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Interaction of Cd/Zn hyperaccumulating plant (*Sedum alfredii*) and rhizosphere bacteria on metal uptake and removal of phenanthrene

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ABSTRACT

The effects of bacteria (*Burkholderia cepacia*) on plant growth, metal uptake, tolerance index and phenanthrene degradation by a hyperaccumulating plant (*Sedum alfredii*) were investigated. It was found that inoculation of bacteria did not enhance plant growth and metal uptake; while both metal translocation factor (up to 84% for Cd and 42% for Zn) and tolerance index (up to 23.2% for shoot and 72% for root) were significantly increased. In addition, inoculation of bacteria also alleviated the reductions of bioaccumulation factor and phytoextraction efficiency of As, Cu and Zn with the increasing proportions of polluted soil applied, while they were even increased for Cd and Pb (up to 31.2 and 124%, respectively). Up to 96.3% of phenanthrene was removed in the treatment with both plant and bacteria at the end of the experiment. A positive correlation between metal and P accumulation in plants was observed, it is suggested that high P uptake is directly involved in metal detoxification and leading to an increased P requirement. With the assistance of bacteria, *S. alfredii* could be able to withstand higher metal concentrations and it could also provide a practical tool for phytoremediation.

1. Introduction

Heavy metals and polycyclic aromatic hydrocarbons (PAHs) are two of the most abundant and toxic pollutants found in polluted soils [1]. Bioremediation is one of the most promising methods of removing PAHs from contaminated environments [2]. However, it is often difficult to generate sufficient microbial biomass in natural soils to achieve an acceptable rate of movement of tightly bound hydrophobic PAHs to the microbes where they can be degraded [3]. To enhance the efficiency of remediation process, one of the methods is to accelerate the removal and degradation rate of PAH through microbial or mechanical process. In addition, it is found that the application of higher plants plays an important role in increasing the amount of microbial biomass in contaminated soils [4]. To fulfill the above requirements and remediate the heavy metal and PAH contaminated soil, phytoextraction is one aspect of in situ plant-based technologies. It is commonly recognized that using metal hyperaccumulating plants for phytoextraction of metals is a feasible remediation technology for the decontamination of metal-polluted soils [5]. Although using hyperaccumulating plants for remediation of persistent contaminants may have advantages over other methods, many limitations exist for this technology. One serious limitation is that metal availability is usually lower in non-treated (EDTA) soils [6]. Therefore, it might limit the phytoremediation efficiency. In addition, the number of microorganisms is depressed in most contaminated soils, so there are not enough bacteria either to facilitate contaminant degradation or to support plant growth [7].

In spite of the abovementioned problems, phytoremediation may still be possible through proper use of soil bacteria. This entails using metal-tolerant, mobilizing and PAH degrading bacteria. Many microorganisms in the soil are able to solubilize 'unavailable' forms of heavy metal-bearing minerals by excreting organic acids [8,9]. In addition, many soil bacteria are tolerant to heavy metals and play important roles in their mobilization [10,11]. The presence of rhizosphere bacteria increased concentrations of Zn in *Thlaspi caerulescens* [12] and Ni in *Alyssum murale* [13].

There is lack of information on the relationships among hyperaccumulating plants and PAH degrading bacteria on soil contaminated by both heavy metals and PAHs. Xu et al. [14] demonstrated the degradation of phenanthrene and pyrene from soil by the application of three plant species, but PAH degrading bacteria was not examined. Siciliano and Greer [15] showed that inoculation

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of grasses *Bromus erectus*, *Lolium perenne* and *Anthoxanthum odoratum* with *Pseudomonas* sp. strain I4 increased the culturable soil heterotrophic population and also reduced trinitrotoluene (TNT) levels in soil, but heavy metals were not studied. Maliszewska-Kordybach and Smreczak [16] found that contamination of soils with PAHs and heavy metals had deleterious influences on soil microbial activity and plant development in their early stage of growth, but no hyperaccumulating plant was involved and heavy metal uptake and PAH degradation were not monitored.

There is currently a dearth of information on using the combination of rhizodegradation and phytoextraction. In addition, the interactions of introduced microbes, heavy metals, PAHs and metal hyperaccumulating plants on soil co-contaminated with heavy metal and PAHs have not been investigated. It is an area of research that has not been covered to any extent. Therefore, this experiment aims to study the interaction of microbes and metal hyperaccumulating plants on soil contaminated with both heavy metals and PAHs. The main objectives were to study the effects of bacteria on the (1) growth of *Sedum alfredii*; (2) metal uptake and accumulation by *S. alfredii*; (3) translocation factor, metal tolerance, bioaccumulation factor and phytoextraction efficiency of *S. alfredii* and (4) degradation of phenanthrene contaminated soil during a 3-month greenhouse study.

2. Materials and methods

2.1. Chemicals

Phenanthrene was obtained from SIGMA Chemical Co. (Germany) with purities higher than 98%. It represented typical PAH compounds with three benzene rings.

2.2. Preparation of phenanthrene and metals contaminated soil

Control soil without any detectable PAHs was collected from Loi Tung village, New Territories, Hong Kong. The basic physicochemical properties of the soil are listed in Table 1. The control and heavy metal polluted soil samples were air-dried and sieved through a 2 mm mesh before sterilization (121 °C for 2 h) by autoclaving (model: SS-325, TOMY, Seiko, Tokyo) to eliminate indigenous microorganisms. Two hundred mg/L of phenanthrene was spiked into the soil according to the method described by Brinch et al. [17]. Phenanthrene powder was dissolved in acetone and added to 25% (w/v) of the soil samples. After evaporation of the acetone, the spiked soil was then thoroughly mixed with multi-metal polluted soil (QZ) from an ancient Pb/Zn mine located at Qu Zhou City, Zhejiang Province at four different rates: 0% (PS0), 10% (PS10), 20% (PS20) and 40% (PS40) (w/w), respectively. Afterwards, the spiked soil samples were covered with aluminium foil and equilibrated in the dark for 3 weeks prior to the experiment. Initial concentrations of phenanthrene and metalloid/heavy metals (As, Cd, Cu, Pb and Zn) in soil were analyzed by gas chromatography/mass spectrometry and inductively coupled plasma atomic emission spectrometry (ICP-AES, Fisons ARL Accuris) before starting the experiment, respectively. For details, please refer to Section 2.5 which showed the details of plant tissues and soil analysis.

2.3. Isolation and inoculation of metal tolerant and phenanthrene degrading bacteria

The bacterium used in this experiment was isolated from a metal mine (Pb/Zn) in Hunan Province, China [18]. The bacterium strain was further enriched by using phenanthrene as a sole carbon source [19]. The bacterium *Burkholderia cepacia* was grown in LB medium for 24 h on an incubation shaker (model no: 4628-1, Lab-line, Barnstead, U.S.A.) at 200 rpm. The culture was centrifuged at 8000 rpm for 10 min (Beckman Advanti J25 I, U.S.A.), then washed with 0.85% sterilized NaCl twice and resuspended in deionized water. The centrifuged bacterial cells were then transferred into sterilized peat moss (from Gartengold Company in Germany) as the microbial inoculum. Finally, the inoculum was transferred into the soil with an initial concentration of approximately 3.0×10^8 CFU/g dry soil [11].

2.4. Experimental design

The experiment consisted of four levels of multi-metal pollution (PS0, PS10, PS20 and PS40; 200 mg/kg phenanthrene was added into each levels of polluted soils), and four treatments: control (CK), bacterial inoculation (B), *S. alfredii* cultivation (P) and bacteria + *S. alfredii* cultivation (B + P), resulting in 16 sets with four replicates each. Mature plants collected from Qu Zhou multi-metal mine were grown on plotting soil and propagated in a greenhouse, and new shoots of the plants were selected and grew in 1/10 hoagland solution [20] for 2 weeks for initiation of new roots. Roots of each plant surface were sterilized with 30 mg/L of chloramphenicol (SIGMA) for 3 min to minimize microbial growth [21], followed by washing with distilled water. All pots were placed randomly on the same bench in a greenhouse, with temperature control (25–28 °C), and supplemented with additional illumination (with a light intensity of 250 μmol/m^2/s, under a 14/10 h-light/dark cycle).

2.5. Plant tissues and soil analysis

After 3 months, plants were harvested and washed thoroughly with deionized water, and the roots of intact plants were immersed in 20 mM Na_2-EDTA for 15 min to remove the metal ions adhered to the root surfaces [22]. The plants were then separated into roots and shoots, oven-dried (70 °C) to a constant weight, grounded and passed into powder for metal analysis. We used all harvested root samples and 0.25 g of shoot samples for metal digestion. Grounded plant materials were digested with conc. HNO_3 [23]. Phosphorus (P) concentration in the shoot and root was measured in digests using the molybdenum blue methods [24].

Soil pH and electrical conductivity (EC; solid:distilled water = 1:5, ORION meter) were measured in a water solution of soil (soil:distilled water, 1:5) after shaking for 2 h at 200 rpm. Water extractable organic carbon (EOC) was analyzed using Total Organic Carbon analyzer (solid:distilled water = 1:10, TOC-5000A, Shimadzu, Japan). The concentrations of water-soluble nitrogen (N) and total kjeldahl N (TKN) were measured using berthelot reaction [24]. The concentrations of water-soluble, 0.5 M $NaHCO_3$-extractable and total P (TP) were measured using molybdenum blue method [24,25]. The concentrations of total, water-soluble and diethylenetriaminepentaacetic acid (DTPA)-extractable heavy metals in soils were determined with ICP-AES after extracting with conc. 50% HF:70% HNO_3, 1:2 (v/v) by microwave extraction [26], deionized water [27] and 1 M DTPA (1.976 g DTPA; 14.29 g triethanolamine; 1.47 g $CaCl_2 \cdot 2H_2O$ and dissolved in 980 ml deionized H_2O, and made up to 1 L, pH 7.30) [28], respectively. Blanks and standard reference materials of soil and plant (NIST 2711 Montana Soil and NIST 1570a Spinach, U.S. Department of Commerce, National Institute of Standards and Technology, U.S.A.) were used for quality control and the recovery rates were within 100 ± 10% after comparing the metal concentrations of standard reference materials to its certificate.

Table 1
Basic physicochemical properties of soil mixed with different proportions of polluted soil from a multi-metal mine.

	pH	Electrical conductivity (EC, μS/cm)	Extractable organic carbon (EOC, mg/kg)	Nitrogen (N, mg/kg)		Total metal concentration (mg/kg)				
				Total kjeldahl N	Water-soluble N	As	Cd	Cu	Pb	Zn
PS0	6.43 ± 0.02	364 ± 5	720 ± 3	1890 ± 114	34.5 ± 0.4	66.0 ± 3	5.03 ± 0.18	12.1 ± 0.7	74.1 ± 4.3	257 ± 11
PS10	6.68 ± 0.08	480 ± 10	811 ± 5	1850 ± 121	37.9 ± 0.7	294 ± 19	41.3 ± 2.5	44.2 ± 2.4	2240 ± 175	2000 ± 113
PS20	6.73 ± 0.09	495 ± 19	612 ± 5	1840 ± 106	34.8 ± 1.9	481 ± 15	72.4 ± 4.8	76.4 ± 4.6	4600 ± 262	3740 ± 200
PS40	6.93 ± 0.01	585 ± 8	512 ± 3	2340 ± 133	37.6 ± 0.9	898 ± 57	96.0 ± 6.3	141 ± 8	8640 ± 417	8360 ± 413

	Phosphorus (P, mg/kg)			Water-soluble metals (mg/kg)				
	Total P	Water-soluble P	Olsen-extractable P	As	Cd	Cu	Pb	Zn
PS0	1200 ± 52	7.52 ± 0.23	86.4 ± 2.0	<0.05	0.0192 ± 0.0011	0.114 ± 0.004	<0.05	0.993 ± 0.014
PS10	1390 ± 86	2.73 ± 0.15	61.7 ± 2.2	0.538 ± 0.023	0.0683 ± 0.0036	0.237 ± 0.001	0.894 ± 0.033	3.87 ± 0.01
PS20	1570 ± 92	1.70 ± 0.06	69.7 ± 2.3	0.751 ± 0.046	0.139 ± 0.005	0.226 ± 0.007	1.25 ± 0.02	5.03 ± 0.01
PS40	1970 ± 104	0.81 ± 0.04	54.5 ± 1.4	0.823 ± 0.039	0.214 ± 0.004	0.221 ± 0.010	1.82 ± 0.03	6.59 ± 0.03

PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
Detection limit (mg/kg): As = 0.05, Cd = 0.005, Cu = 0.005, Pb = 0.05 and Zn = 0.005.
Values are expressed as mean ± standard deviation with $n = 4$.

Tolerance index (TI) was calculated by the following equation [29]:

$$\text{Tolerance index}\,(\%) = \frac{\text{growth in soil} + \text{metal}}{\text{growth in soil} - \text{metal}} \times 100$$

For soil phenanthrene analysis, soil samples were extracted according to EPA Standard Method 3540C [30]. All the samples were extracted for 18 h with acetone and dichloromethane (v:v, 1:1, 80 ml) in a Soxhlet apparatus. Florisil column was used for purifying the concentrated extract (EPA Standard Method 3620B) [31]. The eluant was evaporated to less than 2 ml prior to analysis. Phenanthrene concentrations were analyzed using gas chromatography/mass spectrometry, based on the EPA Standard Method 8270C [32]. A certified reference material (soil CRM104-100, Resource Technology Corporation, US) was used for quality control and the recovery rates were within 100 ± 10% after comparing the phenanthrene concentration of standard reference materials to its certificate.

2.6. Measurement of soil microbial activity

The determination of the dehydrogenase activity was based on the use of soluble tetrazolium salt as an acceptor [33]. Samples of 1.0 g of soil were weighed in test tubes, mixed with 2 ml of 9.88 mM iodonitrotetrazolium chloride (INT) solution and 1.5 ml of 1 M Tris buffer (pH 7), and incubated for 2 h at 40 °C. After incubation, iodonitrotetrazolium formazan (INTF) formed by reduction of INT was extracted with 10 ml of tetrahydrofuran and measured at 464 nm using a UV–visible spectrophotometer (PharmaSpec UV-1700, Shimadzu, Japan). Blanks were treated in the same manner with soil samples, which were sterilized by autoclaving (121 °C, 20 min) to inhibit microbial activity. The acid phosphatase activities were analyzed as described by Alef and Nannipieri [34]. Samples of 1.0 g of soil were weighted in test tubes, mixed with 0.25 ml of toluene, 4 ml of modified universal buffer at pH 6.5 and 1 ml of 15 mM p-nitrophenyl phosphate solution, and incubated for 1 h at 37 °C. After incubation, 1 ml of 0.5 M calcium chloride and 4 ml of 0.5 M sodium hydroxide were added into the tubes and mixed. For the control, 1 ml of p-nitrophenyl phosphate solution was added before filtration (Whatman No. 42 filter paper) and the absorbance was measured at 400 nm using a UV–visible spectrophotometer.

2.7. Statistical analyses

The bioaccumulation factor was defined as the ratio of the metal concentrations in shoot to water-soluble metal concentrations in soils [35] and phytoextraction efficiency was calculated based on the ability of the root to transport heavy metals to shoot (the amount of heavy metals in the shoot expressed/g root dry weight [36]). The translocation factor (TF) for metals within a plant was expressed by the ratio of [metal]shoot/[metal]root which showed the metal translocation properties from roots to aboveground parts [37]. The significant difference between soil parameters, soil metal concentrations and bacterial inoculation was tested by using Fisher's protected least significant difference (LSD) after an analysis of variance (ANOVA). In addition, to compare the effects of contaminated soil and bacteria on soil parameters, soil enzymatic activities, biomass and metal concentrations of plant, two-way ANOVA was tested, statistical results (Tables S1–10) can be found in the supplementary material. All statistical analyses including Pearson's partial correlation analysis were carried out using SPSS 11.0.0. Unless otherwise indicated, all treatment means were tested for significant difference at $P < 0.05$. Means and standard deviations were calculated based on four replicates.

3. Results

3.1. pH, EC and EOC

Table 2 shows the effects of *S. alfredii* and bacterial inoculation on soil pH, EC and EOC mixed with different proportions of the polluted soil. The addition of polluted soil significantly ($P < 0.001$) increased the pH values (Table S1). In addition, the pH values increased significantly ($P < 0.001$) in treatment B by 0.33–1.11 (pH unit), treatment P by 0.35–0.59 and treatment B + P by 0.75–1.61 when compared to treatment CK. However, the pH values of all treatments decreased when compared with the initial pH (Table 1). Different treatments (especially B and B + P) posed significant ($P < 0.001$, Table S1) effects on soil EC but no significant effect was detected in the addition of polluted soil. A marked increase of EC was observed in treatment B by 17.9–84.5%, while EC decreased in treatment P by 63.1–82.7%. However, EC only decreased by 3.41–28.4% in treatment B + P. Tables 2 and S1 show that the addition of polluted soil had significant ($P < 0.001$ for 1st and 2nd month and $P < 0.05$ for 3rd month) effects on soil EOC concentration, decreasing by 22.5–52.0%, 7.86–49.8% and 25.7–45.8 during the 1st,

Table 2
Influence of *S. alfredii* and bacterial inoculation on soil physicochemical parameters after 3 months' growth in soil with different proportions of polluted soil.

Treatment	pH	Electrical conductivity (EC, μS/cm)	Extractable organic carbon (EOC, mg/kg)			Nitrogen (N, mg/kg)		Phosphorus (P, mg/kg)		Total P
			1st month	2nd month	3rd month	Water-soluble N	Total kjeldahl N	Water-soluble P	Olsen-P	
PS0										
CK	4.37 ± 0.14 a	967 ± 72 b	663 ± 43 a	529 ± 79 a	360 ± 23 a	3.27 ± 0.11 a	1852 ± 86 a	25.6 ± 1.3 b	91.7 ± 11.6 b	1140 ± 87 a
B	5.48 ± 0.11 c	1640 ± 138 c	1160 ± 97 c	746 ± 21 a	788 ± 42 b	4.52 ± 0.58 a	3063 ± 184 b	45.6 ± 1.1 d	131 ± 4 c	1248 ± 92 a
P	4.91 ± 0.19 b	167 ± 12 a	736 ± 88 ab	668 ± 28 a	321 ± 18 a	2.87 ± 0.07 a	1836 ± 249 a	18.8 ± 0.7 a	60.0 ± 5.7 a	1162 ± 110 a
B + P	5.98 ± 0.34 d	934 ± 61 b	897 ± 52 b	810 ± 197 a	452 ± 22 ab	3.23 ± 0.26 a	2887 ± 38 b	39.2 ± 1.6 c	119 ± 2 c	1630 ± 257 a
PS10										
CK	5.07 ± 0.12 a	1380 ± 94 bc	513 ± 33 a	487 ± 38 a	285 ± 13 a	6.68 ± 1.19 b	1898 ± 49 a	8.29 ± 2.07 a	37.0 ± 1.3 a	1356 ± 86 a
B	5.70 ± 0.08 bc	1880 ± 115 c	713 ± 49 b	711 ± 55 b	522 ± 45 b	4.06 ± 0.56 ab	2722 ± 256 b	22.5 ± 1.4 b	104 ± 4 b	1670 ± 68 a
P	5.67 ± 0.29 b	268 ± 84 a	778 ± 90 b	554 ± 60 a	309 ± 27 a	2.34 ± 0.27 a	1977 ± 167 a	11.1 ± 0.6 a	34.9 ± 0.5 a	1516 ± 121 a
B + P	6.02 ± 0.10 c	990 ± 328 ab	1090 ± 16 c	856 ± 73 b	397 ± 44 a	3.11 ± 0.06 ab	2956 ± 168 b	23.6 ± 0.3 b	106 ± 1 b	2221 ± 187 b
PS20										
CK	5.45 ± 0.27 a	1300 ± 163 b	541 ± 39 ab	265 ± 24 a	380 ± 57 a	3.33 ± 1.24 a	2039 ± 120 a	3.07 ± 0.26 a	29.0 ± 0.8 a	1460 ± 69 a
B	6.05 ± 0.12 b	1540 ± 125 b	542 ± 51 ab	636 ± 46 b	499 ± 81 a	3.06 ± 0.11 a	3275 ± 145 b	15.1 ± ± 0.3 c	75.5 ± 1.3 b	2024 ± 176 b
P	5.89 ± 0.07 b	303 ± 24 a	346 ± 29 a	312 ± 16 a	336 ± 28 a	1.38 ± 0.32 a	2013 ± 111 a	5.50 ± 0.56 b	27.7 ± 1.4 a	1438 ± 102 a
B + P	6.20 ± 0.21 b	991 ± 57 b	722 ± 33 b	570 ± 10 b	379 ± 12 a	2.86 ± 0.58 a	2876 ± 207 b	15.6 ± 0.4 c	77.4 ± 5.1 b	2130 ± 296 b
PS40										
CK	5.86 ± 0.06 a	1270 ± 147 b	318 ± 13 b	269 ± 24 a	195 ± 11 a	3.26 ± 0.86 ab	2148 ± 89 a	0.763 ± 0.060 a	20.9 ± 1.2 a	1418 ± 110 a
B	6.19 ± 0.14 b	2340 ± 127 c	554 ± 14 d	415 ± 11 b	382 ± 10 c	3.55 ± 0.37 b	3141 ± 90 b	5.03 ± 0.34 b	52.7 ± 1.4 b	1914 ± 126 c
P	6.21 ± 0.17 b	467 ± 36 a	297 ± 8 a	297 ± 12 a	324 ± 29 b	1.40 ± 0.06 a	2255 ± 178 a	1.42 ± 0.17 a	20.7 ± 0.4 a	1527 ± 135 ab
B + P	6.61 ± 0.09 c	996 ± 227 ab	492 ± 5 c	615 ± 30 c	202 ± 13 a	2.23 ± 0.08 ab	3094 ± 46 b	6.54 ± 0.39 c	54.0 ± 1.9 b	1750 ± 90 bc

PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
CK: control; B: bacterial inoculation; P: *S. alfredii* cultivation; B + P: bacteria + *S. alfredii* cultivation.
Values are expressed as mean ± standard deviation with $n = 4$.
For each treatment, different letters in the same column indicate significant difference within treatments at the level of $P < 0.05$.

2nd and 3rd month, respectively. In addition, different treatments (especially B) posed significant ($P < 0.001$, Table S1) effects on soil EOC, increasing by 0.12–74.4%, 41.0–140% and 31.4–119% during the 1st, 2nd and 3rd month, respectively.

3.2. Soil N, P and acid phosphatase activity

Total and bioavailable soil nutrients (N and P) are shown in Table 2. Addition of polluted soil and different treatments significantly ($P < 0.001$, Table S1) decreased the water-soluble N, especially in treatment P. However, the reduction of water-soluble N was alleviated with the inoculation of bacteria. On the other hand, addition of polluted soil did not significantly decrease TKN in soil, while it was significantly ($P < 0.001$) increased in treatment B and B + P, by 43.4–65.4% and 41.0–55.9%, respectively.

Water-soluble and Olsen-P ($NaHCO_3$ extractable, available inorganic P) were significantly decreased by the addition of polluted soil ($P < 0.001$, Table S1), but increased significantly ($P < 0.001$) with the inoculation of bacteria in treatments B and B + P. Water-soluble and Olsen-P increased in treatment B by 78.6–557% and 42.6–180%; in treatment B + P by 108–362% and 97.89–203%, respectively. Similarly, inoculation of bacteria also posed a significant effect on TP, which was increased by 23.2–38.6%, and the addition of polluted soil had no significant ($P > 0.05$) effect on TP.

The influence of *S. alfredii* and bacterial inoculation on soil acid phosphatase activity under different proportions of polluted soil is shown in Fig. 1a. Soil phosphatase activity decreased significantly ($P < 0.001$) with the addition of polluted soil. No enzyme activity was detected in the control under both 20 and 40% of polluted soil. However, different types of treatments had significant ($P < 0.001$) effect on enzyme activities, especially in treatment B + P, which had the highest enzyme activity among different proportions of polluted soils. The relationships between water-soluble P, Olsen-P and activities of acid phosphatase are further illustrated in Fig. 1b and c. Water-soluble P ($R = 0.68$, $P < 0.001$) and Olsen-P ($R = 0.57$, $P < 0.001$) in soil were positively correlated with acid phosphatase ($P < 0.001$).

3.3. Plant biomass and P uptake

No visual symptoms of metal toxicity were observed throughout the experiment. The biomass and P uptake of *S. alfredii* (on a dry weight basis) are shown in Table 3. The addition of polluted soil had no significant effect on shoot biomass. Conversely, root biomass was found to increase significantly ($P < 0.05$, Table S2), especially in 40% polluted soil. Although no dramatic decrease was detected in shoot biomass in higher proportions of polluted soil, it was significantly ($P < 0.05$, Table S2) reduced with the inoculation of bacteria by 15.5–22.1%. For P uptake by *S. alfredii*, the addition of higher proportions of polluted soil had no effect on P uptake in shoot and root. However, inoculation of bacteria significantly ($P < 0.01$, Table S2) enhanced the uptake of P in root by 4.61–74.0%, but no significant difference ($P > 0.05$, Table S2) was detected in shoot which only increased by 5.65–11.3%.

3.4. Metal uptake, translocation factors and tolerance index

The effects of adding polluted soil and bacterial inoculation on metal uptake by *S. alfredii* are illustrated in Table 4. In general, metal uptake in both shoot and root increased significantly (Table S3) due to the addition of polluted soil. However, significant reduction of metal uptake in the treatment of *S. alfredii* with bacterial inoculation was observed, especially for Cd, Pb and Zn (for Cd in shoot and root by 11.4–24.1% and 38.1–50.5%; Pb by 58.6–62.0% and 38.7–83.2%; Zn by 24.6–45.4% and 40.0–44.4%, respectively).

Translocation factor and tolerance index of *S. alfredii* are shown in Table 4. No significant (Table S3) effect of increasing

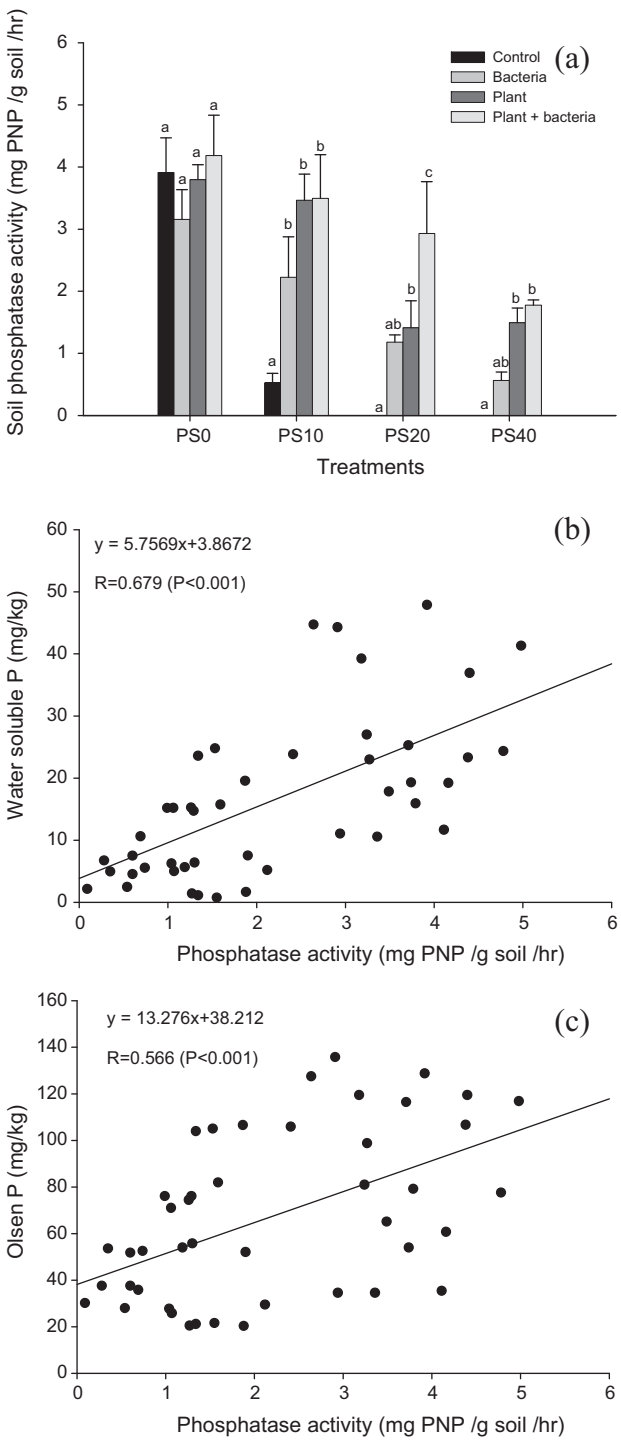

Fig. 1. Influence of *S. alfredii* and bacterial inoculation on (a) soil acidic phosphatase activity with different proportions of polluted soil and relationship of (b) soil water-soluble P and (c) soil Olsen-P with soil acid phosphatase activities after 3 months' growth in soil. PNP: p-nitrophenol; PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil; values are expressed as mean ± standard derivation with $n = 4$ and for each treatment, different letters in the same column indicate significant difference within treatments at the level of $P < 0.05$ in (a).

proportions of polluted soil on translocation factor for metals was observed, except for Zn. However, bacterial inoculation posed different effects on metal translocation factor. For Cu, bacterial inoculation decreased the translocation factor by 10.3–25.6%. On the contrary, for Cd, Pb and Zn, it increased by 24.6–84.0%, 1.4–100% and 10.6–42.0%, respectively. The addition of polluted soil and

Table 3
Influence of bacterial inoculation on plant P, shoot and root biomass of *S. alfredii* after 3 months' growth in soil with different proportions of polluted soil.

Treatment	Biomass (g)		Phosphorus (P, mg/kg)	
	Shoot	Root	Shoot	Root
PS0				
P	0.599 ± 0.047	0.0552 ± 0.0067	2160 ± 180	3270 ± 189
B + P	0.506 ± 0.118	0.0464 ± 0.0171	2330 ± 210	3420 ± 141
PS10				
P	0.690 ± 0.112	0.0483 ± 0.0054	2020 ± 236	3260 ± 179
B + P	0.479 ± 0.094	0.0410 ± 0.0067	2130 ± 189	5670 ± 420
PS20				
P	0.545 ± 0.038	0.0564 ± 0.0072	2150 ± 192	3410 ± 372
B + P	0.451 ± 0.071	0.0461 ± 0.0153	2360 ± 257	5840 ± 297
PS40				
P	0.644 ± 0.097	0.0993 ± 0.0154	1890 ± 81	3240 ± 156
B + P	0.502 ± 0.092	0.0468 ± 0.0081	2110 ± 156	5430 ± 392

PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
P: *S. alfredii* cultivation; B + P: bacteria + *S. alfredii* cultivation.
Values are expressed as mean ± standard deviation with $n = 4$.

bacteria together had no significant effect on the tolerance indices. Nevertheless, bacterial inoculation increased the tolerance indices of shoot and root by 7.32–23.2% and 52.0–72.0%, respectively.

3.5. Bioaccumulation factor and phytoextraction efficiency

Table 5 shows that the addition of polluted soil and bacteria significantly (Cd: $P < 0.01$; Zn: $P < 0.05$, Table S4) decreased the bioaccumulation factor of Cd and Zn, while factors of Cu and Pb were only decreased significantly (Cu: $P < 0.001$; Pb: $P < 0.01$, Table S4) by the inoculation of bacteria. A dramatic decrease in the factor was observed with the increasing proportions of polluted soil used, especially for Cd and Zn without bacterial inoculation. The bioaccumulation factor for Cd and Zn decreased in the absence of bacteria by 30.7–55.0% and 12.5–45.5%, respectively. However, the reduction alleviated with the inoculation of bacteria, for Cd and Zn by 16.2–34.7% and 15.9–29.8%. For phytoextraction efficiency (Table 5), Only Pb significantly ($P < 0.001$, Table S4) decreased with bacterial inoculation and the addition of polluted soil had a significant reduction ($P < 0.05$, Table S4) on Zn. In general, phytoextraction efficiency of Cd, Cu, Pb and Zn decreased with the increasing proportions of polluted soils added into the treatments. However, the

Table 4
Influence of bacterial inoculation on metals uptake, translocation factor and tolerance index of *S. alfredii* after 3 months' growth in soil with different proportions of polluted soil.

Treatment	Total metal (mg/kg)									
	As		Cd		Cu		Pb		Zn	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
PS0										
P	<0.05	20.9 ± 1.2	119 ± 28	37.6 ± 5.6	9.82 ± 0.76	22.0 ± 2.7	<0.05	<0.05	10,240 ± 1220	4280 ± 301
B + P	<0.05	<0.05	132 ± 15	18.6 ± 4.8	8.74 ± 1.05	8.69 ± 1.55	<0.05	<0.05	5590 ± 736	2570 ± 327
PS10										
P	<0.05	84.9 ± 10.7	311 ± 29	139 ± 19	9.91 ± 1.22	26.9 ± 3.4	54.6 ± 7.2	347 ± 72	18,680 ± 2226	10,110 ± 1476
B + P	<0.05	81.2 ± 6.5	236 ± 37	83.1 ± 14.9	8.87 ± 1.28	33.5 ± 4.6	20.7 ± 3.8	58.3 ± 10.6	12,100 ± 972	6010 ± 781
PS20										
P	<0.05	93.2 ± 10.9	399 ± 55	181 ± 29	12.2 ± 0.8	30.8 ± 3.8	85.1 ± 12.9	542 ± 131	19,630 ± 3540	12,710 ± 2826
B + P	<0.05	127 ± 24	303 ± 78	90.0 ± 12.3	10.8 ± 1.8	31.6 ± 6.5	33.0 ± 2.4	145 ± 17	13,630 ± 1816	7070 ± 405
PS40										
P	<0.05	68.7 ± 14.2	398 ± 65	181 ± 27	10.9 ± 1.6	22.2 ± 3.3	127 ± 17	485 ± 101	15,780 ± 2064	12,010 ± 925
B + P	<0.05	86.7 ± 16.4	352 ± 44	112 ± 13	9.59 ± 1.34	19.3 ± 0.9	52.5 ± 6.6	298 ± 33	11,910 ± 1315	6700 ± 857

Treatment	Translocation factor ($Metal_{shoot}/Metal_{root}$)					Tolerance indices (%)	
	As	Cd	Cu	Pb	Zn	Shoot	Root
PS0							
P	n.a.	3.17 ± 0.38	0.632 ± 0.059	0.126 ± 0.028	2.62 ± 0.24	n.a.	n.a.
B + P	0.0938 ± 0.0122	5.83 ± 0.67	0.474 ± 0.077	0.254 ± 0.066	2.55 ± 0.29	n.a.	n.a.
PS10							
P	0.0741 ± 0.0068	2.33 ± 0.22	0.477 ± 0.065	0.190 ± 0.061	1.92 ± 0.25	123 ± 23	87.4 ± 10.6
B + P	n.a.	2.91 ± 0.14	0.432 ± 0.061	0.272 ± 0.035	2.30 ± 0.44	132 ± 34	150 ± 28
PS20							
P	n.a.	2.45 ± 0.36	0.485 ± 0.078	0.173 ± 0.069	1.80 ± 0.35	95.0 ± 18.4	105 ± 13
B + P	n.a.	3.81 ± 0.88	0.401 ± 0.082	0.109 ± 0.009	1.99 ± 0.31	117 ± 11	160 ± 25
PS40							
P	0.544 ± 0.084	2.29 ± 0.25	0.504 ± 0.075	0.281 ± 0.047	1.36 ± 0.26	116 ± 25	185 ± 40
B + P	n.a.	3.27 ± 0.72	0.568 ± 0.049	0.284 ± 0.019	1.94 ± 0.47	133 ± 17	182 ± 27

PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
P: *S. alfredii* cultivation; B + P: bacteria + *S. alfredii* cultivation.
Detection limit (mg/kg): As = 0.05, Cd = 0.005, Cu = 0.005, Pb = 0.05 and Zn = 0.005.
Values are expressed as mean ± standard deviation with $n = 4$.
n.a.: not available.

Table 5
Influence of bacterial inoculation on bioaccumulation factor and phytoextraction efficiency of *S. alfredii* after 3 months' growth in soil with different proportions of polluted soil.

Treatment	Bioaccumulation factor					Phytoextraction efficiency				
	As	Cd	Cu	Pb	Zn	As	Cd	Cu	Pb	Zn
PS10										
P	n.a.	10,960 ± 2087	38.1 ± 4.7	39.1 ± 6.4	4085 ± 553	n.a.	5250 ± 681	171 ± 40	930 ± 173	317,700 ± 26,600
B + P	n.a.	6410 ± 516	30.8 ± 4.1	36.5 ± 13.5	3240 ± 240	n.a.	3380 ± 543	136 ± 23	298 ± 79	181,100 ± 16,260
PS20										
P	n.a.	7590 ± 1107	43.7 ± 5.2	49.1 ± 6.5	3580 ± 428	n.a.	3900 ± 537	122 ± 21	829 ± 132	196,300 ± 39,550
B + P	n.a.	5370 ± 895	34.7 ± 5.9	25.0 ± 3.2	2730 ± 300	n.a.	3670 ± 419	127 ± 14	410 ± 58	165,600 ± 20,110
PS40										
P	17.1 ± 2.8	4940 ± 574	39.2 ± 6.6	69.0 ± 5.4	2226 ± 105	n.a.	3000 ± 238	79.8 ± 8.6	930 ± 122	117,600 ± 28,220
B + P	n.a.	4180 ± 329	29.6 ± 3.8	23.2 ± 3.0	2274 ± 166	n.a.	4430 ± 512	113 ± 32	667 ± 97	144,700 ± 20,510

P: *S. alfredii* cultivation; B + P: bacteria + *S. alfredii* cultivation.
PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
Values are expressed as mean ± standard deviation with $n = 4$.
n.a.: not available.

phytoextraction efficiency of Cd and Pb increased by 8.7–31.2% and 37.6–123.8% in treatment B + P, respectively. For Cu and Zn, the reduction of phytoextraction efficiency was alleviated by the inoculation of bacteria. The phytoextraction efficiency of Cu and Zn decreased by 28.6–53.3% and 38.2–63.0%, respectively, with the increasing proportions of polluted soil. On the contrary, the efficiency for Cu and Zn only decreased 6.6–16.9% and 8.6–20.1% in treatments with bacterial inoculation, respectively.

3.6. Heavy metal extractability and total metals in soil

Table 6 shows the effects of *S. alfredii* with bacteria on water-soluble, DTPA-extractable and total metals at the end of the experiment. The addition of polluted soils and different types of treatments posed significant effects on the concentrations of water-soluble metals. In general, the concentrations of water-soluble metals increased with the increasing proportions of polluted soils, while inoculation of bacteria posed different effects. Treatments B and B + P decreased water-soluble concentrations of Cd, Pb and Zn significantly ($P < 0.001$, Table S5), however, concentrations of water-soluble As and Cu increased with the inoculation of bacteria ($P < 0.001$). The addition of polluted soils and different treatments also posed significant effects ($P < 0.001$, Table S5) on the concentration of DTPA-extractable metals. Concentrations of Pb increased significantly ($P < 0.001$) in treatments B and B + P, while concentrations of DTPA-extractable Cd, Cu and Zn decreased with inoculation of bacteria ($P < 0.001$).

The concentrations of total metals in soil after harvesting the plant are shown in Table 6. The addition of polluted soil significantly ($P < 0.001$, Table S5) increased the total metal concentrations in soil, and different types of treatments significantly ($P < 0.001$) decreased total As, Cu, Pb and Zn in soil. In general, treatments B, P and B + P decreased ($P < 0.001$) the total metal concentrations in soil. For example, treatment P decreased 14.2–31.0% of Cd, 13.1–34.6% of Cu, 4.8–24.6% of Pb and 20.9–26.5% of Zn accordingly when compared to the control.

3.7. Soil dehydrogenase activity

The effects of *S. alfredii* and bacterial inoculation on soil dehydrogenase activities during the experimental period are illustrated in Fig. 2. The addition of polluted soil significantly ($P < 0.001$ for 1st and 2nd month, $P < 0.05$ for 3rd month, Table S6) decreased enzyme activities in soil, while enzyme activities were significantly increased in the treatments with bacteria during the 1st month. During the 2nd and 3rd month, it was observed that soil dehydrogenase activities were significantly ($P < 0.001$, Table S6) enhanced in treatments B and B + P.

3.8. Phenanthrene removal in soil

The removal of phenanthrene from soil is shown in Fig. 3. The addition of polluted soil significantly ($P < 0.01$ for 1st month and $P < 0.001$ for 2nd and 3rd month, Table S6) decreased the removal rate of phenanthrene from soil. However, treatments B, P and B + P significantly ($P < 0.001$) enhanced the removal of phenanthrene in soil. Throughout the pot trial, the removal of phenanthrene from soil was dramatic, with the loss of 66.8–79.5%, 92.5–95.6%, 75.5–86.5% and 92.2–96.3% phenanthrene in treatments CK, B, P and B + P accordingly at the end of experiment.

4. Discussion

4.1. Effects of bacteria on soil pH, EC and EOC

At the end of the experimental period, there has been a decrease in soil pH in all control treatments (without bacterial inoculation and plant cultivation) compared to the beginning of experiment and it might be due to nitrification of the peat moss bacterial inoculums applied [38]. On the other hand, soil pH in each treatment was higher than the control. Lorenz et al. [39] and Luo et al. [40] also found an increase in soil pH during the growth of radish (*Raphanus sativus*) and *T. caerulescens*, which was probably due to the plants taking up N predominantly in the form of NO_3-N, with concurrent excretion of OH^- ions in order to maintain electrical neutrality within their roots [41]. Soil EC and EOC were significantly increased, especially in treatment with bacterial inoculation. It indicated that heterotrophic bacteria, which requires organic supplements for growth and energy supply, may contribute to the increase of EC and EOC because they decompose the organic matter and then release soluble low-molecular-weight organic compounds, such as organic acids, into the soil [42]. EC is useful in monitoring the mineralization process of organic matter, and for that reason the increase of EC could be caused by the decomposition of organic matter in soil. In addition, EC could also serve as a measure for both cations and anions soluble nutrients in soil [43]. Therefore, a similar pattern of concentrations of EC, EOC and water-soluble nutrients could be found. The bacterial metabolic activities led to an increase in EOC and EC, which has an essential function in accumulation and mobility of metals as well as in delaying their circulation in soils [44]. Although EOC and EC concentration increased significantly in the present study, the mobility of metals decreased, due to precipitation effect of high P concentration in the soil.

Table 6
Influence of *S. alfredii* and bacterial inoculation on soil water-soluble, DTPA-extractable and total metals after 3 months' growth in soil with different proportions of polluted soil.

Treatment	Water-soluble metal (mg/kg)					DTPA extractable metal (mg/kg)				
	As	Cd	Cu	Pb	Zn	As	Cd	Cu	Pb	Zn
PS0										
CK	0.451 ± 0.108 ab	<0.05	0.164 ± 0.045 ab	<0.05	2.92 ± 0.38 b	<0.05	0.224 ± 0.014 a	1.36 ± 0.09 b	5.95 ± 1.41 a	47.3 ± 2.8 c
B	0.724 ± 0.217 b	<0.05	0.189 ± 0.023 b	<0.05	1.10 ± 0.30 ab	<0.05	0.218 ± 0.028 a	1.17 ± 0.11 ab	9.19 ± 2.88 a	40.2 ± 3.2 bc
P	0.195 ± 0.048 a	<0.05	0.080 ± 0.005 a	<0.05	0.594 ± 0.046 a	<0.05	0.380 ± 0.020 b	1.35 ± 0.08 b	13.8 ± 4.2 a	27.0 ± 5.6 a
B + P	0.519 ± 0.133 ab	<0.05	0.138 ± 0.036 ab	<0.05	0.737 ± 0.097 a	<0.05	0.247 ± 0.019 a	1.06 ± 0.16 a	14.6 ± 5.0 a	34.8 ± 3.5 ab
PS10										
CK	0.910 ± 0.154 a	0.191 ± 0.051 b	0.243 ± 0.066 a	0.582 ± 0.009 a	16.8 ± 3.5 b	<0.05	5.67 ± 0.33 c	2.91 ± 0.15 b	166 ± 6 a	312 ± 15 b
B	0.803 ± 0.097 a	0.0841 ± 0.0280 a	0.241 ± 0.033 a	0.458 ± 0.077 a	6.13 ± 0.89 a	<0.05	4.40 ± 0.19 ab	2.45 ± 0.14 a	231 ± 20 b	245 ± 7 a
P	1.07 ± 0.14 ab	0.0320 ± 0.0023 a	0.255 ± 0.014 a	1.43 ± 0.23 b	4.60 ± 0.38 a	<0.05	4.88 ± 0.30 b	3.72 ± 0.30 c	282 ± 32 bc	270 ± 39 a
B + P	1.46 ± 0.18 b	0.0440 ± 0.0058 a	0.285 ± 0.020 a	0.751 ± 0.167 a	3.77 ± 0.61 a	<0.05	4.29 ± 0.14 a	3.38 ± 0.18 c	331 ± 14 c	244 ± 19 a
PS20										
CK	0.728 ± 0.093 a	0.258 ± 0.045 b	0.185 ± 0.025 a	0.494 ± 0.007 a	21.4 ± 2.8 b	<0.05	9.45 ± 2.10 a	3.60 ± 0.13 c	146 ± 7 a	404 ± 10 bc
B	1.25 ± 0.29 ab	0.0670 ± 0.0072 a	0.316 ± 0.014 b	0.439 ± 0.050 a	7.22 ± 0.45 a	<0.05	7.88 ± 0.58 a	2.63 ± 0.22 a	183 ± 24 a	339 ± 25 ab
P	0.870 ± 0.15 ab	0.0620 ± 0.0081 a	0.283 ± 0.012 b	1.72 ± 0.21 b	5.44 ± 0.51 a	<0.05	9.66 ± 0.50 a	3.96 ± 0.24 d	179 ± 9 a	412 ± 25 c
B + P	1.34 ± 0.06 b	0.0580 ± 0.0064 a	0.309 ± 0.026 b	1.45 ± 0.35 b	4.99 ± 0.40 a	<0.05	6.75 ± 1.62 a	3.17 ± 0.18 b	290 ± 38 b	326 ± 40 a
PS40										
CK	0.467 ± 0.0712 a	0.287 ± 0.047 b	0.114 ± 0.016 a	0.652 ± 0.019 a	24.2 ± 4.6 b	<0.05	16.7 ± 0.50 c	4.07 ± 0.10 bc	97.5 ± 5.8 a	533 ± 10 c
B	0.755 ± 0.140 ab	0.163 ± 0.010 a	0.319 ± 0.025 b	0.574 ± 0.075 a	11.1 ± 3.3 a	<0.05	15.0 ± 1.08 b	3.35 ± 0.09 a	146 ± 7 c	472 ± 24 ab
P	0.874 ± 0.287 ab	0.093 ± 0.009 a	0.284 ± 0.020 b	3.07 ± 0.19 b	7.44 ± 1.55 a	<0.05	15.1 ± 0.29 b	4.38 ± 0.33 c	124 ± 15 b	501 ± 20 b
B + P	0.982 ± 0.144 b	0.092 ± 0.012 a	0.330 ± 0.051 b	2.40 ± 0.70 b	5.23 ± 0.02 a	<0.05	13.3 ± 0.45 a	3.72 ± 0.21 ab	206 ± 8 d	463 ± 18 a

Treatment	Total metal (mg/kg)				
	As	Cd	Cu	Pb	Zn
PS0					
CK	67.7 ± 24.5 a	4.29 ± 1.36 a	8.10 ± 1.76 ab	77.6 ± 10.5 a	214 ± 12 b
B	25.8 ± 9.2 a	4.09 ± 0.88 a	12.8 ± 0.83 c	75.5 ± 18.9 a	210 ± 5 b
P	33.9 ± 5.5 a	3.10 ± 0.71 a	5.55 ± 1.40 a	59.6 ± 8.4 a	139 ± 21 a
B + P	21.9 ± 4.6 a	2.33 ± 0.96 a	10.1 ± 1.59 bc	66.7 ± 17.1 a	194 ± 10 b
PS10					
CK	229 ± 56 a	21.5 ± 6.4 a	51.9 ± 3.1 b	2588 ± 182 b	1355 ± 179 b
B	134 ± 13 a	16.8 ± 1.6 a	35.5 ± 1.0 a	1596 ± 39 a	1045 ± 21ab
P	168 ± 5 a	18.5 ± 3.5 a	33.9 ± 0.9 a	1953 ± 239 ab	996 ± 66 a
B + P	233 ± 65 a	20.7 ± 6.9 a	42.4 ± 7.4 ab	2368 ± 365 ab	1268 ± 167 ab
PS20					
CK	353 ± 37 a	35.9 ± 4.6 a	69.3 ± 9.5 ab	4420 ± 409 b	2492 ± 116 b
B	334 ± 85 a	40.9 ± 11.2 a	70.6 ± 5.2 ab	4008 ± 82 ab	2047 ± 142 a
P	316 ± 62 a	35.9 ± 4.4a	58.9 ± 4.6 a	3431 ± 446 a	1837 ± 252 a
B + P	310 ± 25 a	42.9 ± 6.8 a	79.9 ± 6.3 b	3668 ± 241 a	2507 ± 111 b
PS40					
CK	709 ± 17 b	79.9 ± 12.3 b	136 ± 16 b	7619 ± 963 a	4930 ± 1051 b
B	522 ± 20 a	52.5 ± 7.7 a	109 ± 6 a	6626 ± 193 a	3397 ± 141 a
P	565 ± 79 a	55.1 ± 6.9 a	119 ± 11 ab	7252 ± 182 a	3899 ± 568 ab
B + P	578 ± 17 a	50.5 ± 5.4 a	125 ± 4 ab	7323 ± 200 a	4076 ± 317 ab

PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil.
CK: control; B: bacterial inoculation; P: *S. alfredii* cultivation; B + P: bacteria + *S. alfredii* cultivation.
Detection limit (mg/kg): As = 0.05, Cd = 0.005, Cu = 0.005, Pb = 0.05 and Zn = 0.005.
Values are expressed as mean ± standard derivation with $n = 4$.
For each treatment, different letters in the same column indicate significant difference within treatments at the level of $P < 0.05$.

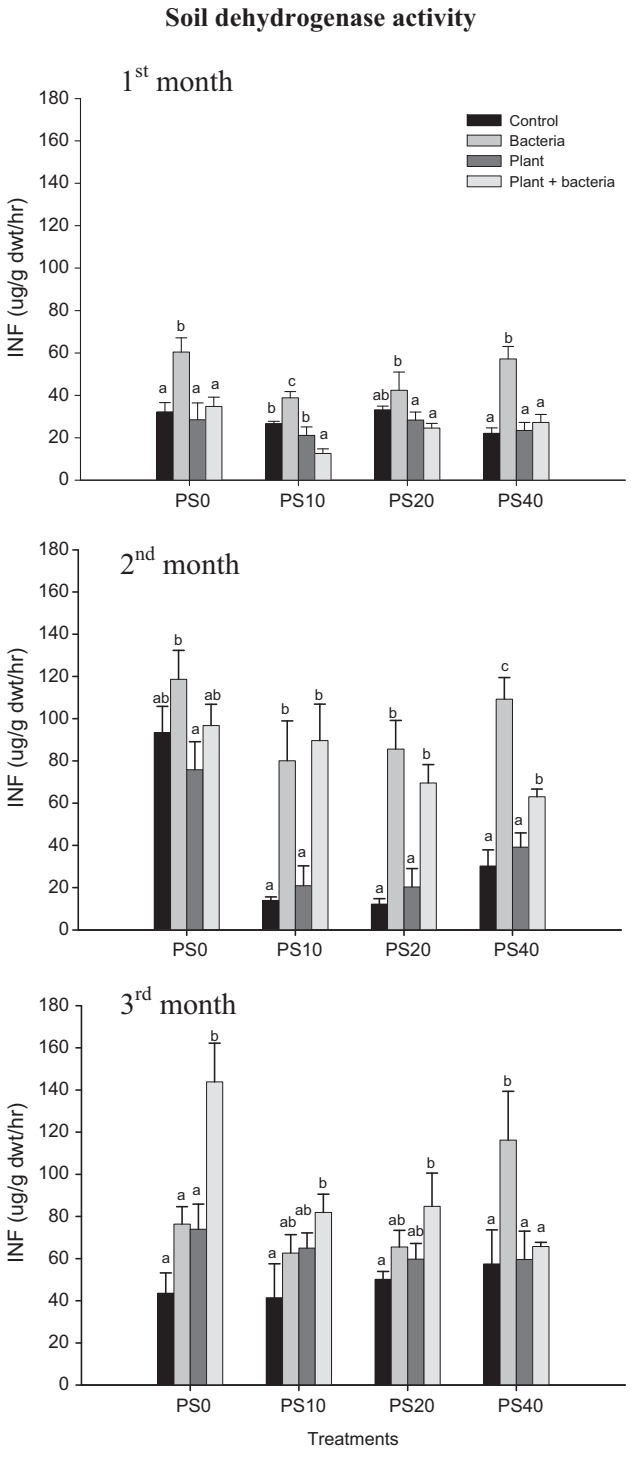

Fig. 2. Influence of *S. alfredii* and bacterial inoculation on soil dehydrogenase activity in soil under different proportions of polluted soil. INF: iodonitrotetrazolium chloride; PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil; values are expressed as mean ± standard derivation with $n = 4$ and for each treatment, different letters in the same column indicate significant difference within treatments at the level of $P < 0.05$.

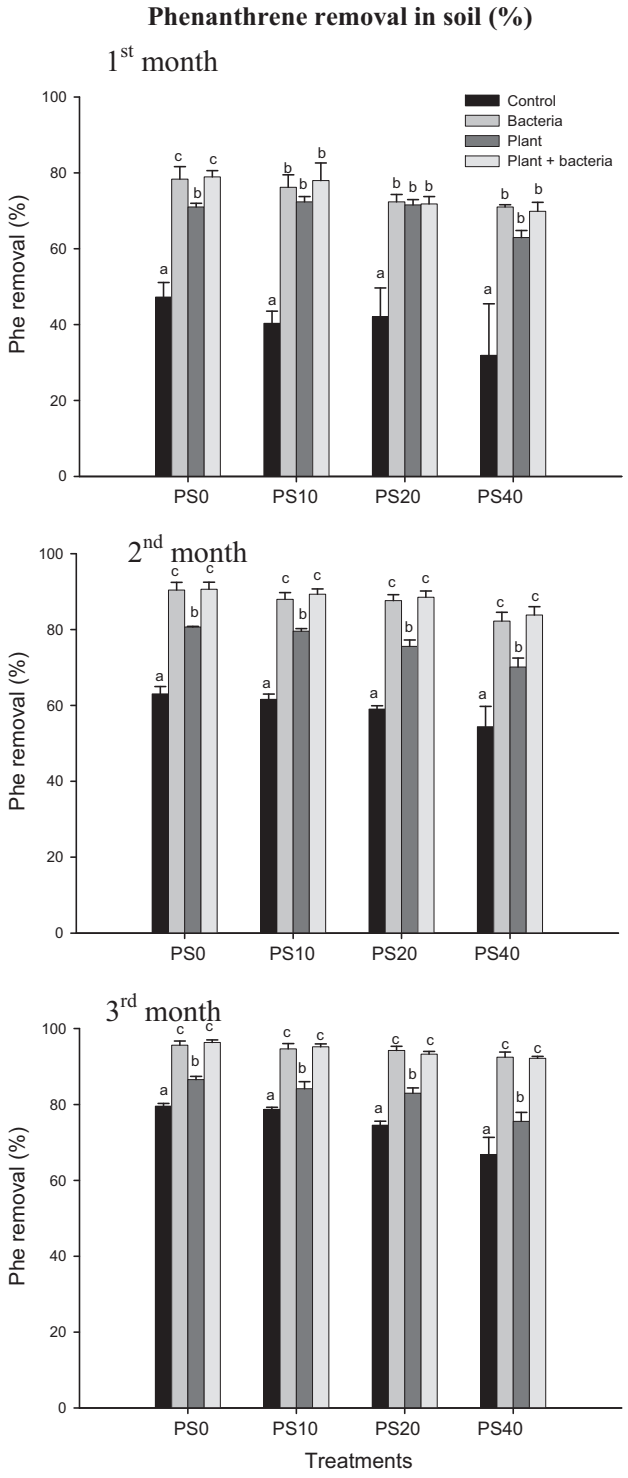

Fig. 3. Influence of *S. alfredii* and bacterial inoculation on phenanthrene removal in soil (%) under different proportions of polluted soil. PS0: 0% polluted soil; PS10: 10% polluted soil + 90% control soil; PS20: 20% polluted soil + 80% control soil; PS40: 40% polluted soil + 60% control soil; values are expressed as mean ± standard derivation with $n = 4$ and for each treatment, different letters in the same column indicate significant difference within treatments at the level of $P < 0.05$.

4.2. Effects of bacteria on soil N and P

In the present study, different treatments and addition of polluted soil caused an increase in TKN but a decrease in water-soluble N, especially in treatment P, it may be caused by the uptake and utilization of plant or leaching along with water application during the whole experimental period. Mine tailings and metal contaminated soils are poor in major nutrients such as N and P [45]. Therefore, the role of microorganisms is very important, as they help to promote nutrient circulation and reduce the need for chemical fertilizers. Biological N-fixation provides a major source of N for plants. Therefore, N and P concentrations were significantly

increased in treatments with bacterial inoculation, as *B. cepacia* possess P-dissolving and N_2-fixation capabilities [11]. Some of the bacteria may solubilize inorganic P due to excretion of organic acids [46]; organic acid production was also detected with the inoculation of *B. cepacia* in a previous work [18]. Furthermore, growth and metabolic activity of soil microorganisms are limited by nutrient availability [47]. Mineral nutritional factors can affect the number of bacteria in the rhizosphere [48]. The increased concentrations of N and P in the soil might be due to the presence of *B. cepacia*, therefore the number of bacteria in rhizosphere of *S. alfredii* should have increased due to the enrichment of available N and P in soil.

In the present study, higher acid phosphatase was also observed in the treatment with plant and bacteria, therefore the available P in soil was increased. This is in line with Tarafdar and Marschner [49] who revealed that plant-produced phosphatase is exclusively acid phosphatase. Acid phosphatase could be produced by bacteria, fungi, yeasts and protozoa, and therefore enhanced acid phosphatase activity in the rhizosphere may be produced directly by plant roots or indirectly via stimulation of the microbial biomass [50].

4.3. Effects of bacteria on available metals

Microbial activity strongly influences metal speciation and transport in environment [51]. Plant roots and their free-living and symbiotic microbial populations can significantly alter the physicochemical characteristics of the rhizosphere by metabolic activities, e.g., metabolite excretion, resulting in a geochemical environment that is markedly different from the bulk soil [52]. The present study revealed that inoculation of *B. cepacia* prompted the release of available N and P into soil. However, the enhanced concentrations of P altered the bioavailability of metals in soil. A negative correlation between water-soluble metals and available P (water-soluble and Olsen-P) was observed (Table S7). The decrease in water-soluble Cd, Pb and Zn concentrations caused by bacterial inoculation might be attributed to a dissolution–precipitation process due to the increasing P in soil solution, as a consequence of functional release by bacteria [53]. Phosphorus has been shown to immobilize and reduce Pb bioavailability [54,55]. Chaney et al. [56] found that addition of P fertilizer inhibited the uptake of some heavy metals, such as Pb, due to metal precipitation as pyromorphite and chloropyromorphite. Pyromorphites are highly stable Pb phosphate minerals under natural conditions [57] and Brown et al. [55] also showed that P reduced Cd availability in soil.

4.4. Effects of bacteria on plant biomass and metal uptake

Microbial inoculations in polluted soils can help the plant through toxin decomposition as well as promoting plant growth [58]. Microorganisms may also interact symbiotically with roots to enhance the potential for metal uptake [59]. Therefore, inoculation of *B. cepacia* should have a positive effect on *S. alfredii* through the detoxification of metals and promote plant growth, however our results showed that the potential of metal uptake was not enhanced.

The present study shows that inoculation of *B. cepacia* did not enhance plant biomass and metal uptake of *S. alfredii*, when compared to the plant treatment without bacterial inoculation. Although in a previous work [18] we observed an obvious increase in shoot and root biomass with the inoculation of *B. cepacia* in the hydroponic experiment, both metal uptake (by *S. alfredii*) and plant biomass were reduced in the present study. This might be due to the elevated P concentration in soil, which decreased metal bioavailability (Table S7) and eventually decreased the metal uptake by plant. In addition, a negative correlation between soil N, P and metal uptake in plant was obtained (Table S7). Excessive P application to *S. alfredii* might inhibit growth and Zn uptake [60]. In other studies, significant increase of biomass and metal uptake was observed in As and Cd/Zn hyperaccumulators with increasing concentrations of metals in soils or nutrient solutions [61,62]. In order to explain the consequences of reduced plant biomass and metal uptake, the relationships between plant biomass, soil nutrients, metal uptake and metal availability in soil were investigated. Positive correlations between plant biomass and metal uptake by plant (Table S8), between metal availability and plant metal concentrations were obtained (Table S8). Based on these results, it is suggested that the amount of available metals in soil would significantly affect metal uptake in plant and plant biomass. Metal contents in plant depended on metal availability in soil and the microbial activities in the rhizosphere [63].

4.5. Effects of bacteria on P uptake

B. cepacia increased P uptake in both shoot and root in all treatments. Increased nutrient uptake by plants inoculated with bacteria has been attributed to the production of plant growth regulators by the bacteria at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil [64]. The production of phytohormones and vitamins by rhizosphere bacteria has been revealed in different studies [65,66]. Rhizosphere bacteria *Pseudomonas* spp., *Azospirillum* spp., *Pantoea* spp. and *Agrobacterium* spp. increased plant growth and nutrient uptake in maize, wheat and legumes [67], and inoculation of maize and wheat with *Azotobacter* and *Azospirillum* increased plant growth, nutrient uptake and yield [68].

4.6. Effects of bacteria on metal tolerance

In polluted areas, plants are more dependent on microbial activity because microorganisms are able to enhance their metabolic activity to combat stress [69]. Depending on the level of environmental damage, the activity and diversity of soil organisms are increased to a threshold value prior to the loss of function. The bioaccumulation factor is one of the most important factors determining the feasibility of phytoextraction [70]. In the present study, although bacterial inoculation did not enhance metal uptake by the plant, it was observed that metal tolerance in plants had increased and bioaccumulation factor reduction (Cd and Zn) was less severe with increasing proportions of polluted soil. Furthermore, bacterial inoculation on *S. alfredii* alleviated the reduction of phytoextraction efficiency (Cu and Zn) and increased the phytoextraction efficiency for Cd and Pb by 8.8–31.2% and 37.7–124%, respectively. A positive correlation between metal and P accumulated in plants was observed (Table S9). One of the possible explanations is that P uptake is directly involved in Zn detoxification by the host [71], leading to an increased P requirement in the presence of high shoot metal concentrations. If this is the case, bacterial inoculation could play an important role in metal detoxification by increasing available P in soil and P uptake in plant. It has also been suggested that phytotoxicity caused by Cd could be alleviated by increased absorption of major nutrients such as N and P [72]. Bacteria could protect plants against the inhibitory effects of metals through siderophore production and P solubilizing isolates, which might have helped plant root proliferation and enhanced the uptake of soil minerals such as Fe and P by the host plant [73].

Metal phytoextraction is a promising alternative approach to the ex situ decontamination techniques applicable to slightly or moderately contaminated soils [74]. With the assistance of *B. cepacia*, *S. alfredii* might be able to withstand higher metal concentrations in polluted soils, without decreasing phytoextraction efficiency. Doelman [75] reported that the efficiency of phytoremediation of heavy metal contaminated sites is closely related to the presence

of higher proportions of metal resistant microbial populations in the soil, which likely resulted in better nutritional assimilation and protection for plants. These beneficial effects, together with the suggested interrelationship of microbial metal resistances, indicated that inoculation with microbes might have some potential in aiding plants thrive in heavily metal contaminated soils.

4.7. Effects of bacteria on enzymatic activities

Soil enzymes could behave differently when exposed to different pollutants, such as heavy metals, pesticides and PAHs [76]. In polluted soils, the size of microbial population is dramatically reduced. The activity of dehydrogenase (oxireductase enzyme) is an indication of microbial metabolism in soil [77]. In addition, glucosidase have a key role in regulating nutrient availability through the mineralization processes [78]. Figs. 1 and 2 show that the addition of phenanthrene and increasing proportions of polluted soil weakened the activities of acid phosphatase and dehydrogenase. The enzymatic activities were affected by the presence of heavy metals and PAHs, but bacterial inoculation increased enzymatic activities, when compared to treatments without bacterial inoculation. Lipophilic compounds like PAHs have a narcotic mode of toxic action and they may affect the permeability and structure of bacteria by interacting with lipophilic components of their cytoplasmic membranes [79]. Hence, heavy metals may penetrate more easily into microbial cells and affect their functions in PAH contaminated soil. A similar combined toxic effects of heavy metals (Zn, Cd and Cu) and phenanthrene toward soil bacteria was reported by Gogolev and Wilke [80].

A negative correlation between water-soluble metals and soil enzymes (acid phosphatase and dehydrogenase) was observed (Table S10). High levels of metals could negatively affect soil microbial biomass [81], and resulted in a negative effect on dehydrogenase activity, which is similar to Cd effect on the microbial biomass [69]. However in the present study, soil enzymatic activities increased with bacteria and plant cultivation. It was revealed that plant root exudates enhanced the development of a higher diversity of bacteria in soil and were efficient for supporting bacterial growth [82,83]. Such stimulating effects reflected better biological function and fertility of the inoculated soil. Thus, the effectiveness of different treatments types on metal tolerance and phytoextraction efficiency could be the result of an indirect effect through changes on microbial composition in the rhizosphere [84].

4.8. Effects of bacteria and plants on phenanthrene removal

It was observed that bacteria enhanced the removal of phenanthrene from soil, i.e. the removal of phenanthrene in treatment B + P was more efficient than CK. The presence of bacteria could increase the degradation rate of phenanthrene, and it also plays an important role in enhancing plant tolerance to stressful conditions [85]. A negative correlation between soil dehydrogenase and phenanthrene concentration in soil was observed (Table S10). As a result of the high enzyme activities, the phenanthrene concentration decreased. Therefore, the presence of *B. cepacia* protected plants from metal and PAH toxification, leading to a further increase in enzymatic activities, which is important for enhancing the degradation of phenanthrene in soil.

5. Conclusion

The present study elucidated the interactions between a hyperaccumulating plant and bacteria on soil co-contaminated with heavy metal and phenanthrene. Though bacterial inoculation can improve soil N and P nutrition, metal availability in soil decreased. In addition, inoculation of bacteria did not enhance metal uptake and accumulation by *S. alfredii*. This might be due to the precipitation effects of elevated P concentration in soil. The present study indicated that inoculation of bacteria enhanced metal tolerance of *S. alfredii* and increased its phytoextraction efficiency for Cd and Pb by 8.8–31.2% and 37.7–124%. The bacteria used in this study also increased the removal of phenanthrene. Therefore, the use of integrated methods to enhance remediation process may be an optimal solution for the clean up of mixed persistent contaminants and heavy metals from the environment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.01.055.

References

[1] S. Baran, J.E. Bielinska, P. Oleszczuk, Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons, Geoderma 118 (2004) 221–232.
[2] S.C. Wilson, K.C. Jones, Bioremediation of soil contaminated with polycyclic aromatic hydrocarbons (PAHs): a review, Environ. Pollut. 81 (1993) 229–249.
[3] M. Alexander, Biodegradation and Bioremediation, Academic Press Inc., San Diego, USA, 1999.
[4] I. Raskin, R.D. Smith, D.E. Salt, Phytoremediation of metals: using plants to remove pollutants from the environment, Curr. Opin. Biotechnol. 8 (1997) 221–226.
[5] A.J.M. Baker, S.P. McGrath, C.M.D. Sidoli, R.D. Reeves, The possibility of *in situ* heavy metal decontamination of polluted soil using crops of metal-accumulating plants, Resour. Conserv. Recycl. 11 (1994) 41–49.
[6] P. Römkens, L. Bouwman, J. Japenga, C. Draaisma, Potentials and drawbacks of chelate-enhanced phytoremediation of soils, Environ. Pollut. 116 (1) (2002) 109–121.
[7] S.D. Siciliano, J.J. Germida, Bacteria inoculants of forage grasses enhance degradation of 2-chlorobenzoiz acid in soil, Environ. Toxicol. Chem. 16 (1997) 1098–1104.
[8] B.E. Kalinowski, L.J. Liermann, S.L. Brantley, X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende, Geochim. Cosmochim. Acta 107 (2000) 225–231.
[9] W.C. Li, Z.H. Ye, M.H. Wong, Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii*, Plant Soil 326 (2009) 453–467.
[10] R. Idris, R. Trifonova, M. Puschenreiter, W.W. Wenzel, A. Sessitsch, Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*, Appl. Environ. Microbiol. 70 (5) (2004) 2667–3277.
[11] W.C. Li, M.H. Wong, Effects of bacteria on metal bioavailability, speciation and mobility in different metal mine soils: a column study, J. Soil Sediment 10 (2010) 313–325.
[12] S.N. Whiting, M.P. de Souza, N. Terry, Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*, Environ. Sci. Technol. 35 (2001) 3144–3150.
[13] R.I. Abou-Shanab, J.S. Angle, T.A. Delorme, R.L. Chaney, P. van Berkum, H. Moawad, K. Ghanem, H.A. Ghozlan, Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*, New Phytol. 158 (2003) 219–224.
[14] S.Y. Xu, Y.X. Chen, W.X. Wu, K.X. Wang, Q. Lin, X.Q. Liang, Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation, Sci. Total Environ. 363 (2006) 206–215.
[15] S.D. Siciliano, C.W. Greer, Plant-bacterial combinations to phytoremediate soil contaminated with high concentrations of 2,4,6-trinitrotoluene, J. Environ. Qual. 29 (2000) 311–316.
[16] B. Maliszewska-Kordybach, B. Smreczak, Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination, Environ. Int. 28 (2003) 719–728.
[17] U.C. Brinch, F. Ekelund, C.S. Jacobsen, Method for spiking soil samples with organic compounds, Appl. Environ. Microbiol. 68 (2002) 1808–1816.
[18] W.C. Li, Z.H. Ye, M.H. Wong, Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant *Sedum alfredii*, J. Exp. Bot. 58 (2007) 4173–4182.
[19] J.W.C. Wong, K.M. Lai, C.K. Wan, K.K. Ma, M. Fang, Isolation and optimization of PAH-degradative bacteria from contaminated soil for PAHs bioremediation, Water Air Soil Pollut. 139 (2002) 1–13.
[20] D.R. Hoagland, D.I. Arnon, The Water-culture Method for Growing Plants Without Soil, California Agricultural Experiment Station Circular No. 347, 1950.

[21] G.V. Subbarao, N. Ae, T. Otani, Genotypic variation in iron and aluminum-phosphate solubilizing activity of pigeonpea root exudates under P deficient conditions, Soil Sci. Plant Nutr. 43 (1997) 295–305.
[22] X.E. Yang, V.C. Baligar, D.C. Martens, R.B. Clark, Cadmium effects on influx and transport of mineral nutrients in plant species, J. Plant Nutr. 19 (1996) 643–656.
[23] A.R. Marin, S.R. Pezeshki, P.H. Masscheleyn, H.S. Choi, Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants, J. Plant Nutr. 16 (1993) 865–880.
[24] A.L. Page, R.H. Miller, D.R. Keeney, Methods of Soil Analysis—Chemical and Microbiological Properties, 2nd edition, ASA, Inc./SSSA, Inc., Madison, WI, USA, 1982.
[25] S.R. Olsen, L.E. Sommers, Phosphorus, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis, Part 2, SSSA, Madison, WI, 1982, p. 403.
[26] USEPA, Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically based Matrices, U.S. Environmental Protection Agency, Washington, DC, 1996.
[27] L.Q. Ma, A.L. Choate, G.N. Rao, Effects of incubation and phosphate rock on lead extractability and speciation in contaminated soils, J. Environ. Qual. 26 (1997) 801–807.
[28] W.L. Lindsay, W.A. Norvell, Development of DTPA test for zinc, iron, manganese, and copper, Soil Sci. Soc. Am. 42 (1978) 421–428.
[29] D.A. Wilkins, The measurement of tolerance to edaphic factors by means of root growth, New Phytol. 80 (1978) 623–633.
[30] USEPA, Method 3540C, Soxhlet Extraction, U.S. Environmental Protection Agency, Washington, DC, 1996.
[31] USEPA, Method 3620B, Florisil Cleanup, U.S. Environmental Protection Agency, Washington, DC, 1996.
[32] USEPA, Method 8270C, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), U.S. Environmental Protection Agency, Washington, DC, 1996.
[33] J.T. Trevors, Dehydrogenase in soil: a comparison between the INT and TTC assay, Soil Biol. Biochem. 16 (1984) 673–674.
[34] K. Alef, P. Nannipieri, Methods in Applied Soil Microbiology and Biochemistry, Academic Press, London, 1995.
[35] S.P. McGrath, F.J. Zhao, E. Lombi, Phytoremediation of metals, metalloids, and radionuclides, Adv. Agron. 75 (2002) 1–56.
[36] F.Y. Wang, X.G. Lin, R. Yin, Role of microbial inoculation and chitosan in phytoextraction of Cu, Zn, Pb and Cd by *Elsholtzia splendens*—a field case, Environ. Pollut. 147 (1) (2006) 248–255.
[37] E. Stoltz, M. Greger, Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings, Environ. Exp. Bot. 47 (2002) 271–280.
[38] F. Yan, S. Schubert, Soil pH changes after application of plant shoot materials of faba bean and wheat, Plant Soil 220 (2000) 279–287.
[39] S.E. Lorenz, R.E. Hamon, P.E. Holm, H.C. Domingues, E.M. Sequeira, Cadmium and zinc in plants and soil solutions from contaminated soils, Plant Soil 189 (1997) 21–23.
[40] Y.M. Luo, P. Christie, A.J.M. Baker, Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of *Thlaspi caerulescens* grown in a Zn/Cd-contaminated soil, Chemosphere 41 (2000) 161–164.
[41] M.J. Hedley, P.H. Nye, R.E. White, Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings, II. Origin of the pH change, New Phytol. 91 (1982) 31–44.
[42] K. Bosecker, Bioleaching: metal solubilization by microorganisms, FEMS Microbiol. Rev. 20 (1997) 591–604.
[43] S. De Neve, J. Van De Steene, R. Hartman, G. Hofman, Using time domain reflectometry for monitoring mineralization of nitrogen from soil organic matter, Eur. J. Soil Sci. 51 (2000) 295–304.
[44] A.M. Ure, C.M. Davidson, Chemical Speciation in the Environment, Blackie Academic & Professional, London, 1995.
[45] M. Puschenreiter, G. Stöger, E. Lombi, O. Horak, W.W. Wenzel, Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: rhizosphere manipulation using EDTA and ammonium sulphate, J. Plant Nutr. Soil Sci. 164 (2001) 615–621.
[46] E. Hoberg, P. Marschner, R. Lieberei, Organic acid exudation and pH changes by *Gordonia* sp. and *Pseudomonas fluorescens* grown with P adsorbed to goethite, Microbiol. Res. 160 (2005) 177–187.
[47] G.E. Welbaum, A.V. Sturz, Z. Dong, J. Nowak, Managing soil microorganisms to improve productivity of agro-ecosystems, Crit. Rev. Plant Sci. 23 (2004) 175–193.
[48] P. Marschner, J. Gerendas, B. Sattelmacher, Effect of N concentration and N source on root colonization by *Pseudomonas fluorescens* 2-79RLI, Plant Soil 215 (1999) 135–141.
[49] J.C. Tarafdar, H. Marschner, Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus, Soil Biol. Biochem. 26 (1994) 387–395.
[50] S.A. Hebrien, J.L. Neal, Soil pH and phosphatase activity, Commun. Soil Sci. Plan. 21 (1990) 439–456.
[51] C. Lors, C. Tiffreau, A. Laboudigue, Effects of bacterial activities on the release of heavy metals from contaminated sediments, Chemosphere 56 (2004) 619–630.
[52] M.A. Whitelaw, T.J. Harden, K.R. Helyar, Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*, Soil Biol. Biochem. 31 (1999) 655–665.
[53] S.C. Wu, Y.M. Luo, K.C. Cheung, M.H. Wong, Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: a laboratory study, Environ. Pollut. 144 (3) (2006) 765–773.
[54] R. Melamed, X. Cao, M. Chen, L.Q. Ma, Field assessment of lead immobilization in a contaminated soil after phosphate application, Sci. Total Environ. 305 (2003) 117–127.
[55] S.L. Brown, R. Chaney, J. Hallfrisch, J.A. Ryan, W.R. Berti, *In situ* soil treatments to reduce the phyto- and bioavailability of lead, zinc, and cadmium, J. Environ. Qual. 33 (2004) 522–531.
[56] R.L. Chaney, S.L. Brown, Y.M. Li, J.S. Angle, T.I. Stuczynski, W.L. Daniels, C.L. Henry, G. Siebelec, M. Malik, J.A. Ryan, H. Compton, Progress in risk assessment for soil metals, and *in situ* remediation and phytoextraction of metals from hazardous contaminated soils, in: U.S-EPA Phytoremediation: State of Science, Boston, MA, May 1–2, 2000.
[57] K.A. Maenpaa, J.V.K. Kukkonen, M.J. Lydy, Remediation of heavy metal-contaminated soils using phosphorus: evaluation of bioavailability using an earthworm assay, Arch. Environ. Contam. Toxicol. 43 (2002) 389–398.
[58] G. Puppi, R. Azcón, G. Höflich, Management of positive interactions of arbuscular mycorrhizal fungi with essential groups of soil microorganisms, in: S. Gianinazzi, H. Schüepp (Eds.), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems, Birkhäuser Verlag, Basel, Switzerland, 1994, pp. 201–215.
[59] B.R. Glick, Phytoremediation: synergistic use of plants and bacteria to clean up the environment, Biotechnol. Adv. 21 (2003) 383–393.
[60] W.Z. Ni, Q. Sun, X. Yang, Growth and zinc accumulation of *Sedum alfredii* Hance—a Zn hyperaccumulator as affected by phosphorus application, Bull. Environ. Contam. Toxicol. 72 (2004) 756–762.
[61] A.O. Fayiga, L.Q. Ma, X.D. Cao, B. Rathinasabapathi, Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata* L., Environ. Pollut. 132 (2004) 289–296.
[62] D.M. Deng, W.S. Shu, J. Zhang, H.L. Zou, Z. Lin, Z.H. Ye, M.H. Wong, Zinc and cadmium accumulation and tolerance in populations of *Sedum alfredii*, Environ. Pollut. 147 (2006) 381–386.
[63] A. Vivas, B. Biro, J.M. Ruiz-Lozano, J.M. Barea, R. Azcon, Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity, Chemosphere 62 (9) (2006) 1523–1533.
[64] G. Hoflich, R. Metz, Interactions of plant-microorganism associations in heavy metal containing soils from sewage farms, Bodenkultur 48 (1997) 239–247.
[65] S. Dobbelaere, J. Vanderleyden, Y. Okon, Plant growth-promoting effects of diazotrophs in the rhizosphere, Crit. Rev. Plant Sci. 22 (2003) 107–149.
[66] M. Lucy, E. Reed, B.R. Glick, Application of free living plant growth-promoting rhizobacteria, Anton. Leeuw. Int. J. G. 86 (2004) 1–25.
[67] G. Höflich, W. Wiehe, G. Kühn, Plant growth stimulation with symbiotic and associative rhizoshpere microorganisms, Experientia 50 (1994) 897–905.
[68] R.M. Boddey, J. Döbereiner, Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future, Fert. Res. 42 (1995) 241–250.
[69] J.L. Moreno-Ortego, L. Falchini, G. Renella, L. Landi, P. Nannipieri, Use of the ecological dose (ED_{50}) to assess to toxicity of Cd on soil microbial and biochemical parameters, in: R.P. Dick (Ed.), Enzymes in the Environment: Activity Ecology & Applications, Granada, Spain, July 12–15, 1999, p. 129.
[70] S.P. McGrath, F.J. Zhao, Phytoextraction of metals and metalloids from contaminated soils, Curr. Opin. Biotechnol. 14 (2003) 277–282.
[71] R.F.M. Van Steveninck, M.E. Van Steveninck, D.R. Fernando, Heavy metal (Zn, Cd) tolerance in selected clones of duckweed (*Lemna minor*), Plant Soil 146 (1992) 271–328.
[72] E. Sieverding, Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems, Bremer Verlag, Germany, 1991, p. 371.
[73] A. Gupta, J.M. Meyer, R. Goel, Development of heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization, Curr. Microbiol. 45 (2002) 323–327.
[74] S.P. McGrath, F.J. Zhao, E. Lombi, Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils, Plant Soil 232 (2001) 207–214.
[75] P. Doelman, Resistance of soil microbial communities to heavy metals, in: V. Jensen, A. Kjoelles, L.H. Soerensen (Eds.), Microbial Communities in Soil, Elsevier, London, 1985, pp. 369–384.
[76] R. Boopathy, Factors limiting bioremediation technologies, Bioresour. Technol. 74 (2000) 63–67.
[77] J. Skujins, Extracellular enzymes in soil, Crit. Rev. Microbiol. 4 (1976) 383–421.
[78] B.P. Degens, G.P. Sparling, L.K. Abbott, Increasing the length of hyphae in a sandy soil increases the mount of water-stable aggregates, Appl. Soil Ecol. 3 (1996) 149–159.
[79] J. Sikkema, J.A.M. De Bont, B. Poolman, Interactions of cyclic hydrocarbons with biological membrane, J. Biol. Chem. 269 (1994) 8022–8028.
[80] A. Gogolev, B.M. Wilke, Combination effects of heavy metals and fluoranthene on soil bacteria, Biol. Fert. Soils 25 (1997) 274–278.
[81] C. García, T. Hernández, J.A. Pascual, J.L. Moreno, M. Ross, Soil microbial activity as biomarker of degradation and rehabilitation processes, in: R.P. Dick (Ed.), Enzymes in the Environment: Activity Ecology & Applications, Granada, Spain, 1997, p. 124.
[82] A. Cébron, B. Louvel, P. Faure, C. France-Lanord, Y. Chen, C.J. Murrell, C. Leyval, Root exudates modify bacterial diversity of phenanthrene degraders in PAH-polluted soil but not phenanthrene degradation rates, Environ. Microbiol. 13 (3) (2011) 722–736.
[83] D. Técher, P. Laval-Gilly, S. Henry, A. Bennasroune, P. Formanek, C. Martinez-Chois, M. D'Innocenzo, F. Muanda, A. Dicko, K. Rejšek, J. Falla, Contribution of Miscanthus × giganteus root exudates to the biostimulation of PAH degradation: an in vitro study, Sci. Total Environ. 409 (20) (2011) 4489–4495.

[84] A. Medina, A. Probanza, F.J. Gutierrez Mañero, R. Azcón, Interactions of arbuscular-mycorrhizal fungi and *Bacillus* strains and their effects on plant growth, microbial rhizosphere activity (thymidine and leucine incorporation) and fungal biomass (ergosterol and chitin), Appl. Soil Ecol. 22 (2003) 15–28.

[85] X.D. Huang, Y. El-Alawi, D.M. Penrose, B.R. Glick, B.M. Greenberg, A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils, Environ. Pollut. 13 (3) (2004) 465–476.