18.10
Cd49Pb700	5.61	10.12	101.79	38.70	43.10	648.00	33.40
Cd77Pb1100	5.64	10.09	101.82	39.10	42.70	964.00	49.70

Cd0Pb0 denotes 0 mg/kg Cd and 0 mg/kg Pb. *OM* organic matter, *AN* available nitrogen, *AP* available phosphorus, *AK* available potassium, *A-Pb* available lead, *A-Cd* available cadmium peptone liquid medium and continuously cultured with shaking at 28 ℃ for 24 h. The liquid culture medium was centrifuged for 25 min and washed with sterile water 2–3 times after the supernatant was drained. Then the bacterial suspension was prepared using the sterilized liquid medium, and the amount of bacteria was determined using the cell counting method and then diluted it proportionally to obtain the target bacterial solution (107 cfu/mL).

Sample collection and determination

Collection and determination of Sedum alfredii Hance rhizosphere soil

The potted soil was poured out, and the plant roots were shaken until free of soil. The soil was scraped and collected by using a spoon. *Sedum alfredii* Hance shoots were collected. The available Pb and Cd content in rhizosphere soil was determined through the DTPA extraction method and atomic absorption spectrometry. The speciation of Pb and Cd in rhizosphere soil was determined by using the Tessier five-step extraction method and atomic absorption spectrometry.

Biomass determination of Sedum alfredii Hance

The shoots of *Sedum alfredii* Hance were collected and washed clean with tap water and deionized water. Moisture on the surfaces of the plants was removed with absorbent paper. The fresh weights of the plants were taken. After incubation at 105 ℃ for 30 min, the plants were dried at 75 ℃, weighed to obtain their dry weights, and pulverized for preservation. After the removal of rhizosphere soil, the roots of *Sedum alfredii* Hance were cleaned and dried at 75 ℃ after 30 min of clearance at 105 ℃. The samples were weighed to obtain their dry weights and then crushed for preservation.

Determination of Pb and Cd content in Sedum alfredii Hance

A 0.1000 g plant sample was passed through a 0.5-mm screen and subjected to HNO_3–H_2O_2 digestion and atomic absorption spectrometry.

Data processing and analysis

Data processing, analysis, and chart construction were performed by using Excel, SPSS, Origin, and other software. SPSS program was used for ANOVA, and the Duncan method was used for multiple comparisons. For the ANOVA results obtained through SPSS, different lowercase letters indicated significant differences ($P < 0.05$) between treatments, whereas the same lowercase letters indicated no significant difference ($P > 0.05$).

Results

Rhizosphere soil pH and effective state Pb and Cd content

From the results in Table 2, it can be seen that the rhizosphere soil pH of *Sedum alfredii* Hance ranged from 5.54 to 5.75, and there was a significant effect on the rhizosphere soil pH of *Sedum alfredii* Hance with resistant bacteria ($P < 0.05$). In the presence of Pb and Cd contamination, the rhizosphere soil pH was reduced under the addition of bacteria, and the more bacteria, the more acidic the rhizosphere soil. In the experimental group, the differences between treatments were not significant ($P > 0.05$), except for EJ2 (Cd77Pb1100 mg/kg, 200 ml bacterial suspension) treatment of *Sedum alfredii* Hance where rhizosphere soil pH was significantly lower than EJ0 (Cd77Pb1100 mg/kg, 0 ml bacterial suspension) treatment ($P < 0.05$). Among the Pb and Cd contamination treatments, the pH value of rhizosphere soil decreased the most under B (Cd14Pb200 mg/kg, 200 ml of bacterial suspension) and C (Cd28Pb400 mg/kg, 200 ml of bacterial suspension) treatments.

Table 2 Effective state Pb and Cd concentrations and pH in rhizosphere soil of *Sedum alfredii* Hance after 60 days (at harvest) of treatment with different Pb and Cd concentrations

Treatment		pH	A-Pb-R (mg/kg)	A-Cd-R (mg/kg)
CK	J0	5.75 ± 0.01a	0.011 ± 0.02a	0.01 ± 0.01a
	J1	5.71 ± 0.02b	0.009 ± 0.01a	0.02 ± 0.03a
	J2	5.66 ± 0.01c	0.010 ± 0.03a	0.01 ± 0.02a
A	J0	5.62 ± 0.03a	49.290 ± 1.73a	3.78 ± 0.01a
	J1	5.60 ± 0.05a	50.400 ± 3.60a	2.84 ± 0.04b
	J2	5.58 ± 0.01a	43.000 ± 2.05b	2.76 ± 0.02c
B	J0	5.60 ± 0.03a	109.150 ± 2.64a	6.68 ± 0.07a
	J1	5.56 ± 0.03a	108.550 ± 0.99a	5.46 ± 0.03b
	J2	5.55 ± 0.03a	103.600 ± 0.44b	5.41 ± 0.04b
C	J0	5.59 ± 0.03a	196.520 ± 0.01a	11.46 ± 0.06a
	J1	5.55 ± 0.06a	192.450 ± 1.84a	8.43 ± 0.26b
	J2	5.54 ± 0.02a	192.600 ± 4.37a	8.11 ± 0.87b
D	J0	5.60 ± 0.08a	208.600 ± 2.05a	24.09 ± 2.66a
	J1	5.57 ± 0.08a	210.650 ± 4.32a	21.62 ± 1.01a
	J2	5.56 ± 0.06a	212.350 ± 4.58a	20.13 ± 2.01a
E	J0	5.62 ± 0.01a	255.700 ± 8.72a	38.89 ± 0.81a
	J1	5.59 ± 0.03ab	256.800 ± 0.90a	33.5 ± 2.65b
	J2	5.58 ± 0.02b	258.050 ± 3.01a	30.64 ± 0.01b

A-Pb-R available Pb concentration in rhizosphere soil, *A-Cd-R* available Cd concentration in rhizosphere soil. The results showed that rhizosphere soil pH and available Pb and Cd contents under different treatments at harvest. The numbers after different letters in the same column of each treatment were significantly different ($P < 0.05$)

The experimental results showed (Table 2) that the effective state Pb content of rhizosphere soil of *Sedum alfredii* Hance plants ranged from 43 mg/kg to 258.05 mg/kg. From the results, it is clear that at low Pb and Cd concentrations (i.e., treatments A, B, and C), addition of bacteria was able to reduce the effective rhizosphere Pb content; whereas, in high Pb and Cd concentration treatments (D and E), the presence of resistant bacteria inhibited the uptake of Pb by the plants, and it is clear from the results of D and E that the effective rhizosphere Pb content did not increase significantly after a gradient of Pb and Cd concentration at high Pb and Cd concentrations. The variation of Cd was different from that of Pb. The effective Cd content that was found in the rhizosphere soil of *Sedum alfredii* Hance was markedly reduced after the addition of bacteria, and the differences between treatments reached significant levels except for treatment D ($P < 0.05$). The experimental results showed that the maximum reduction of effective state Cd in the rhizosphere soil was under treatment C, where J2 was reduced by 43.53% compared to J0; however, the maximum reduction was in treatment E, where J2 was reduced by 8.25 mg/kg compared with J0.

Percentage of various forms of Pb and Cd in rhizosphere soil

The influence of different treatments on the percentages of Pb species in rhizosphere soil is shown in Fig. 1. The results showed that the other four forms of Pb, except for organically bound Pb, were present at large proportions in rhizosphere soil. Exchangeable, carbonate-bound, and Fe–Mn oxide-bound Pb accounted for large proportions of Pb content under each treatment. The percentage of exchangeable Pb ranged from 30.76 to 47.13% and that of carbonate-bound Pb ranged from 16.76 to 26.81%. The percentages of Fe–Mn oxide-bound, organic-bound, and residual Pb fell were in the ranges of 15.07–22.4%, 1.02–8.38%, and 10.91–21.82%, respectively. Under the same conditions of Pb and Cd contamination, the exchangeable Pb content of the rhizosphere soil without bacterial treatment was higher than that of the rhizosphere soil with bacterial treatment, and the exchangeable Pb content of the rhizosphere soil under J2 (200 ml of bacterial suspension) was lower than that under J1 (100 ml of bacterial suspension). Also, as exchangeable state content decreased, residual state content increased. The presence or absence of bacteria had a negligible effect on the contents of carbonate-bound, Fe–Mn oxide-bound, and

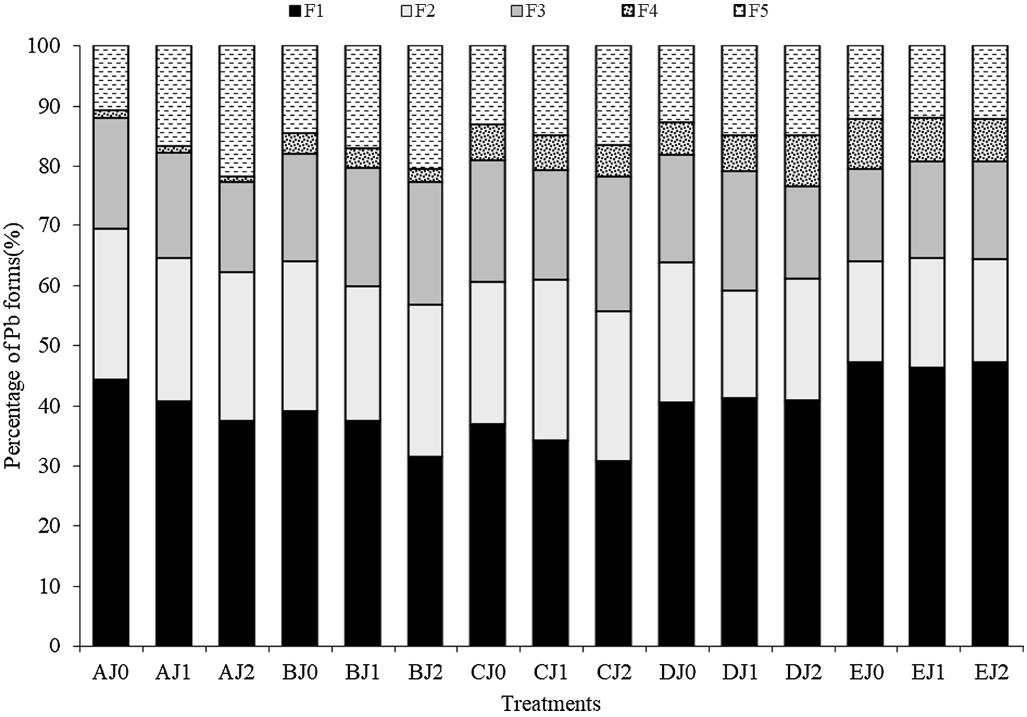

Fig. 1 Proportion of each form of Pb in rhizosphere soil after 60 days of planting in *Sedum alfredii* Hance. This figure represents the distribution of each form of Pb in the rhizosphere soil of *Sedum alfredii* Hance at harvest. AJ0 represents Cd7Pb 100 mg/kg, 0 mL of bacterial suspension; AJ1 represents Cd7Pb 100 mg/kg, 100 mL of bacterial suspension; AJ2 represents Cd7Pb 100 mg/kg, 200 mL of bacterial suspension. The rest is the same. F1, F2, F3, F4, F5 are different forms of Pb, exchangeable, bound to carbonates, bound to Fe–Mn Oxides, bound to organic matter, residual

organic-bound Pb under the same Pb and Cd contamination treatments. In addition, the organic bound state Pb content increased overall with increasing Pb and Cd concentrations.

The percentages of Cd forms in rhizosphere soil are shown in Fig. 2. Among the five forms, the exchangeable form accounted for the largest proportion, followed by the carbonate-bound form. The highest percentage of Cd was in the exchangeable state in all treatments, which was above 50% and increased with the increasing concentration of Pb and Cd treatments. The carbonate bound state was in the range of 14 to 40%; the Fe–Mn oxide-bound state was in the ranged of 7 to 18%; and the remaining two forms accounted for less than 1%. The test results showed that the addition of bacteria increased the exchangeable Cd content from 0.97 to 9.85%; and reduced the Fe–Mn oxide-bound Cd content from 2.32 to 34.91% than without the addition of bacteria. Comparing from the same treatment, the exchangeable state content of Cd in the rhizosphere soil with the addition of bacteria was higher than that without the addition of bacteria, and it increased with the increase of the amount of addition. Meanwhile, the proportion of Fe–Mn oxide-bound state of rhizosphere soil Cd was correspondingly reduced. For the organic bound state and residue state, the effect of adding bacteria and not adding bacteria on their contents was not significant. Under the same Pb and Cd contamination conditions, the effect of adding bacteria and not adding bacteria on the content of carbonate bound state was also not significant, and the most affected state was Fe–MnO bound state.

Effects of "double-resistant" bacteria on the biomass of *Sedum alfredii* Hance

The effects of different Pb and Cd concentrations on the biomass of *Sedum alfredii* Hance are shown in Table 3. The test results showed that the growth of *Sedum alfredii* Hance was significantly affected by the resistant bacteria ($P < 0.05$). Under the effect of resistant bacteria, the fresh weight of the aboveground parts increased by 27.21% to 31.48%, the dry weight of shoots increased by 16.99% to 28.58%, and the dry weight of the underground parts increased by 16.98% to 43.40%. However, the aboveground fresh weight of *Sedum alfredii* Hance weas all lower in the Pb and Cd contamination treatment than CK. The aboveground fresh weight of *Sedum alfredii* Hance was the highest under B treatment and the highest fresh weight was 179.68 g/pot under J2 condition.

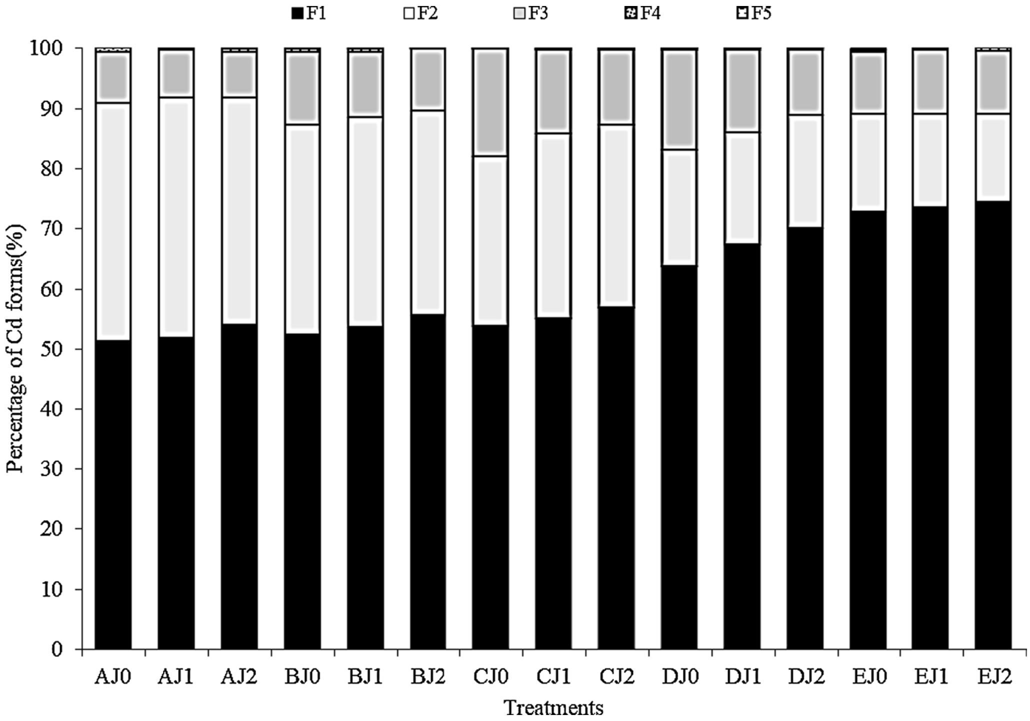

Fig. 2 Proportion of each form of Cd in rhizosphere soil after 60 days of planting in *Sedum alfredii* Hance. This figure represents the distribution of each form of Cd in the rhizosphere soil of *Sedum alfredii* Hance at harvest. AJ0 represents Cd7Pb 100 mg/kg, 0 mL of bacterial suspension; AJ1 represents Cd7Pb 100 mg/kg, 100 mL of bacterial suspension; AJ2 represents Cd7Pb 100 mg/kg, 200 mL of bacterial suspension. The rest is the same. F1, F2, F3, F4, F5 are different forms of Pb, exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter, residual

Table 3 Changes in biomass of various parts of *Sedum alfredii* Hance under different Pb and Cd concentration treatments

Treatment		S-F-W (g/pot)	S-D-W (g/pot)	U-D-W (g/pot)	T-D-W (g/pot)
CK	J0	134.58 ± 2.81b	10.30 ± 0.79b	0.43 ± 0.03b	10.73b
	J1	170.11 ± 2.18a	13.22 ± 1.69a	0.62 ± 0.04a	13.84a
	J2	173.15 ± 1.46a	14.53 ± 1.64a	0.76 ± 0.04a	15.29a
A	J0	139.53 ± 1.63b	11.64 ± 0.64b	0.40 ± 0.06b	12.04b
	J1	174.94 ± 2.13a	14.12 ± 0.98a	0.68 ± 0.08a	14.80a
	J2	176.61 ± 1.84a	14.47 ± 0.36a	0.75 ± 0.02a	15.22a
B	J0	140.98 ± 1.64b	11.11 ± 0.14b	0.46 ± 0.08b	11.57b
	J1	176.58 ± 1.32a	13.74 ± 0.70a	0.82 ± 0.07a	14.56a
	J2	179.68 ± 1.62a	13.96 ± 0.21a	0.87 ± 0.09a	14.83a
C	J0	136.11 ± 2.45c	13.47 ± 2.01b	0.69 ± 0.02b	14.16b
	J1	167.47 ± 1.16b	22.32 ± 0.34a	0.84 ± 0.06a	23.16a
	J2	170.66 ± 1.98a	23.44 ± 0.81a	0.92 ± 0.06a	24.36a
D	J0	132.92 ± 1.46c	12.63 ± 0.32b	0.65 ± 0.05b	13.28b
	J1	163.59 ± 2.35b	13.84 ± 0.74b	0.88 ± 0.06a	14.72b
	J2	167.80 ± 2.17a	19.78 ± 1.36a	0.96 ± 0.04a	20.74a
E	J0	129.49 ± 1.61c	11.11 ± 0.11c	0.63 ± 0.08b	11.74c
	J1	143.05 ± 2.24b	13.47 ± 0.73b	0.69 ± 0.08b	14.16b
	J2	147.54 ± 2.02a	19.12 ± 1.35a	0.86 ± 0.07a	19.98a

The data in Table 3 represent the biomass of each part of *Sedum alfredii* Hance at different Pb and Cd concentration treatments. Each abbreviation in the table indicates: *S-F-W* shoot fresh weight, *S-D-W* shoot dry weight, *U-D-W* underground dry weight, *T-D-W* total dry weight. The numbers after different letters in the same column of each treatment were significantly different ($P < 0.05$)

However, the aboveground dry weight of *Sedum alfredii* Hance plants was the highest under CJ1 and CJ2 conditions. In addition, the effect of resistant bacteria on the dry weight of aboveground and underground parts of *Sedum alfredii* Hance was significant with or without Pb and Cd contamination ($P < 0.05$), and the dry weight of *Sedum alfredii* Hance under the added bacteria condition was higher than those without the added bacteria.

Changes in the effective state of Pb and Cd in soils

The changes in different treatments on the available concentrations of Pb and Cd in the soil are shown in Fig. 3. The results showed that the available concentrations of Pb and Cd in the soil decreased with time. Under treatments A, B, and C, the addition of bacteria could decrease the available concentrations of Pb in the soil (Fig. 3A). And the addition of 200 ml of bacterial liquid resulted in a considerable reduction than that 100 ml of bacterial liquid. The treatments D and E were the opposite. The difference is that the available concentrations of Cd under the addition of bacteria reduced faster (Fig. 3B). The total decrease in available Cd concentrations in soil increased with the increase of the pollution concentration of Pb and Cd, while Pb only has this trend under the first three treatments (i.e., A, B, and C). Figure 3A and C revealed that the trend of the available concentrations of Pb and Cd was the same in the soil. The results also showed that the reduction of Pb available concentrations in soil was 2.54–24.75 mg/kg, and the available concentration of Cd was 0.99–8.52 mg/kg.

Pb and Cd levels in *Sedum alfredii* Hance plants

The effects of different treatments on the concentrations of Pb and Cd in the above- and belowground parts of *Sedum alfredii* Hance are shown in Fig. 4. At lower Pb and Cd contamination concentrations (A, B, and C), resistant bacteria could promote the accumulation of Pb in the above- and belowground parts of *Sedum alfredii* Hance and increased with the addition of bacteria (Fig. 4A, C). D and E treatments showed the opposite results. At lower Pb and Cd concentrations, Pb concentration in the shoot of *Sedum alfredii* Hance increased by 0.16 to 5.36 mg/kg in the presence of bacteria, and significantly decreased them by 27.34 to 122.42 mg/kg at higher Pb and Cd concentrations ($P < 0.05$). As with the aboveground Pb concentration, there was a "low promotion and high inhibition" phenomenon of Pb uptake by resistant bacteria in *Sedum alfredii* Hance (Fig. 4C). At low Pb and Cd contamination concentrations, the root Pb concentrations increased by 0.56 to 1.23 mg/kg, 9.40 to 9.56 mg/kg, and 15.68 to 16.82 mg/kg, respectively, while at high Pb and Cd contamination in D and E, the root Pb concentrations decreased by 40.04 to 51.39 mg/kg, 4.42 to 9.96 mg/kg, respectively, after bacterial addition. Between treatments, the highest reduction was between J2 and J0 (30.14%) in D treatment ($P < 0.05$).

The addition of resistant bacteria could promote the uptake of Cd in *Sedum alfredii* Hance and increase the Cd content in the above- and belowground parts of the plant (Fig. 4B, D). When 100 ml of bacterial solution was added, the Cd concentration in the aboveground part of *Sedum alfredii* Hance was increased from 3.41 to 19.12%; when 200 ml of bacterial solution was added, it was increased by 5.51 to 21.92%. The Cd concentration in the roots of *Sedum alfredii* Hance increased by 0.49 to 4.8 mg/kg with the addition of bacteria. The largest increase was between AJ2 and AJ0, and AJ2 increased by 23% compared with AJ0. Compared with no addition, the root Cd concentration of *Sedum alfredii* Hance increased from 0.49 to 3.2 mg/kg with 100 ml of bacterial solution, and from 1.17 to 4.80 mg/kg with 200 ml of bacterial solution. Under the same Pb and Cd pollution conditions, the root Cd concentration of *Sedum alfredii* Hance increased with the addition of more bacteria, but the difference was not significant ($P > 0.05$).

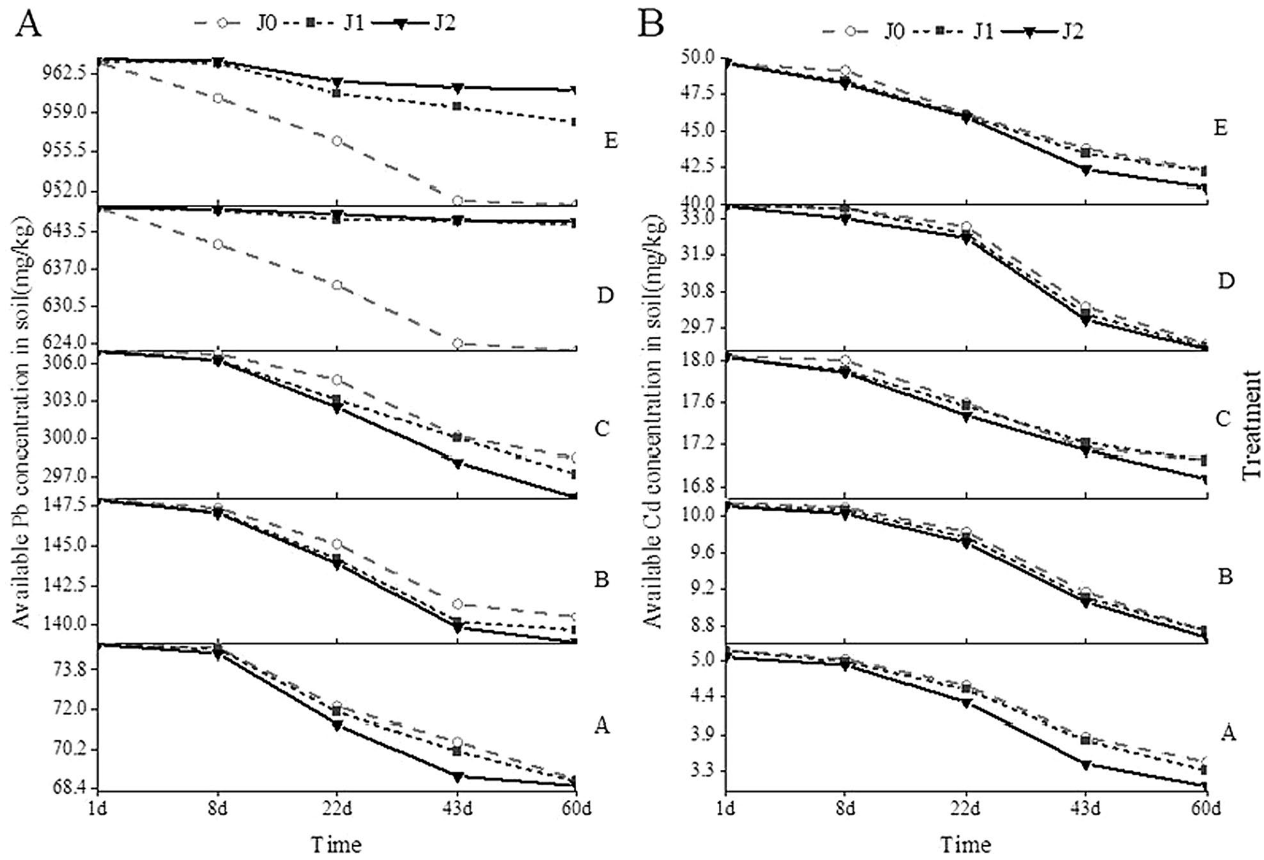

Fig. 3 Processes of soil effective state Pb and Cd content from day 1 to day 60 under different Pb and Cd concentration treatments. This figure shows the variation of Pb and Cd concentration in soil from day 1 to 60. Left graph (**A**) is the change process of soil available Pb content with time in each treatment; right graph (**B**) is the change process of soil available Cd content with time in each treatment. J0 represent no bacterial suspension, J1 means adding 100 ml bacterial suspension and J2 means adding 200 ml bacterial suspension

Enrichment factors and transport coefficients of Pb and Cd in *Sedum alfredii* Hance

The root enrichment factor is the ratio of the content of a certain substance in plant roots to that in rhizosphere soil, which can reflect the strength of the root system's ability to enrich this substance. The effects of different treatments on the enrichment factor and transport coefficient of *Sedum alfredii* Hance are shown in Fig. 5. As noted from Fig. 5A, it can be seen that resistant bacteria can improve the enrichment of Pb in *Sedum alfredii* Hance under the lower Pb and Cd concentration pollution conditions. Under Pb and Cd contamination conditions, resistant bacteria had a significant effect on Pb enrichment in *Sedum alfredii* Hance roots only in treatment B ($P < 0.05$). Similarly, the resistant bacteria were able to significantly enhance the enrichment of Cd in the root system of *Sedum alfredii* Hance (Fig. 5B) ($P < 0.05$). On the whole, the Cd enrichment factor of *Sedum alfredii* Hance roots ranged between 1.02 and 2.29 under Pb and Cd contamination treatment, and resistant bacteria could increase the Cd enrichment factor of *Sedum alfredii* Hance roots by 0.28 to 0.93. The results also showed that under all treatments, the Cd enrichment factor shown by the roots of *Sedum alfredii* Hance treated with 200 ml of bacterial liquid was significantly higher than shown by the roots without bacterial treatment ($P < 0.05$).

The transport coefficient of plants refers to the ratio of the concentration of a certain substance in the shoot of a plant to the concentration of this substance in the root. It can directly indicate the capability of plant roots to transport a substance to aboveground components. The experimental results showed that the transport capacity of Pb by the root system of *Sedum alfredii* Hance was increased by the resistant bacteria at low Pb and Cd treatment and decreased at high Pb and Cd concentration treatment (Fig. 5C). Compared with J0, J1 treatment increased the Pb transport coefficient of *Sedum alfredii* Hance by – 0.41 to 0.25; J2 increased it by – 0.53 to 0.09. Cd transport was slightly different from Pb, and *Sedum alfredii* Hance roots showed a low and then high

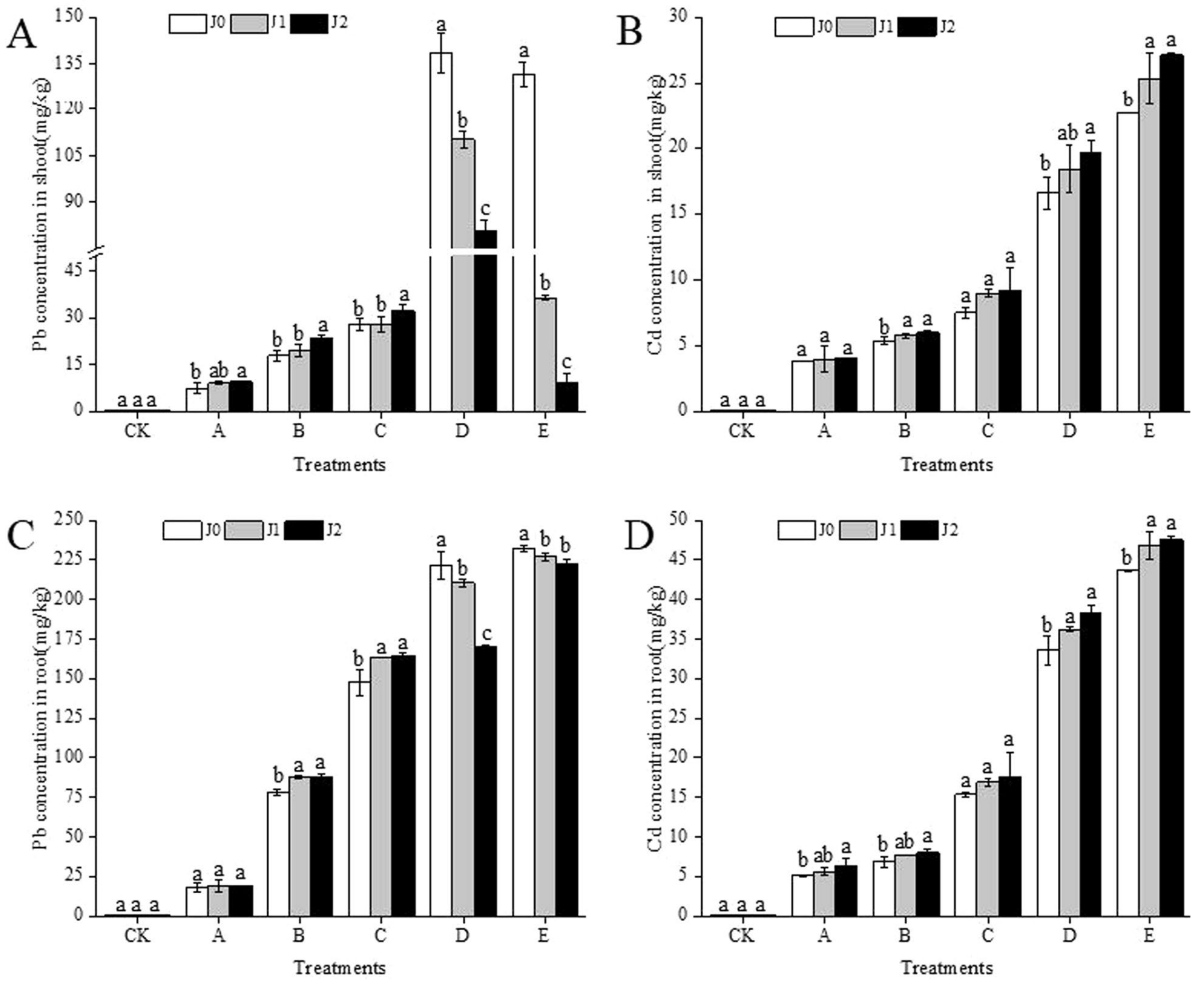

Fig. 4 Uptake of Pb and Cd by *Sedum alfredii* Hance under different Pb and Cd concentration treatments. This figure represents the changes of Pb and Cd contents in aboveground and underground parts of *Sedum alfredii* Hance at different Pb and Cd concentration treatments. **A** shows the Pb content in the shoot of plants under different treatments, **C** corresponds to underground; **B** shows the Cd content in the shoot of plants under different treatments, **D** corresponds to underground. Different letters indicate a significant difference at $P < 0.05$

transport of Cd (Fig. 5D). The effect of resistant bacteria on Cd transport did not differ significantly under the Pb and Cd contamination treatment, with none reaching a significant level ($P > 0.05$). The Cd transfer coefficient was largely above 0.5, with little overall variation.

Discussion

All microscopic organisms in the soil that are invisible to the naked eye or cannot be clearly seen are generally referred to as soil microorganisms. Soil microorganisms, the dominant subsurface life form, can produce metabolites, such as plant hormones (Meena et al. 2017), siderophores (Chen et al. 2019), organic acids (Kateryna et al. 2018), and enzymes (Gilmore et al. 2015), that promote plant growth and enhance heavy-metal enrichment. In recent years, the interaction between the rhizosphere and microbial systems of hyperaccumulative plants has become a new area in the research on improving the bioremediation capacity of contaminated soil and has gained attention worldwide. Previous studies on the bioremediation of heavy-metal pollution in soil found that some *Bacillus* species can reduce soil pH and Cd bioavailability and thus can be combined with plants for the amelioration of heavy-metal-contaminated soil via extraction. In addition, the roots of hyperaccumulative plants can secrete organic acids. The accumulation of these acids in the rhizosphere environment results in the formation of a local highly acidic environment that can

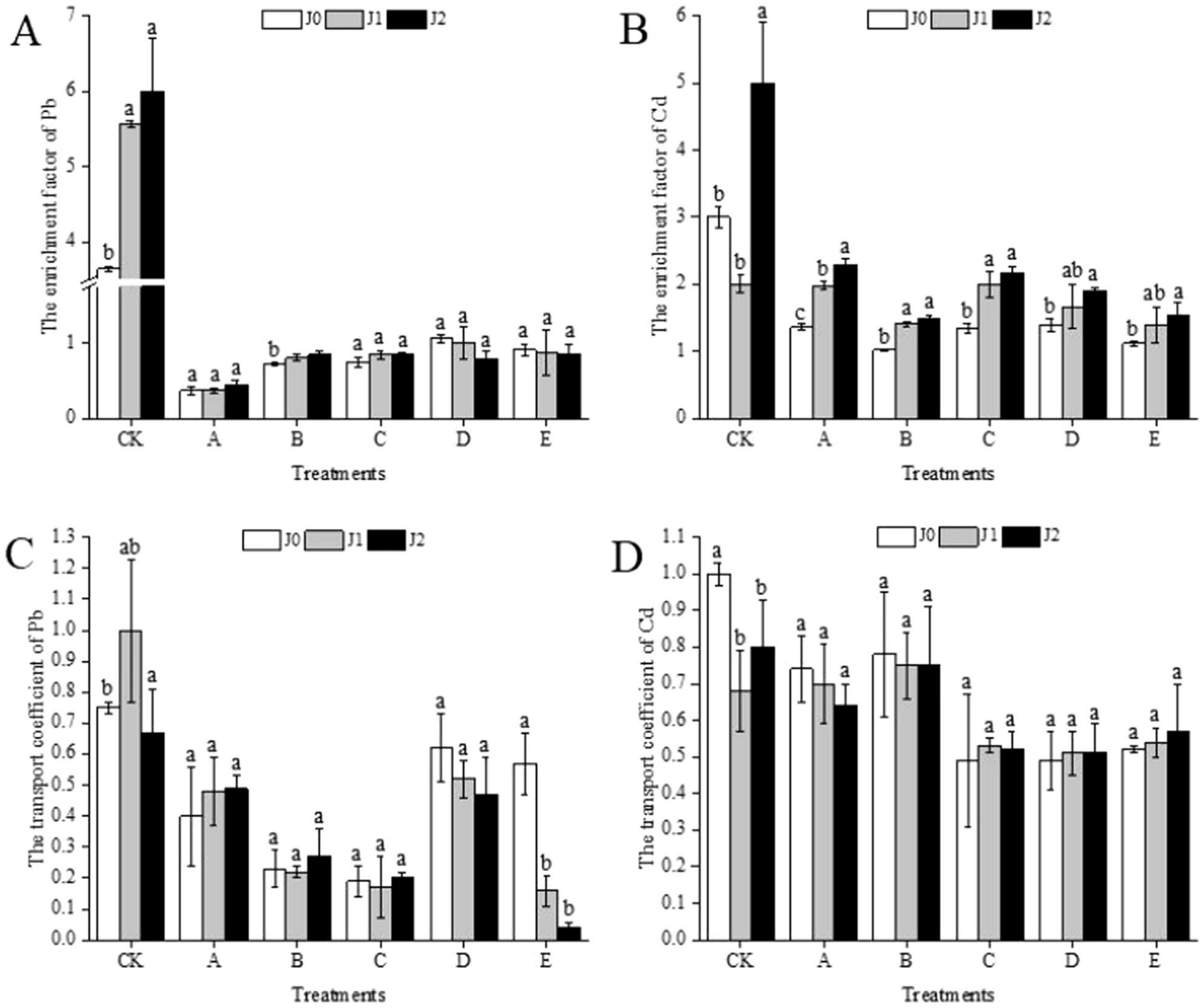

Fig. 5 Effects of different Pb and Cd concentration treatments on root enrichment factors and plant transport coefficients of *Sedum alfredii* Hance. The Pb and Cd enrichment factor is determined by the ratio of Pb and Cd content in the root system to Pb and Cd content in the rhizosphere soil. Pb and Cd transport coefficient is obtained from the ratio of Pb and Cd concentration aboveground and Pb and Cd content in the root system. EF: enrichment factor; TC: transport coefficient. **A** shows the Pb enrichment of plants under different treatment conditions, and **B** shows the enrichment of Cd; **C** shows the transport of Pb to plants under different treatment conditions, and **D** shows the transport of Cd. Different letters indicate a significant difference at $P < 0.05$

transform Cd in the soil from an inert bound state into an active state (Lin et al. 2018). For example, Yang et al. (2013) showed that *Bacillus glia* can reduce rhizosphere pH and increase available Cd content. Consistent with previous studies, this study showed that the pH of rhizosphere soil decreased and that the content of available Cd increased after the addition of resistant bacteria (Fig. 6).

The fugacity of heavy metals in soil is one of the main factors affecting the efficiency of phytoremediation, and their fugacity is influenced by factors such as soil pH, soil parent material and inter-root environment (Li et al. 2016; Wu et al. 2018). *Bacillus megaterium*, a P-solubilizing and K-promoting bacterium, can increase the content of available P and promote plant growth by decomposing soft phospholipids and desorbing P in soil. It can also activate heavy metals in soil through its secretions (Jiang et al. 2019). Jeong et al. (2012) found that inoculation with *B. megaterium* can improve the mobility and bioavailability of Cd in soil and increases the extraction rate of plants by 2 times. In this study, we found that the addition of resistant bacteria could effectively increase the exchangeable state of Cd in the rhizosphere soil of *Sedum alfredii* Hance, which is consistent with the results of Jeong et al. (2012). The reason for this may be

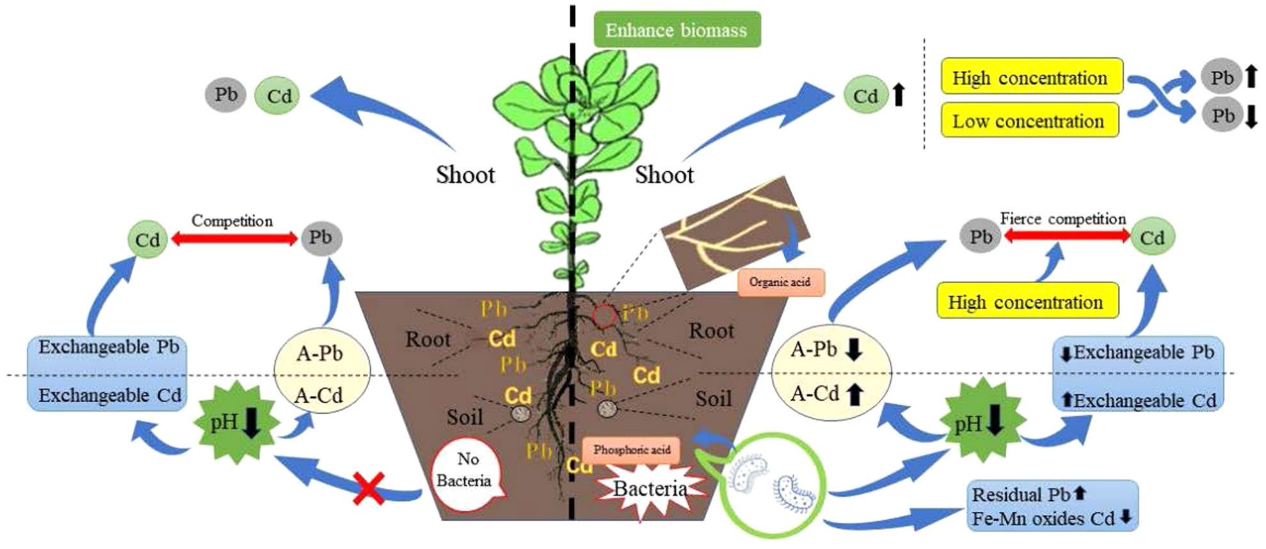

Fig. 6 Mechanism of resistant bacteria affecting Pb and Cd absorption by *Sedum alfredii* Hance. The graph on the right shows the changes in the fugitive patterns of heavy metals Pb and Cd in the soil and the Pb and Cd content of *Sedum alfredii* Hance with the addition of resistant bacteria. The left side of the figure shows the change of Pb and Cd without the addition of resistant bacteria. The upward arrows indicate an increase and the downward the opposite. A-Pb indicates the effective state of Pb; A-Cd indicates the effective state of Cd

that resistant bacteria have a better mineralolytic effect and increase the soil cation content. These cations would compete with Cd ions for adsorption sites and reduce the adsorption of Cd to the soil, thus increasing the effective state of Cd in the soil. Furthermore, the increase in K content in the soil improved the ionic strength of the soil solution and decreased the adsorption of Cd in the soil (Naidu et al. 1997; Appel and Ma 2002). The findings of this work also suggested that the addition of resistant bacteria could effectively reduce the exchangeable Pb content in soil likely because resistant bacteria could release P and K. This effect improved the cation exchange capacity (CEC) of the soil. High CEC is indicative of the presence of numerous negative charges that can increase the amount of Pb ions adsorbed by static electricity in the soil. *Bacillus* can release other elements into the soil while releasing P and K. The dissolution of phosphoric acid and phosphate in the P-releasing process of *Bacillus* metabolism induces the transformation of some Pb species into species that are difficult to dissolve (Cao et al. 2009). This effect results in the replacement of Ca on the surfaces of inorganic minerals by Pb and the transformation of exchangeable Pb in the soil into stable Fe–Mn-bound Pb (Brown et al. 2004), thus reducing the content of available Pb in the soil. However, it is also possible that in the presence of tolerant bacteria, the production of transporter proteins for Pb in *Sedum alfredii* Hance decreased and more transporter proteins related to Cd were synthesized. Because the uptake of cadmium by *Sedum alfredii* Hance increased significantly after the addition of resistant bacteria, the uptake of Pb decreased gradually, and its inhibition effect was more obvious under the conditions of high concentration of Pb and Cd pollution (Figs. 4, 6). The mechanism of this effect needs to be further explored.

Through the analysis of Pb and Cd species distribution in the rhizosphere soil of *Sedum alfredii* Hance, this study found that after the addition of resistant bacteria, the content of exchangeable Pb in the soil decreased, whereas the proportion of residual Pb increased. At the same time, the content of exchangeable Cd in the soil increased and the proportion of Fe–Mn oxide-bound Cd decreased. These results could be attributed to the secretion of organic acids and other substances by the resistant bacteria that dissolve Fe–Mn oxide-bound Cd in the soil (Jeong et al. 2012; Li et al. 2011a, b). Moreover, metabolic activities, such as P dissolution, by the resistant bacteria induce the transformation of heavy-metal species in soils. Elzahabi and Yong (2001) demonstrated that in the soil system, an increase in pH increases the amount of negative charges on the surfaces of clay minerals, hydrated oxides, and organic matter, thus strengthening the adsorption capacity for heavy metal ions and reducing the concentration of heavy-metal ions in the solution. The reduction in pH increases the content of soluble and exchangeable heavy metals in the soil such that the amount of negative charges carried by soil colloids decreases and the competitive effect of H^+ is enhanced (Lin et al. 1998). Therefore, under the same Pb and Cd contamination condition, the amount of resistant bacteria increased, as did the exchangeable and carbonate-bound states of Cd. The action of resistant bacteria increased the conversion of exchangeable Pb into residual Pb in the soil (Fig. 6). This resistant bacterium inhibits the uptake and utilization of Pb by *Sedum alfredii* Hance probably by reducing the bioefficacy of Pb and thus the enrichment of Pb by *Sedum alfredii* Hance.

The most obvious alterations in plants after heavy-metal poisoning are variations in plant growth, such as reduced biomass and stunted growth (Jamali et al. 2014). Zlobin et al. (2019) showed that in higher plants, the heavy metal Cd can reduce plant biomass by inhibiting photosynthesis. Chiao et al. (2019) found that Cd can affect the physiological and biochemical activities of the cell membrane and enzyme system of roots, thereby inhibiting the division of root tip cells and the elongation of roots. Li et al. (2017a, b) also found that Cd ions can cause intracellular ion imbalance and impair physiological metabolic function by preempting the entry of Ca ions into plant cells via channels. Many earlier studies have pointed out the weak promotion and intense inhibition effects exerted by Cd on plant growth. This study demonstrated that with the increase in Pb and Cd concentration, the biomass of plants first increased, then decreased, and finally reached the maximum value under treatment B. The present results were consistent with previous results (Guo et al. 2019). In addition, inoculation with specific strains can also promote plant growth. For example, *Bacillus* can produce many secretions, such as auxin (Venkatachalam et al. 2014), gibberellin (Chen et al. 2016), and cytokinin (Adhikari et al. 2001), that have significant growth-promoting effects on plants. Guo et al. (2014) found that the growth of tomato seedlings in the presence of *Bacillus polymyxa* is more vigorous than that in the absence of the bacteria. In this study, the biomass (including aboveground fresh and dry weight as well as root dry weight) of *Sedum alfredii* Hance was significantly increased after inoculation with resistant bacteria, and this result indicated that the addition of resistant bacteria could promote the growth of *Sedum alfredii* Hance. The growth of *Sedum alfredii* Hance was significantly better than that of the control group after the addition of resistant bacteria, likely because the microbial community structure in the rhizosphere soil of *Sedum alfredii* Hance had been altered to a certain extent after the addition of resistant bacteria, and the interaction between the metabolic activities of resistant bacteria and the growth of *Sedum alfredii* Hance resulted in the production of organic acids and root exudates that reduced the soil pH, adjusted the living and growing environment of soil microbes and *Sedum alfredii* Hance, and improved root activity. The related mechanism of these effects still needs further study.

Microorganisms, as the most active life-forms in soil, can significantly affect the uptake of heavy metals by plants by factors such as their abundance, secretion species, and community diversity. Liu et al. (2015) found that *Clitocybe* can effectively promote the absorption of Cu and Cd by plants. Giridhar et al. (2014) demonstrated that the nonsymbiotic endophytic fungi PDR1-7 can promote Pb absorption in the roots of *Pinus sylvestris*. Li et al. (2017a, b) found that the extracellular polymeric substances secreted by white rot fungi play an important role in enriching low amounts of Pb in the soil. Bacteria have the same role as fungi. Sinha and Mukherjee (2008) found that in zucchini cultivation, inoculating plant growth-promoting rhizobacteria that are capable of secreting siderophores can reduce the Cd content in zucchini shoots and roots, thus preventing the toxic effects of excessive Cd on plants. The results of this study showed that the Cd content in the roots and shoots of *Sedum alfredii* Hance significantly increased after the addition of resistant bacteria, and there was also a significant difference in plant cadmium content between J2 (200 mL bacterial solution) and J1 (100 mL bacterial solution). This result was consistent with the above research results. However, in this study, the Pb content in the aboveground part and roots of *Sedum alfredii* Hance increased at lower Pb and Cd contamination concentrations, and the increment was more after the addition of resistant bacteria; whereas, it was significantly lower after the addition of resistant bacteria at higher Pb and Cd contamination conditions. The reason may be that the resistant bacteria promoted the growth of *Sedum alfredii* Hance while reducing the effective state concentration of Pb in soil. At high Pb and Cd concentrations, the biomass of plants decreased because of the enhancement in the stress effect of Pb and Cd on *Sedum alfredii* Hance. Meanwhile, the limiting effect of resistant bacteria on Pb absorption by *Sedum alfredii* Hance absorption became increasingly marked.

The root enrichment factor and plant transport coefficient are two important indexes of heavy-metal uptake by plants. They are crucial representatives of the capability of plants to accumulate, transport, and distribute heavy metals. The results of this study showed that the addition of resistant bacteria could improve the enrichment factor and transport coefficient of Cd in *Sedum alfredii* Hance. These results were in agreement with those obtained above. Resistant bacteria might promote the enrichment and transport of Cd by increasing the levels of NRAMP3, NRAMP4, IRT1, and other proteins in *Sedum alfredii* Hance (Abedi and Mojiri 2020). However, the accumulation and transport of Pb in *Sedum alfredii* Hance showed low increments and decrements likely because resistant bacteria increased the formation of iron membranes in roots that consequently reduced the capability of the roots of *Sedum alfredii* Hance to absorb Pb and also reduced the amount of the heavy metal Pb transported to the soil (Zhang 2018).

The rhizosphere refers to the soil microenvironmental domain around plant roots. It is the portal through which soil moisture, mineral nutrients, and heavy metals enter the roots and leads to the shoot of plants. It is also the region wherein soil microorganisms and roots have the most direct and intense influence on the soil when carrying out life activities and metabolism. During plant growth, the environment of the rhizosphere microecological zone can be altered through ion exchange, root exudates, and root decomposition (Darrah 1991). Microorganisms can coordinate with root exudates to stimulate plant roots to secrete additional H^+ that replace the heavy-metal ions that are adsorbed on the surfaces of soil particles, thus affecting their migration and bioavailability. They can also affect the physical and chemical balance of heavy-metal compounds in soil through the secretion of citric acid and succinic acid. Cellular vesicles with containment and barrier effects and cell walls with retention and fixation are important mechanisms for plants to cope with heavy metal stress (Fu et al. 2011; Wang et al. 2007). Nedelkpska and Doran (2000) showed that proteins and coordination groups in plant cell walls bind to Cd ions and form precipitates in subcells, thus forming the first barrier to the entry of Cd ions into cell walls. In addition, the storage of heavy metals in the soluble components of plant root cells has a role in detoxification (Fu et al. 2011; Xu et al. 2010).

Conclusions

In this study, we combined "double-resistant" bacteria with Pb and Cd super-enriched plant—*Sedum alfredii* Hance to remediate Pb and Cd complex contaminated soil and explored its remediation effect and mechanism. The results showed that the resistant bacteria had significant effect on *Sedum alfredii* Hance to remediate Cd contamination, and in the presence of the resistant bacteria, a competitive relationship was created between Pb and Cd, while the enrichment of Pb by *Sedum alfredii* Hance was inhibited. Resistant bacteria were able to lower the rhizosphere soil pH, alter the rhizosphere zone environment by lowering the inter-root soil pH, activate the heavy metal Cd, and promote the accumulation of Cd in the aboveground part and roots of *Sedum alfredii* Hance. In addition, the resistant bacteria could alleviate the stress exerted by combined Pb and Cd pollution on *Sedum alfredii* Hance by enhancing the growth and biomass accumulation of the plant. Resistant bacteria promoted Cd uptake and inhibited Pb enrichment in *Sedum alfredii* Hance by increasing the exchangeable Cd content and decreasing the exchangeable Pb content. The results of this study can provide a theoretical basis for further in-depth research on the mechanism of enhanced Pb and Cd composite contaminated soil remediation by "double-resistant" bacteria in *Sedum alfredii* Hance, and also provide a theoretical basis and technical reference for the practical application of remediating Pb and Cd-composite contaminated soil.

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Authors' contributions
DHJ designed this study. TDM conducted the experiments and curation the data, writing—original draft. DDS and SHX conducted the experiments. XJH and ZGH assist in the experiments. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets and codes used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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