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Full Length Research Paper

Evaluation of inoculation of plant growth-promoting rhizobacteria on cadmium and lead uptake by canola and barley

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Plant growth promoting bacteria are important for plant growth and heavy metal uptake of metal-polluted soils. In this study, to isolate (cadmium and lead) Cd and Pb-resistant bacteria, seven strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were plated onto Tryptone Soya Broth supplemented with different concentrations of $Pb(No_3)_2$ and $CdCl_2$ (0, 30, 60 mg L^{-1}). Three heavy metals resistant isolated strains included *P. putida* strain 11 (*P.p.*11*)*, *P. putida* strain 4 (*P.p.*4*)* and *P. fluorescens* strain 169 (*P.f.*169*)* were investigated. The bacteria were able to produce indole-3-acetic acid (IAA), siderophores and 1-aminocyclopropane-1-carboxylic deaminase (ACCD). They were inoculated to canola and barly seeds in a soil artificially contaminated with Cd (10 and 20 mg kg^{-1}) and Pb (300 and 600 mg kg^{-1}) in a pot experiment. Results showed that the inoculation with these PGPR increased shoot dry matter of canola. An increase in Cd and Pb uptake was observed in canola inoculated with *P.p.*11 and *P.f.*169. Translocation factor indicated that inoculated canola with *P.f.*169 and barley with all of the three strains had abilities of Cd and Pb phytoextraction in the contaminated soil respectively. The bacterial strains protected the plants against the inhibitory effects of cadmium and lead probably due to production of IAA, siderophore and ACCD activity.

Key words: 1-aminocyclopropane-1-carboxylic deaminase (ACC deaminase), canola, heavy metals, phytoextraction, *Pseudomonas*.

INTRODUCTION

Heavy metals are continuously being added to soils through various agricultural and industrial such as the use of agrochemicals and the long-term deposition of urban sewage sluge on agricultural soils, waste disposal, waste incineration and vehicle exhausts. All these sources cause accumulation of these elements in agricultural soils and pose a threat to food safety and potential health risks (Dell Amico et al., 2008).

Unlike organic compounds, heavy metals can not be degraded and the clean up usually requires their removal (Lasat, 2002), so it has led to an increased in developing systems that can remove or neutralize its toxic effects in soil, sediments and wastewater (Valls and Lorenzo, 2002). Phytoremediation is emerging as a potential cost-effective solution for the remediation of contaminated soils (Blaylock et al., 1997), but is generally time-consuming (Baker et al., 2000). In general, the ideal plant species to remediate a heavy metal-contaminated soil should be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest (Ebbs et al., 1997).

The interface between microbes and plant roots (rhizosphere) is considered to greatly influence on the growth and survival of plants (Rajkumar and Freitas, 2008). Metal-tolerant plant–microbe associations have

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Table 1. Characteristics of the soil used in the pot experiments.

Soil name	pH	$Caco_3$ (%)	EC (ds m^{-1})	OM (%)	P[a]	K[a]	Total content of elements (mg kg^{-1})					
							Mn	Cu	Fe	Zn	Cd	Pb
Typic haplocalcid	7.8	37.5	5	0.85	15	125	354.5	25	185.5	149.5	4.5	38

[a]The datas are given as phosphorous and potassium concentration is available content (mg kg^{-1}).

been the objective of particular attention due to the potential of microorganisms for bioaccumulating metals from polluted environment or its effects on metal mobilization/immobilization and consequently enhancing metal uptake and plant growth (Glick, 2010). Several of the plant-associated bacteria have been reported to accelerate phytoremediation in metal-contaminated soils by promoting plant growth and health, and they play a significant role in accelerating phytoremediation (Kuffner et al., 2008; Compant et al., 2010; Grandlic et al., 2008; Dary et al., 2010; Kidd et al., 2009).

The mechanisms enabling those plant growth-promoting rhizobacteria (PGPR) to promote the growth of the host plant and metal accumulation process may be included: (a) synthesize some compounds such as indole-3-acetic acid (IAA), siderophores, organic acids and 1-aminocyclopropane-1-carboxylic (ACC) deaminase, (b) stimulate certain metabolic pathways like nitrogen fixation and phosphate uptake, and (c) affect the metal mobility and availability to plant (Idris et al., 2004).

With respect to the negative effects of Cd and Pb, attempting to alleviate the intense effects of them with the use of bacterial inoculation has a great significance. As there are very little data on the effects of fluorescent pseudomonads containing plant growth promoting properties on heavy metal uptake by plant in Iran contaminated soils, this research is of particular interest. The objective of this research was to test the effect of fluorescent pseudomonads including *Pseudomonas fluorescence* and *Pseudomonas putida* on plant biomass production, Cd and Pb translocation from root to shoot and Cd and Pb-uptake by barley and canola in Cd and Pb contaminate soils.

MATERIALS AND METHODS

Isolation of cadmium and lead tolerant bacteria

To isolate and characterize fluorescent pseudomonads, 28 combined soil samples from the Iranian provinces were collected. Using the method of King et al. (1954), 70 isolates with high levels of fluorescence were selected and purified on a King B medium (peptone 20 g; $MgSO_4$ 1.5 g; K_2HPO_4 1.5 g; glycerol 10 ml; agar 20 g and distilled water 1 L). After physiological and biochemical tests 25 isolates of *P. putida* and 25 isolates of *P. fluorescens* were identified, and some were randomly selected and kept for the following experiments on a slanted medium of King B.

To screen the isolates as being able to utilize ACC as the sole N-source, the method of Glick et al. (1995) was used with some modifications. Seven strains were determined as the strains to utilize ACC as the sole N-source thus, these strains with the ability to utilize ACC as the sole N-source due to the production of ACC deaminase were isolated. Further, Salkowski indicator was used to measure the isolates' ability to produce auxin (Benizri et al., 1998) and siderophore production was analysed on chrome azurol S agar plates (Schywn and Nielands, 1987).

To isolate Cd and Pb-resistant bacteria, seven strains were plated onto TSB (Tryptone Soya Broth) agar medium supplemented with different concentrations of $Pb(NO_3)_2$ (0, 30, 60 mgL^{-1}) and of $CdCl_2$ (0, 30, 60 mgL^{-1}). After incubation at 28°C for 4 d the content of light absorption at 405 nm wavelength was determined. The bacteria showed higher resistance to Cd and Pb toxicity were selected for further study.

Bacterial suspension preparation

For inoculation, the selected bacterial strains were grown in TSB medium on a shaker at 250 rpm at 30°C for 24 h. Bacterial cells in the exponential phase were harvested by centrifugation at 1500g, 10 min, and the pellets were washed twice with sterile distilled water, and recentrifuged. Bacterial suspensions in distilled water were adjusted to get an inoculum density of Ca 10^5 colony forming units (CFU) m L^{-1} (Sheng et al., 2008).

Preparation of Cd and Pb-contaminated soil

The soil was firstly collected from the upper terrace of Zayanderood River in Isfahan, Iran. The soil was air-dried and sieved (2 mm) to remove plant materials and stones. The soil was classified as Fine Loamy Mixed Typic haplocalcid. Total concentration of Cd and Pb in the soil was measured with an atomic absorption spectrometer (AAS) (Perkin Elmer 2380; Carter, 1993). The soil artificially was contaminated with aqueous solution of $CdCl_2$ (0, 10, 20 mg kg^{-1}) and of $Pb(No_3)_2$ (0, 300, 600 mg kg^{-1}). The total heavy metals concentrations and some selected soil properties are listed in Table 1. Before using, the amended soils were left in the greenhouse for a month to stabilize metal.

Pot experiments

A mixture of 5 kg (dry weight) of artificially contaminated soil and sand was put into plastic pots. Seeds of barley (Karondarkavir CV.) and canola (Sarigol CV.) were surface-sterilized with a solution of 1.5% (v/v) sodium hypochlorite for 10 min and washed with sterile water. Six seeds were sown in each pot at a depth of approximately 2 cm below the soil surface.

5 m L^{-1} of the bacterial suspension was added to every seed on each pot for the inoculation treatment while the uninoculated treatment received sterile water as a control. The pots were placed in a greenhouse at 25°C and were moistened with sterile water and maintained at 60% of its water holding capacity (WHC).

Table 2. Characteristics of bacterial strains.

Bacterial strains	ACCD activity	IAA production (mg kg^{-1})	Siderophore production (mm)
P. putida strain108	+	8.9	1.7
P. putida strain11	+	7.67	1.6
P. putida strain159	+	6.79	2.1
P. putida strain 4	+	9.6	1.9
P. fluorescens strain196	+	6.8	1.7
P. fluorescens strain169	+	5.8	1.8
P. fluorescens strain79	+	6.1	1.5

P. : *Pseudomonas.*

A factorial experiment on the basis of a completely randomized block design with three replicates were performed. The experimental factors included two plants (canola and barley), two heavy metals (Cd, Pb), three levels of 0, 10, 20 and 0, 300, 600 mg kg-1 which were prepared using $CdCl_2$ and $Pb(No_3)_2$ sources respectively, and four strains of bacteria, *P.p.*4, *P.p.*11 and *P.f.*169, and a treatment without bacteria (as the control treatment).

Analysis of biomass and contents of cadmium and lead

After 8 weeks, plant samples were collected, divided into above-aground shoots and roots and washed three times with deionized water. To determine the dry weight, shoots and roots of plants were oven-dried separately at 75°C for 24 h and were ground to 0.5 mm for analysis. Then 0.2 g of shoots and 0.1 g of roots were digested with the H_2So_4/H_2O_2 method (Harmon and Lajtha, 1999). The digested solution was diluted with deionized water to a volume of 50 mL^{-1} in a flask. The concentrations of Cd and Pb were measured with an AAS. In addition, the available and total Cd and Pb concentrations in the soil were extracted by diethylene triamine pentaacetic acid (DTPA) (Lindsay and Norvell, 1978) and digestion in a mixture of concentrated HNo_3 and HCl (Carter, 1993) were determined by AAS respectively.

Two uptake amounts (UA) in aerial and root, and translocation factor (TF) as defined in Equation (1) and (2) which were computed from the treatment concentrations will be used to discuss the results from this study.

$$UA = \text{concentration} * \text{biomass} \quad (1)$$

$$TF = \frac{\text{Carial}}{\text{Croot}} \quad (2)$$

Statistic analysis

Statistical analysis was done with SAS software. Analysis of variance (ANOVA) followed by post hoc Fisher LSD test. All analyses were performed at the $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Characterization of ACC deaminase-producing fluorescent pseudomonads

Different strains differed in their ability to utilize ACC as the sole N-source. Only seven strains, *P. putida* strain 11 (*P.p.*11), *P. putida* strain 4 (*P.p.*4), *P. fluorescence* strain 169 (*P.f.*169), *P. fluorescence* strain108 (*P.f.*108), *P. fluorescence* strain 196 (*P.f.*196), *P. fluorescence* strain 159 (*P.f.*159) and *P. fluorescence* strain 79 (*P.f.*79), were able to utilize ACC as the sole N-source due to the production of ACC deaminase. The bacteria differed in their ability to produce auxin, ranging from 5.8 to 9.6 mg L^{-1}. As it can be seen in Table 2, the ability to produce siderophore differed between bacteria from 1.5 to 2.1 mm.

Isolation and selection of metal-resistant bacteria

With increase in the Pb level in the TSB medium from 30 to 60 mg L^{-1}, bacterial resistance did not decrease. Three strains *P.p.*11, *P.p.*4 and *P.f.*169 showed more resistance when the concentration of Cd in the medium reached 60 mg L^{-1} (Table 3). Our results indicate that the strains *P.p.*11, *P.p.*4 and *P.f.*169 are tolerant to Cd and Pb, so these strains were selected for future experiments.

Effects of metal-resistant bacteria on the growth of canola and barley

Mean shoot dry matter in canola and barley ranged from 3.76 to 7.02 g pot^{-1} and 1.67 to 4.28 g pot^{-1} respectively. Mean root dry matter in canola and barley ranged from 0.38 to 0.73 g pot^{-1} and 0.23 to 0.56 g pot^{-1} respectively (Table 4). Compared with the plants grown in the absence of Cd and Pb, the non-inoculated plants grown in the Cd and Pb-contaminated soil showed decrease in shoots and roots dry weight. However, plants inoculated with PGPR showed an increase in shoot dry weight despite in the presence of Cd and Pb.

In canola at the concentration of 10 mg Cd kg^{-1} the greatest effect was found for *P.p.*11, *P.f.*169 and at Cd concentration of 20 mg kg^{-1} *P.p.*11 showed the most excellent growth effect. For the Pb-treated pots at the level of 300 mg Pb kg^{-1} shoot dry weight increased when the soil was inoculated with *P.f.*169 in comparison with

Table 3. Bacterial growth in different concentrations of heavy metals (light absorption at 405 nm wave length).

Bacterial strains	Cd (mg kg^{-1})		Pb (mg kg^{-1})		Blank
	30	60	30	60	
P. putida strain108	1.60	1.58	2.80	2.85	2.50
P. putida strain11	2.43	0.17	2.75	2.73	2.63
P. putida strain159	1.83	0.02	2.68	2.66	2.80
P. putida strain 4	2.35	0.22	2.75	2.84	2.88
P. fluorescens strain196	0.45	0.20	2.71	2.74	2.40
P. fluorescens strain169	1.99	0.65	2.69	2.60	2.70
P. fluorescens strain79	0.73	0.02	2.71	2.66	2.72

P. : *Pseudomonas.*

Table 4. Effects of different isolates on the dray weight (g pot^{-1}) of canola and barley, at different concentration of heavy metals (pot experiment, mean of three replicas per treatment).

plant	Bacterial strains	Cd (mg kg^{-1})						Pb (mg kg^{-1})					
		0		10		20		0		300		600	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Canola	*P. p. 4*	0.52b	3.92b	0.61a	4.90b	0.38b	5.61ab	0.62b	4.91b	0.53a	3.91b	0.44a	3.76b
	P. p. 11	0.54ab	6.28a	0. 73a	6.48a	0.58a	6.70a	0.67ab	6.8a	0.56a	5.71ab	0.43a	4.80a
	P. f. 169	0.63a	4.73 b	0.66a	7.02a	0.58a	5.34ab	0.71a	5.63 b	0.52a	6.21a	0.38a	4.40ab
	Control	0.59ab	4.88 b	0. 57a	4.74b	0.51ab	4.21b	0.65ab	5.58 b	0.62a	4.13b	0.46a	3.94ab
Barley	*P. p. 4*	0.42a	3.94 a	0.36a	2.95a	0.35a	1.67a	0.42a	2.99 b	0.31a	2.54a	0.29a	2.49a
	P. p. 11	0.48a	3.27 a	0.42a	2.21a	0.23a	2.14a	0.48a	2.93 b	0.37a	2.39a	0.35a	2.16a
	P. f. 169	0.47a	3.91 a	0.39a	2.00a	0.25a	2.01a	0.47a	4.28 a	0.36a	3.00a	0.29a	2.30a
	Control	0.56a	3.32 a	0.51a	2.68a	0.30a	2.06a	0.51a	3.3 b	0.41a	2.49a	0.34a	2.27a

P. f.: *Pseudomonas fluorescens*, *P. p.*: *Pseudomonas putida.*

the non-inoculation soil (Table 4). The content of shoot dry matter in the canola with *P.p.*169 treatment was the highest (7.02 g pot^{-1}). In both canola and barley the maximum shoot dry matter were obtained in the pots were inoculated with beneficial bacteria. In general, both addition of Cd, Pb and bacteria increased significantly the shoot dry matter of canola (Table 4). There were no obvious differences in root dry weight between the inoculated plants and the plants without inoculation.

Inoculation with the Cd and Pb-resistant bacteria lightened the inhibitory effects of Cd and Pb and stimulated the growth of canola to some extent. This approach was in agreement with previous observation that inoculation of rhizosphere with PGPRs increased plant biomass in *Brassica juncea* grown on contaminated soils (Belimov et al., 2001).

PGPR may interact in several ways with plants to improve their growth. The PGPR with ACC deaminase activity decreased plant stress by efficiently blocking ethylene production (Cheng et al., 2007) and enhanced plant growth. Ethylene is important for plant growth, but an excess of ethylene promoted by stresses like heavy metals can inhibit plant development. Glick et al. (1998), suggested a model in which the rhizosphere bacteria, producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, may reduce plant ethylene and thereby alleviate the stress. ACC is involved in biosynthetic pathway of ethylene, as an intermediate in the conversion of methionine to ethylene (Adams and Yang, 1979). In general, ACC is exuded from plant roots or seeds and then taken up by the ACC-utilizing bacteria (Contesto et al., 2008) and cleaved by ACC deaminase to α-ketobutyrate and ammonia. The bacteria utilize the ammonia evolved from ACC as a sole nitrogen source and thereby decrease ACC within the plant (Penrose and Glick, 2001).

PGPR also enhance plant growth through the synthesis of the plant auxin IAA. In general, the IAA which is produced by microbe, promotes root growth directly by stimulating plant cell elongation or cell division and contributes to plant growth as well plant defense system development (Patten and Glick, 2002; Glick et al.,

Table 5. Influence of bacterial inoculation on above-ground tissue and root metal concentration (mg kg^{-1}) of canola and barley, at different concentration of heavy metals.

Plant	Bacterial strains	Cd (mg kg^{-1})						Pb (mg kg^{-1})					
		0		10		20		0		300		600	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Canola	*P. p. 4*	1.33a	1.73b	27.13a	27.4ab	42.43a	36.6c	0.00a	0.00a	81.3c	7.2b	285.5b	14.76a
	P. p. 11	1.00a	2.53a	26.13a	32.1a	42.86a	45.2b	0.00a	0.00a	126b	8.5ab	358.7a	9.93b
	P. f. 169	1.15a	2.20a	25.06a	26.9ab	37.5a	51.5a	0.00a	0.00a	151.6a	9.43a	312.4ab	14.4a
	Control	0.90a	2.30a	21.32a	24.46b	42.97a	40bc	0.00a	0.00a	75.6c	6.7b	222.4c	9.03b
Barley	*P. p. 4*	0.00a	1.20a	39.2ab	4.66a	77.1ab	5.6b	0.00a	0.00a	60.47b	7.1ab	254.38b	21.53a
	P. p. 11	0.00a	0.93a	49.2a	4.7a	83a	5.6b	0.00a	0.00a	99.33a	10.00a	291.8a	20.00a
	P. f. 169	0.00a	1.20a	51.3a	2.46b	62.7b	4.93b	0.00a	0.00a	103.6a	10.20a	285.86a	14.4ab
	Control	0.00a	0.85a	33.09b	5.4a	76.8ab	7.26a	0.00a	0.00a	58.62b	4.53b	169.8c	12.0b

P. f.: *Pseudomonas fluorescens*, *P. p.*: *Pseudomonas putida.*

1998). According to the IAA level, root elongation changes qualitatively. A low level of IAA promotes primary root elongation whereas a high level of IAA stimulates lateral and adventitious root formation but inhibits the root growth (Xie et al., 1996). Thus, the IAA producing PGPR can facilitate plant growth by altering the plant hormonal balance.

Iron is a necessary cofactor for many enzymatic reactions and hence is an essential nutrient for virtually all organisms. Heavy metals contamination of soil has negative effects on iron nutrition. In contaminated soils, the siderophore producing rhizosphere bacteria might offer a biological rescue system that is capable of chelating Fe^{3+} and making it available to plant roots (Dimkpa et al., 2008a).

In addition, siderophores produced by rhizosphere bacteria bind heavy metal ions and thus enhance their bioavailability in the rhizosphere of plants (Braud et al., 2009). Phytosiderophores typically have a lower affinity for iron than microbial siderophores. As a result, increasing in trace metal uptake by the plants caused by microbial siderophores might enhance the effectiveness of phytoextraction processes of contaminated soil. (Dimkpa et al., 2008a).

Metal uptake

In canola the plants grown in soil inoculated with PGPR, above-grown tissue Cd and Pb concentrations were increased. Significant increases ($p<0.05$) of above-ground tissue Pb concentrations and significant decreases ($p<0.05$) of above-ground tissue Cd concentrations of barley were observed when the soil was inoculated with bacteria in comparison with the non-inoculated soil. Significant increases ($p<0.05$) of root Pb concentration of the both canola and barley and root Cd concentration of barley were obtained when the soil was inoculated with bacteria (Table 5).

The maximum Cd and Pb uptake in the above-ground tissues were obtained in canola compared with barley due to the greater biomass. Inoculation with *P.p.*11 and *P.f.*169 increased the uptake of Cd at the Cd levels of 10 and 20 mg kg^{-1} and inoculation with *P.f.*169 increased the uptake of Pb at the Pb levels of 300 and 600 mg kg^{-1} in the shoot of canola (Table 6).

Pot experiment demonstrated that the application of Cd- and Pb-solubilizing and growth-promoting bacteria could effectively increase the available Cd and Pb in rhizosphere soils and promote the growth of the canola, increasing the Cd and Pb uptakes of the canola even under non-sterile conditions. Studies have demonstrated that bacterial isolates significantly increased the bioavailability of heavy metals in soil (Chen et al., 2005). The same results were reached in our experiment, that the available Cd and Pb concentrations in the heavy metal-contaminated soil of canola were significantly enhanced by *P.f.*169 , *P.p.11*(at the concentration of 10 and 20 mg kg^{-1} Cd) and by *P.f.*169 (at Pb concentration of 300 mg kg^{-1}) (Table 7).

Some studies have demonstrated that heavy metal-resistant bacteria can enhance metal uptake by hyperaccumulator plants (Whiting et al., 2001; De Souza et al., 1999). Microorganism in rhizosphere can affect metal availability and mobility to the plant, which can produce chelators for ensuring metal availability, solubilize metal phosphates, and reduce soil pH (Abou-Shanab et al., 2006). Among the various metabolites produced by PGPR, the siderophores play a significant role in metal mobilization and accumulation (Rajkumar et al., 2010), as these compounds produced by PGPR solubilize unavailable forms of heavy metal-bearing Fe but also form complexes with bivalent heavy metal ions that can be assimilated by root mediated processes (Braud et al., 2009).

There was a higher uptake of Pb within the roots than

Table 6. Influence of bacterial inoculation on above-ground tissue and root metal uptake (mg pot^{-1}) of canola and barley, at different concentration of heavy metals.

Plant	Bacterial strains	Cd (mg kg^{-1})						Pb (mg kg^{-1})					
		0		10		20		0		300		600	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Canola	*P. p. 4*	0.0007a	0.007c	0.016a	0.13b	0.016b	0.2bc	0.00a	0.00a	0.04b	0.02b	0.12a	0.05ab
	P. p. 11	0.0005a	0.016a	0.015a	0.2a	0.025a	0.3a	0.00a	0.00a	0.07a	0.04ab	0.15a	0.04ab
	P. f. 169	0.0007a	0.013ab	0.017a	0.18a	0.017ab	0.27ab	0.00a	0.00a	0.08a	0.05a	0.12a	0.06a
	Control	0.0006a	0.011c	0.015a	0.11b	0.021ab	0.16c	0.00a	0.00a	0.04b	0.02b	0.11a	0.03b
Barley	*P. p. 4*	0.00a	0.0047a	0.014a	0.013a	0.027a	0.009b	0.00a	0.00a	0.18b	0.018a	0.074a	0.053a
	P. p. 11	0.00a	0.003a	0.02a	0.01a	0.019a	0.012ab	0.00a	0.00a	0.037a	0.024a	0.1a	0.043a
	P. f. 169	0.00a	0.0047a	0.02a	0.004b	0.016a	0.01b	0.00a	0.00a	0.037a	0.031a	0.08a	0.031a
	Control	0.00a	0.0028a	0.017a	0.014a	0.023a	0.015a	0.00a	0.00a	0.024ab	0.01a	0.074a	0.026a

P. f.: *Pseudomonas fluorescens*, P. p.: *Pseudomonas putida.*

Table 7. Effects of inoculation with strains on available and total Cd and Pb in soil (mg kg^{-1}) at different concentration of heavy metals.

Plant	Bacterial strains	Cd (mg kg^{-1})						Pb (mg kg^{-1})					
		0		10		20		0		300		600	
		Avail	Total	Avail	Total	Avail	Total	Avail	Total	Avail	Total	Avail	Total
Canola	*P. p. 4*	0.06a	1.83a	2.72b	6.00a	7.14ab	9.16a	3.4a	18.50a	115.00a	151.66b	225.67a	491.67 a
	P. p. 11	0.06a	1.83a	3.21a	6.16a	7.73a	9.33a	2.4a	14.83a	121.66a	148.83b	197.33a	566.7a
	P. f. 169	0.05a	1.83a	3.41a	6.33a	7.82a	9.16a	2.4a	19.33a	116.33a	174.83a	242.67a	541.7a
	Control	0.06a	1.83a	2.62b	5.83a	6.52b	9.00a	2.7a	17.83a	103.00b	147.00b	238.67a	391.67b
Barley	*P. p. 4*	0.06a	2.00a	2.56b	5.83a	6.9ab	9.00a	2.9a	27.16a	110.33b	146.00a	234.66b	341.7a
	P. p. 11	0.06a	2.00a	2.78a	5.83a	7.59a	9.16a	2.3a	19.2c	111.00b	149.00a	233.45b	308.7a
	P. f. 169	0.06a	1.83a	2.54b	6.00a	6.36b	9.00a	2.5a	26.2ab	122.00a	144.83a	268.25a	321.0a
	Control	0.06a	1.66a	2.6ab	5.83a	7.19ab	8.66a	2.7a	20.0bc	109.33b	149.33a	248.66b	350.0a

P. f.: *Pseudomonas fluorescens*, P. p.: *Pseudomonas putida.*

within the shoots of plants grown in Pb-treated soil. As shown in Table 6, the root of canola inoculated with *P.p.*11 and *P.f.*169 showed the highest uptake of Pb. This suggests that Pb from the soil is readily taken into the plant, yet there is a small amount of translocation of this metal into the above- ground tissues.

The inoculation with *P.p.*4 and *P.f.*169 significantly decreased the uptake of Cd in the above- ground tissues of barley (Table 6). In this situation the harvestable parts of barley might be utilized for human or animal consumption. It was shown in some experiments, *Flavobacterium* sp.L30 and *Klebsiella mobilis* CIAM could accumulate soluble free Cd ions in bacterial mass and immobilize free Cd ions extracellularly by binding Cd in complex forms. Since soluble metal is more toxic than bound or percipitated metal, the PGPR reduced significantly Cd toxicity and availability it for barley. The Cd binding resulted in decreasing of Cd uptake by barley plants (Pishchik et al., 2002).

Translocation factor (TF) of plants in Cd and Pb treatments

TF values can describe movement and distribution of heavy metals in plants. TF values were studies in each treatment. In this study, as shown in Table 8, *P.f.*169 enhanced TF values in canola at the Cd level of 20 mg kg^{-1}. In contrast, *P.p.*11 and *P.f.*169 decreased TF in barley at Cd concentration of 10 mg kg^{-1}. The results suggested that canola inoculated with *P.p.*169 had higher potential for Cd remediation of Cd-contaminated soils. The level of Cd in plants might be affected by several physiological factors of plants, including Cd uptake from the solution, xylem translocation from root to shoot and sequestration of Cd (in subcellar compartments or as

Table 8. The effect of different isolates on the translocation factor (TF) of canola and barley, at different concentration of heavy metals.

Plant	Bacterial strains	Cd (mg kg^{-1})			Pb (mg kg^{-1})		
		0	10	20	0	300	600
Canola	*P. p. 4*	1.38b	1.08a	0.87b	0.00a	0.09a	0.05a
	P. p. 11	2.6a	1.23a	1.08ab	0.00a	0.07b	0.03b
	P. f. 169	1.9a	1.09a	1.4a	0.00a	0.06b	0.05a
	Control	2.55a	1.15a	0.93b	0.00a	0.09a	0.04a
Barley	*P. p. 4*	0.00a	0.12ab	0.073a	0.00a	0.13a	0.08a
	P. p. 11	0.00a	0.01b	0.068a	0.00a	0.1a	0.068a
	P. f. 169	0.00a	0.04c	0.08a	0.00a	0. 1a	0.05a
	Control	0.00a	0.16a	0.095a	0.00a	0.07b	0.07a

P. f.: *Pseudomonas fluorescens*, *P. p.*: *Pseudomonas putida.*

organic complexes) (Hart et al., 1998).

The inoculated canola with *P.p.*11, *P.f.*169 (at the level of 300 mg kg^{-1} Pb) and with *P.p.*11 (at the concentration of 600 mg kg^{-1} Pb) significantly decreased TF values. On the other hand, all of the three strains significantly increased translocation of Pb at concentration of 300 mg kg^{-1} in barley compared to the plants which were not inoculated (Table 8), so barley with all of PGPR had better ability of Pb phytoextraction.

Higher TF values may suggest that the plant can take up Cd and Pb from soil and store them in the shoots with great efficiency (Zhou and Song, 2004). Bioaccumulation depends not only on the characteristics of the organism itself, but also on the characteristics of the substance and the environment factors (Niu Zhin-Xin et al., 2007). An explanation for translocation of heavy metals in plants was given by Salt et al. (1995), who said that ABA-induced stormatal closure dramatically reduced Cd or Pb accumulation in shoots of *Indian mustard*. Cellular sequestration of Cd or Pb can have a large effect on the levels of free Cd or Pb in the symplast and, thus, in the potential movement of Cd or Pb through out the plants.

Conclusions

The utilizing of PGPR to lighten heavy-metal contamination was a feasible and effective method for phytoremediation. Our study demonstrated that inoculation with the three ACC-utilizing bacteria was an efficient method for protecting canola and barley seeds from growth inhibition caused by toxic concentrations of Cd and Pb. Pot experiments showed that the application of Cd and Pb-resistant bacteria increased Cd and Pb phytoremediation efficiency. *P.p.*11 and *P.f.*169 strains had the greater effect. Canola with *P.p.*169 showed better ability of Cd phytoextraction at the Cd level of 20 mg kg^{-1} and barley with all of three strains had better potential of Pb phytoextraction at concentration of 300 mg kg^{-1}. In Comparison with control treatments, inoculation with rhizobacteria influenced the root and above-ground biomass of canola at the Pb concentration of 300 mg kg^{-1}, the concentration of 10 and 20 mg kg^{-1} of Cd. Inoculation with rhizobacteria influenced the metals concentration in shoot and root, metals uptake and metals translocation factor (TF) of both plants at the both concentrations of Cd and Pb. The more PGPR properties present in an organism, the more likely the chances of that organism being a successful inoculant strain. Extensive research in the areas of colonization capability, the role of rhizobacteria and plant roots in the uptake of metals and interaction between them is required to ellucidate the mechanisms of PGPR protection against toxic elements in soil to achieve the stabilization, revegetation and remediation of metal-polluted soils.

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