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Investigating the effects of plant growth promoting bacteria and *Glomus Mosseae* on cadmium phytoremediation by *Eucalyptus camaldulensis* L.

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ABSTRACT: This research aims to study the effect of Mycorrizal fungus and Plant-Growth-Promoting Bacteria (PGPB) on Cadmium (Cd) uptake by one-year-old *Eucalyptus Camaldulensis* seedlings. The treatments have involved three levels of heavy metal (0, 30, and 60 mg/kg) for Cd, and three bacterial levels (no bacteria (B0), *Bacillus* (Ba105), and *Pseudomonas* (Ps36, Ps448)), inoculated with mycorrhizal fungus *Glomus mosseae* (M1) and non-inoculated with fungus (M0). Results show that absorption of these elements in plant increased as Cd concentration in soil became more. Inoculation by Ps448 bacteria had an incremental effect on Cd uptake by 90%, compared to the non-inoculated (control) samples. Moreover, inoculation of the plants with mycorrhizal fungus increased Cd uptake by 24%, compared to the control. Also, it has been observed that plant resistance to metal stress and plant growth under such conditions ascended in treatments wherein inoculation happened with mycorrhizal fungus and bacteria. The highest Cd heavy metal uptake has been observed in Eucalyptus (shoots and roots), treatment (C2B2M1) with 648.19 micrograms per one seedling in pot. According to the obtained results, *Eucalyptus* with biological factors (fungi and bacteria) has the ability to clean and purify the contaminated soil with Cd heavy metal.

Keywords: cadmium, *Eucalyptus*, minimum inhibitory concentration, phytoremediation, *Pseudomonas*.

INTRODUCTION

Heavy metals are phytotoxic either at all concentrations or above certain threshold levels. They damage the environment by affecting soil fertility, biomass and crop yields, and ultimately human health (Mudgal et al., 2010; Hamzah et al., 2016). Among heavy metals, cadmium (Cd) has attracted researchers' attention due to its environmental impacts as well as relatively-high mobility in soil and plant systems (Mauskar, 2007). The use of plants with high-biomass, capable to accumulate pollutants in their organs, is the essential perquisites for successful phytoremediation in contaminated soils (Shen et al., 2002). Plants like *Eucalyptus* have been suggested as an eco-friendly, sustainable, and low-cost solution to restore and remediate the soils, contaminated with heavy metal (Dickinson, 2000). *E. camaldulensis* appropriately grows

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in mineral soils with heavy metal pollution. Its massive root system, enables it to accumulate high amounts of heavy metals; therefore, it can be used to clean-up heavy-metal-contaminated soils (Martha, 2006). *Glomus* has been reported as a dominant Arbuscular Mycorrhiza Fungi (AMF) genus in heavy-metal-contaminated soils (Zarei et al., 2008a; 2008b; Ortega-Larrocea et al., 2007; Zarei et al., 2010). Anwesha et al. (2012) found that plants, inoculated with AMF, were more unwavering during stress conditions. Inoculation of the plant with mycorrhizal increased metal extraction, uptake, and translocation efficiencies in heavy-metal-polluted soils (Bahraminia et al., 2016). Plant Growth Promoting Rhizobacteria (PGPR) can increase phytoremediation efficiency as it affects soil pH and supplies iron by siderophore production, decreasing plant pathogens and increasing phosphorus solubility, indole acetic acid production, and metal transfer from soil to plant (Ahmad et al., 2016). Considering the influence of plant as an accompany microorganism in enhanced phytoremediation, current research has been conducted to assess the effect of PGPR and AMF on growth and cadmium phytoremediation of *Eucalyptus* plant.

MATERIALS AND METHODS

The present study has used Plant Growth Promoting (PGP) bacteria, such as *Bacillus mycoides* (Ba105) (native to contaminated soils around Haft Emarat Mine, Arak, Markazi Province) (Motesharezadeh et al., 2008) and *Pseudomonas florescence* (Ps448). Biological tests have been conducted with the aim of evaluating PGP traits as well as determining Minimum Inhibitory Concentration (MIC) of Cd. The PGP tests included solubilization of insoluble mineral phosphate test (Sperber, 1958), siderophore production ability (Alexander & Zuberer, 1991), Hydrogen Cyanide production ability (HCN) (Donate-Correa et al., 2004), and indole acetic acid hormone production (IAA) (Patten & Glick, 2002). In order to evaluate isolates' resistance to Cd, growth medium containing different amounts of Cd (as $CdCl_2.H_2O$) (0, 20, 40, 60, 80, 120, 160, 200, 250, and 300 mg/L) were used. Minimum Inhibitory Concentration (MIC) of Cd was measured, based on Washington and Shutter's Method (1980).

Glomus mosseae inoculant is indigenous to heavy metals contaminated soils (Anguran Mine, Zanjan Province, Iran) (Zarei et al., 2008a; 2008b; 2010). Mycorrhizal inoculum was prepared via trap culture with forage sorghum (*Sorghum bicolor* L.) with spores of *G. mosseae*.

The soil sample, used for *Eucalyptus* cultivation, had been collected from research field of University College of Agriculture and Natural Resources, University of Tehran, located at Dolat Abad, Karaj. Some physical and chemical properties of the soil such as electrical conductivity (EC), pH, organic matter content (Walkley & Black 1934), texture (hydrometric method), total nitrogen (Kjeldahl method), and available amount of micronutrients (Iron, Zinc, Copper, and Manganese), and heavy metals (Cadmium and Lead) were determined via DTPA-extraction method and Shimadzu-670 atomic absorption (Page, 1982) (Table 1). After the initial analysis of the soil, it was found that the concentration of Cd and Pb in this soil was low. Hence, the soil sample, utilized in this study, was artificially contaminated with these metals, in accordance to the experimental design. In our laboratory for each soil testing, detailed standard operating procedures were prepared with a methodology reference. The measurements and analyses were executed by well-trained analysts. For example, in the lab operation, samples got rehomogenized prior to sub-sampling for analysis. Samples, reagents, and equipment were stored, separately. It runs a known reference sample at regular intervals. New standards had been checked against old standards before use with calibration

standards, made when extracting the solution, utilized for the soil samples. As another useful quality control technique, a summary of known sources of error in the lab operation was being written every time for soil testing to inform new employees. Reagent blanks were used in order to detect and quantify analytical bias, accuracy, and precision in soil testing. Blank subtraction was used to correct systematic sources of contamination. In order to contaminate the soil, $CdCl_2.H_2O$ was sprayed on different layers of soil uniformly in three levels, including 0, 30, and 60 mg Cd kg^{-1} soil, which were weighed and dissolved in 200 ml distilled water (Motesharezadeh et al., 2008). In order to accelerate the desirable equilibrium (resulted from adding $CdCl_2.H_2O$ to soil), contaminated pots were incubated for five months at 25±3°C while being wetted and dried, thus the soil was allowed to reach balanced conditions (Yizong et al., 2009).

The current study has been conducted as factorial arrangement, based on completely-randomized design with four replications. Experimental factors included Cd concentration at three levels (0, 30, and 60 mg kg^{-1}), inoculation with PGPB at three levels (without inoculation, Ba105, and Ps448), and inoculation with mycorrhizal fungi at two levels (without inoculation and inoculated with *Glomus mosseae*). After applying the treatments, species of uniform one-year *Eucalyptus Camaldulensis* plant (mean height of 30±2.12 cm, mean stem diameter of 40±0.36 mm) were planted by hand in 4-kg pots, one plant per pot. Plants in greenhouse were maintained at natural conditions for five months at maximum and minimum temperatures of 28 and 15°C, respectively. After the growth period, plant height, stem diameter, root area, root volume and length, leaf area (using leaf area-meter CI-202 Area Meter), and chlorophyll (by SCMR method, using SPAD-502 (Minolta, Japan)) were measured (Songsri et al., 2008; Fageria, 2009).

Plant shoot and root at each pot were cut, then, after drying for 72 hours at a temperature of 65°C, they were weighed. Plant roots were removed and root staining was performed (Kormanik & McGraw, 1982). Samples were dried ash, dissolved in 2M HCl, and finally the concentrations of Cd (using Shimadzu-670 atomic absorption), P (using Schimadzo UV-3100 spectrophotometer), and potassium (via ELEA flame photometer) were determined (Ryan et al., 2001). The data were analyzed by the software program, called SAS v.9.1, and mean comparison tests were carried out by Duncan's multiple range tests at 5% probability level, using MSTAT-C.

RESULTS AND DISCUSSION

Table 2 demonstrates the growth-promoting traits of selected bacterial isolates with the results showing that only Ps448 was able to produce siderophore. Furthermore, based on the obtained results both isolates have presented IAA-production ability on LB medium (containing L-tryptophan precursor; however, by producing 135 ppm auxin, Ba105 had more ability than Ps448). Results of HCN production ability show that only Ps448 had HCN production ability. According to these results, Ps448 and Ba105 had the insoluble mineral P solubilisation ability as they produce 2.93 and 1.33 mm halo diameter to colony diameter ratio, respectively. The outcome of heavy metal resistance test revealed that Ba105 was significantly resistant to Cd-concentration and MIC, having achieved a record of 200 mg/l, while MIC for Ps448 was much lower than Ba105, being 120 mg/L (Table 2). Jung Ho et al. (1998) showed that gram-positive and gram-negative cell walls contain functional groups that are bound to heavy metals. Main components of gram-positive cell walls are peptide glycan, ticoik acid, and ticoronic acid (Zouboulis et al., 2004), all of which have functional groups and when

deprotonated, can efficiently be bound to metal cations. Gram-negative cell walls have less peptide glycan than gram-positive ones, having more complex extra cellular membranes. The extra-cellular membrane of gram-negative bacteria has phospholipids, lypo-proteins, and various proteins (Zouboulis et al., 2004). There are different groups of Cd-resistant bacteria with different Cd-resistance mechanisms (Malik, 2004). Bacterial resistance mechanisms include degradation of metals and complexes, conversion of metals to less toxic compounds and direst distribution and flow to inside cell. Most of heavy metals inside the cells are located on poly-phosphate granules, or bounded with low molecular weight proteins, called Metallothionein (MT) and phytochelatins (Lefcort et al., 2002).

Table 1. Some physico-chemical properties of the studied soil

pH	EC ($dS\ m^{-1}$)	Soil Texture	CEC ($cmol_c\ kg^{-1}$)	Organic matter (%)	Total N (%)	K	P	Fe*	Mn*	Zn*	Cu*	Pb*	Cd*
								($mg\ kg^{-1}$)					
8.2	3.3	Clay loam	26	1.30	0.146	206.78	7.81	8.03	26.54	1.28	2.06	1.61	0.02

*DTPA extractable

Table 2. Characteristics of growth-promoting bacteria

Isolate/trait	MIC for Cd concentrations (mg/l)	Gram staining	Microbial siderophore production	IAA-production	P-solubilisation	HCN-production
Bacillus mycoides (Ba105)	200	+	-	+	+	-
Pseudomonas florescence (Ps448)	120	-	+	+	+	+

+: appropriate response to studied trait, -: lack of appropriate response

Variance analysis showed that Cd concentration and different levels of biological factors had a significant influence on *Eucalyptus* root colonization (Table 3).

Mean comparisons with Duncan's multiple range tests at probability level of 5% showed that the greatest amount of root colonization was related to 0 mg/kg Cd, and by significantly increasing the Cd concentration of the soil, root colonization was decreased (Table 4). What is more, bacterial treatments significantly affected this parameter and percentage of root colonization increased by 38.9%, compared to control in treatment of Ps448. It can be concluded that plant inoculation with mycorrhizal arbuscular fungi raised root colonization, compared to control (Table 4). Decrease in root colonization was considered an adaptive mechanism for heavy metal toxicity (Oudeh et al., 2002) (Table 4). Sensitivity of AM endophytes to higher levels of heavy metals was observed as decaying or delay in colonization ability (Meharg & Cairne, 2000). Weissenhorn and Leyval (1995) reported that in Pb- and Cd contaminated soils root colonization was decreased up to 40%. Bafel (2008) reported that plants, inoculated with mycorrhizal fungi, have more root colonization than non-inoculated ones, showing that increase in heavy metal concentration significantly decreased arbuscular mycorrhizal fungi colonization, so that for 100 mg/kg Pb in soils, inoculation with mycorrhizal arbuscular fungi increased root colonization for 37 to 65%, while increasing metal concentration up to 1000 mg/kg raised root colonization for just 30%.

Table 3. Results of variance analysis of studied traits of eucalyptus at different Cd concentrations and biological factors

SOV	df	Root colonization	Shoot dry weight	Root dry weight	Height	Stem diameter	Leaf Area Index	Phosphorous		Potassium		Cadmium	
								shoot	Root	shoot	Root	shoot	Root
Metal (C)	2	270.35**	0.002ns	1.56ns	1.32ns	0.95ns	3.35*	3.42**	12.31**	8.35**	0.22*	303.47**	107.68**
Bacteria (B)	2	65.54**	3.4*	0.22ns	0.28ns	0.44ns	0.78ns	116.31**	19.81**	2.79ns	6.48**	14.38**	13.02**
Fungi (M)	1	8.15**	15.87**	0.83ns	1.98**	0.50*	3.16ns	3.64ns	2.46ns	8.29**	0.05ns	0.31ns	4.72*
C*B	4	65.54**	1.76ns	0.96ns	18.88ns	5.28ns	1.19*	13.97**	3.59*	2.08ns	0.80ns	8.32ns	3.03*
C*M	2	65.54**	3.48*	0.08*	2.33ns	6.15**	0.64ns	3.00ns	0.18ns	3.93*	0.07ns	2.91**	1.18*
B*M	2	270.35**	8.85**	0.69**	2.95ns	2.38ns	4.11**	1.78ns	0.68ns	5.84**	0.76ns	1.70ns	1.56ns
C*B*M	4	8.15**	1.13ns	1.52ns	7.14**	0.62ns	0.47ns	1.81ns	0.88ns	1.08ns	1.04ns	0.71ns	1.70ns
error	51												

BC: interaction of bacteria and Cd, BM: interaction of bacteria and fungi, CM: interaction of Cd and fungi, BCM: interaction of bacteria, Cd and fungi, and ** and *: significant at 1% and 5% respectively.

Table 4. Mean comparison of the main effects of Cd concentration and biological factors on root colonization percentage

	Root colonization (percent)
Heavy metal concentration	
Control	12.92a
30 mg/kg Cd	10.42b
60 mg/kg Cd	6.33c
Bacteria (B)	
Control (without inoculation)	8.37c
Bacillus 105 (Ba105)	9.66b
Pseudomonas 448 (Ps448)	11.63a
Fungi (M)	
Control (without inoculation)	0b
Inoculation with mycorrhiza	19.77a

Means in each column with different letters have significant difference ($P < 0.05$).

Based on the results, presented in Table 3, Cd concentration did not affect *Eucalyptus* growth parameters; whereas shoot dry weight was significantly increased through inoculation with bacteria ($P<0.05$). Plant inoculation with mycorrhiza raised shoot dry weight and plant height ($P<0.01$) and stem diameter ($P<0.05$), while it had no significant impact on root dry weight (Table 3).

Mean comparison of Duncan's multiple range tests at 5% probability level showed that plant inoculation with mycorrhiza increased shoot dry weight, height, and stem diameter increased, compared to control, by 18, 15, and 5% respectively. The lowest plant height, stem diameter, root, and shoot dry weight were observed in 60 mg kg^{-1} Cd (Table 5). Results of mean comparison tests showed that bacterial inoculation significantly raised plant shoot dry weight and Ps448 treatment caused an 16.2% increase in shoot dry weight, compared to control (without bacterial inoculation) (Fig. 1). The interaction effects of inoculation of biological factors in various Cd levels on

plant height were remarkable. According to the results of mean comparison, the lowest plant height was observed in mycorrhizal inoculation, without any bacterial inoculation and 0 mg/kg Cd treatment (C0B0M1) as well as mycorrhizal inoculation, without any bacterial inoculation and 30 mg/kg Cd (C1B0M1) treatment. While, treatment without mycorrhizal inoculation, and inoculated with Ps448 and 30 mg/kg Cd (C0B0M1) had the highest plant height (Fig. 2).

Results of variance analysis showed that interaction of bacterial and mycorrhizal inoculation significantly affected SPAD ($P<0.01$) and leaf area index ($P<0.05$) (Table 3). Figure 3 illustrates the interaction between bacterial inoculations and mycorrhizal inoculation. Based on the achieved results, the control had the greatest leaf area index, while lack of bacterial inoculation in the presence of mycorrhizal inoculation resulted in the lowest leaf area among other treatments (Fig. 3). For SPAD index, B0M0 treatment (no inoculation of bacteria and mycorrhiza) had the lowest SPAD content, having no considerable difference from other treatments (Fig. 4).

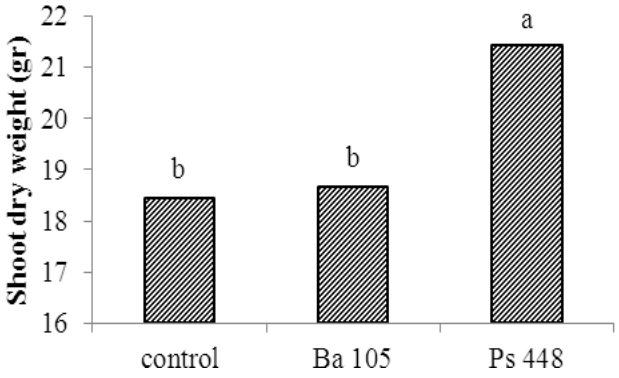

Fig. 1. Effect of bacterial inoculation on shoot dry weight. Bars with same letters have no significant difference ($P<0.05$)

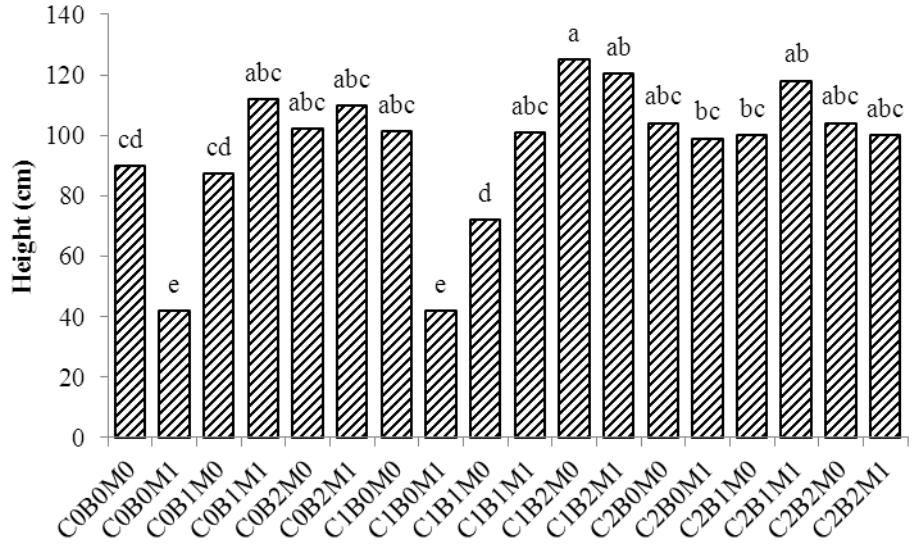

Fig. 2. Effect of interaction between biological factors in soil Cd levels on plant height. Bars with same letters have no significant difference ($P<0.05$)

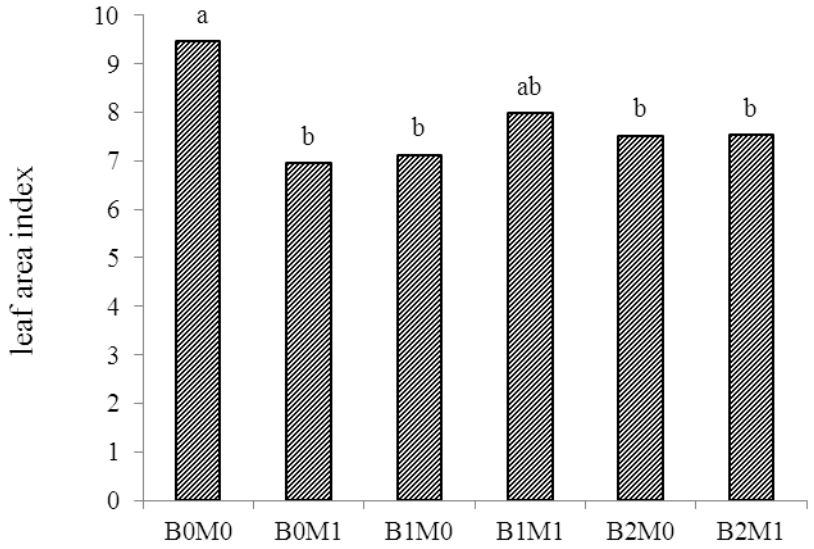

Fig. 3. Effects of interaction between mycorrhizal inoculation and bacteria on leaf area index (B0: without bacteria, Ba105: Ba105, B2: Ps400, M0: without mycorrhiza, M1: mycorrhizal inoculation. Bars with same letters have no significant difference (*P*<0.05))

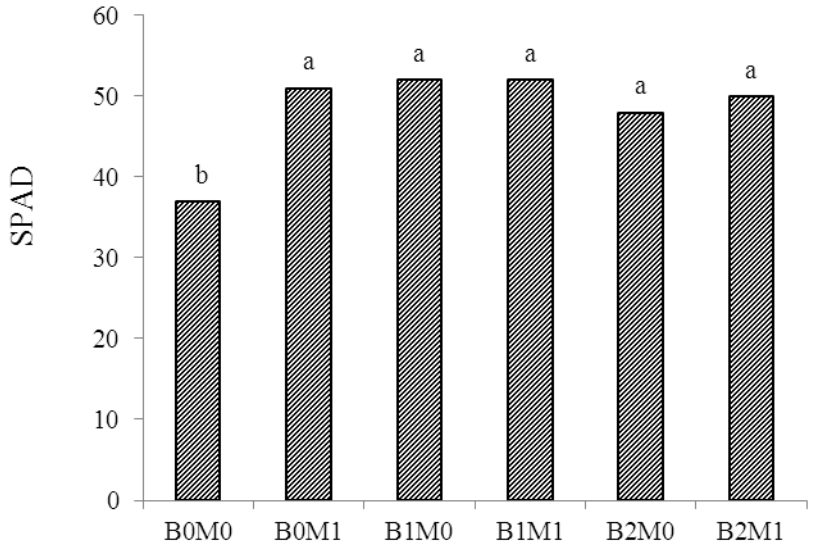

Fig. 4. Effects of interaction between mycorrhizal inoculation and bacteria on leaf area index (B0: without bacteria, Ba105: Ba105, B2: Ps400, M0: without mycorrhiza, M1: mycorrhizal inoculation. Bars with same letters have no significant difference (*P*<0.05)).

Results of variance analysis revealed that the effect of Cd concentration and bacterial inoculation on P uptake in plant root and shoot was significant ($P < 0.01$), whereas inoculation with mycorrhiza had no significant effect on P uptake (Table 3). Results of mean comparisons test demonstrated that control treatment had the greatest P uptake in plant root and shoot. As Cd concentration in soil ascended from 0 to 60 mg/kg, root and soot P declined by 86% and 16%, respectively. Plant inoculation with Ps448 caused 2- and 3-fold increase in P uptake by plant shoot and root, respectively (Table 6). Plant inoculation with Ps448 at Cd concentration of 0 mg/kg had the greatest P uptake in both roots and shoots. Control treatment

(without bacteria) in all Cd-levels had the lowest P uptake (Fig. 5). Based on the results from Table 6, the highest shoot and root K content was observed at 60 and 0 mg/kg Cd level, respectively. The Greatest values of root and shoot K uptake were observed in the treatment without mycorrhizal inoculation (Table 6). With regards to shoot K uptake, only the interaction between mycorrhizal inoculation and soil Cd-level and bacterial and mycorrhizal inoculation was significant (P <0.05 and P <0.01, respectively). Inoculating with Ba105 and mycorrhiza (without bacterial and mycorrhizal inoculation) increased K uptake in plant shoot by 40%, compared to the control. Additionally, plant inoculation in 60 mg/kg Cd resulted in 41% increase in shoot K uptake; however, its difference with mycorrhizal inoculation was not significant at 30 mg/kg Cd (Fig. 6).

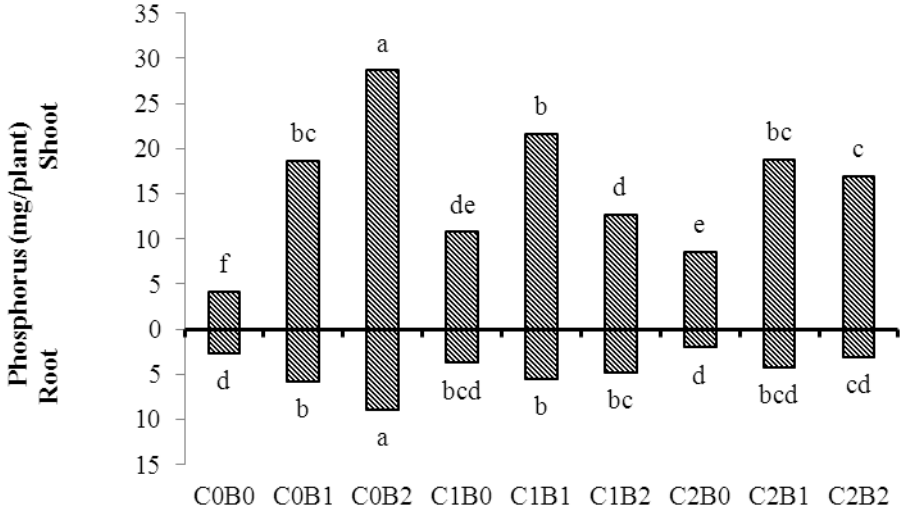

Fig. 5. Effects of interaction between bacterial inoculation and soil Cd level on P uptake (B0: without bacteria, Ba105: Ba105, B2: Ps400, C0: control, C1: 30 mg/kg, C2: 60 mg/kg. Bars with same letters have no significant difference (P<0.05))

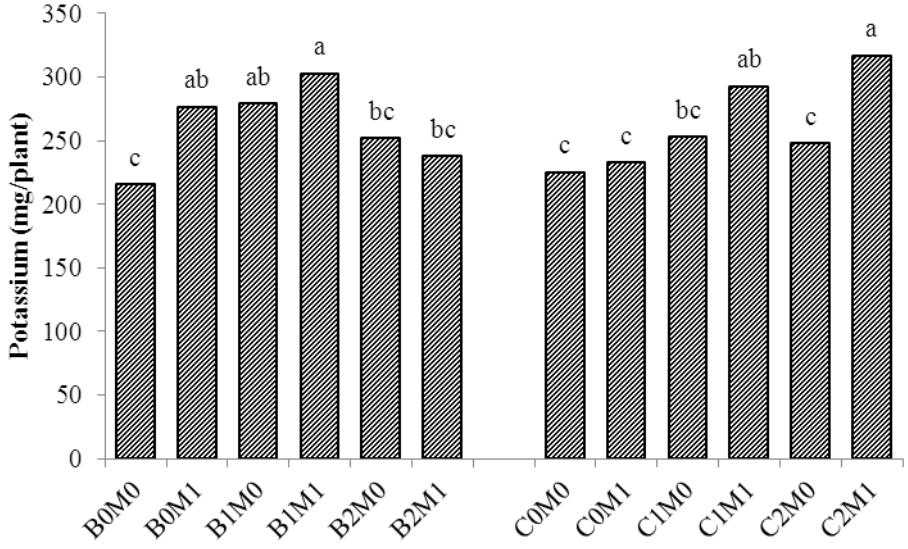

Fig. 6. Effects of interaction between bacterial and mycorrhizal inoculation and soil Cd level on K uptake (B0: without bacteria, Ba105: Ba105, B2: Ps400, C0: control, C1: 30 mg/kg, C2: 60 mg/kg. M0: without mycorrhiza, M1: mycorrhizal inoculation. Bars with same letters have no significant difference (P<0.05)).

Based on the results of variance analysis, increase in soil Cd concentration affected plant shoot and root Cd uptake significantly ($P < 0.01$). Also, inoculation with PGPB significantly affected shoot and root Cd uptake, while mycorrhizal inoculation only affected root Cd uptake ($P < 0.05$, Table 3). Based on mean comparison results, as soil Cd-level in soil increased, Cd uptake in plant shoot and root rose by 48- and 100-fold, compared to the control, respectively (Table 6). Plant inoculation with Ba105 and Ps448 caused to increase in Cd uptake in plant shoot and root. There was no significant difference between Ba105 and Ps448 for Cd-uptake in plant shoot, but plant inoculation with Ps448 resulted in 90% and 25% increase in Cd uptake, compared to the control (without inoculation) and inoculation with Ba105, respectively (Table 6). Mycorrhizal inoculation significantly affected root Cd uptake and plant inoculation caused a 24% increase in root Cd uptake (Table 6).

Effects of interaction between Cd concentrations with mycorrhizal fungi significantly affected shoot ($P < 0.01$) and root ($P < 0.05$) Cd uptake (Table 3). Based on the mean comparison results, the greatest values of Cd uptake in plant shoot and root were observed in mycorrhizal inoculation and Cd concentration of 60 mg/kg. For root Cd uptake, there was not any considerable difference between mycorrhizal inoculation and no-mycorrhizal inoculation; however, plant inoculation with mycorrhiza at all Cd-levels (0, 30, and 60 mg/kg) increased shoot Cd uptake by 100%, 9%, and 14% and root Cd uptake by 26%, 36%, and 16%, respectively (Fig. 7).

Study results have revealed that Cd had negative impacts on growth characteristics (Figs. 1, 2, and 3; Table 5). Shah et al. (2011) discovered that Cd strongly affected plant growth. Decrease in plant biomass could be due to disorder in nutrients and water uptake and transfer in plant shoots. Negative impact of higher concentrations of heavy metals in plant growth can be due to the following reasons: ethylene stress and iron limitation in plant (Glick, 2003). PGPR, having ACC-deaminase activity, efficiently decreased plant ethylene stress by preventing ethylene production (Cheng et al., 2007) while it increased plant growth. It is possible that Microbial siderophore directly increased plant growth by increasing iron availability at soils around roots (Kloepper et al., 1980). Additionally, these siderophore indirectly help plant growth by controlling pathogenic factors, since chelate is available in rhizosphere's iron, making them unavailable for pathogenic fungi and in turn leading to limited fungi growth. Vivas et al. (2006) reported that plants, colonized by native *Glomus mosseae*, can be more efficient for increasing plant root and shoot as well as nutrient uptake, compared to exogenous strains. PGPR, along with AM fungi, can have synergistic effects on increasing plant growth (Barea, 1997), which is resulted from more consumption of nutrients (Barea et al., 2002), preventing the plant's pathogenic fungi (Budi et al., 1999) and increasing its root branches (Gamalero et al., 2004). IAA, produced by bacteria, increased plant growth directly by promoting plant cells, elongation, or cell division, contributing to the increase and amplification of plant defense system (Patten & Glick, 2002).

Cd had an influence on P and K uptake in *Eucalyptus* (Figs. 5 & 6). Phosphorus availability in soils depends on plant's nutritional conditions and amounts as well as soil microbial flora (Khan, 2005). Soil microorganisms contributed to some processes, which ultimately affected P-form conversion and its availability. Microorganisms increased P availability for plants by mineralizing organic P and solubilisation of precipitated phosphates (Chen et al., 2004; Kang et al., 2002). Bafel (2008) showed that by raising heavy

metal concentration, P and N concentration declined in plant shoot. Gholami and Rahemi (2010) showed that increase in heavy metal stress led to a remarkable drop in the amount of potassium in plant shoots, which possibly could be due to osmotic regulation (Mattina et al., 2003). Nutrients' uptake by the roots is selective uptake property of plasma membrane. Cd can change plasma membrane permeability and affect nutrients' transferring processes (Mattina et al., 2003).

Various microorganisms can release P from precipitated P-sources, the dominant species of which include *bacillus*, *pseudomonas*, *peniciliuim*, and *aspergillus* (Fageria, 2009). Bacteria are more efficient than fungi in phosphate solubilisation and have greater population (Alam et al., 2002). Sheng and Xia (2006) showed that organic acids, produced by PGPR, such as citric acid, oxalic, tartaric, and succinic acid could chelate metals, releasing potassium from K-containing minerals. Increasing the P content in plants, inoculated by mycorrhiza, is due to its great affinity to P absorption mechanisms, extensive absorption area of fungal hypha, production of organic acids, and the increase in nutrients availability (Sheng & Xia, 2006).

Arriagada et al. (2007) reported that great parts of metal concentration were accumulated in eucalyptus stem, being harmful to plant's growth and development (Fig. 7). Biro and Takacs (2007) investigated metal phytoremediation by popular and reported the highest metal accumulation in plant roots; however, there was no significant difference between leaf and stem accumulation. Shariat et al. (2010) reported that Cd accumulation in stem, root, and leaves of Eucalyptus occidentalis had no significant difference; as such absorbed Cd in plant root at 15 mM was almost 5-folds greater than the leaves.

PGPB increased nutrients' uptake via various mechanisms (Ansari & Malik, 2007). PGPR (Motesharezadeh et al., 2008) or some fungi like ectomycorrhiza (Marin et al., 2009) and arbuscular mycorrhiza (Zarei et al., 2008a; 2008b) were extensively applied to enhance phytoremediation. Improvement of heavy metal uptake by PGPR is caused by production of auxin and siderophore in Pb- and Cd-contaminated soils, leading to higher nutrient uptake (Kuffner et al., 2008) (Table 6; Fig. 7). Chen et al. (2004) showed that bacteria isolate increased heavy metals bioavailability in the soil. Siderophore, produced by rhizosphere bacteria, was bounded by metal ions; hence it increased their bioavailability in plant rhizosphere (Dobbelaere et al., 2003). Among the various metabolites, produced by PGPR, siderophores play an important role in metal immobilization and accumulation (Dobbelaere et al., 2003). Bacteria's ability to produce siderophore, ACC-deaminase, and IAA led to higher amounts of nutrients like Cd availability for plants; hence it increased Cd uptake and accumulation by plant roots (Vivas et al., 2006) (Tables 2 & 4). Also, by dissolving insoluble phosphates and improving P nutrition, plants were protected from Cd toxic effect (Vivas et al., 2006) (Table 2). These results comply with those of Glassman and Casper (2012). The findings could be useful to improve basic ecological understanding of the context-dependency of plant-soil interactions and are potentially important in restoration of heavy-metal-contaminated sites.

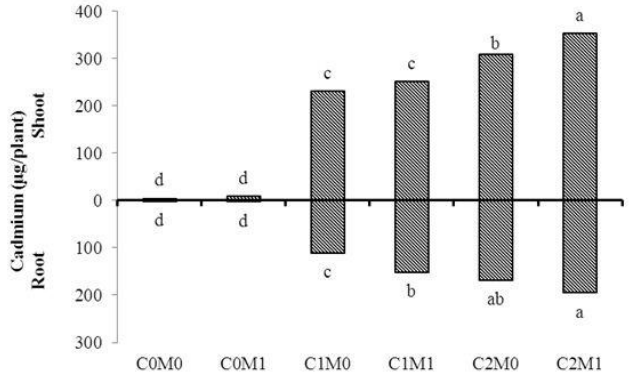

Fig. 7. Effects of interaction between mycorrhizal inoculation and soil Cd level on Cd uptake by eucalyptus (C0: control, C1: 30 mg/kg, C2: 60 mg/kg, M0: without mycorrhiza, M1: mycorrhizal inoculation. Bars with same letters have no significant difference (P<0.05))

Table 5. Results of mean comparisons of the effect of mycorrhizal inoculation and interaction between mycorrhizal inoculation and Cd levels on growth parameters of *Eucalyptus*

	Shoot dry weight (g)	Root dry weight (g)	Height (cm)	Stem diameter (cm)
Fungi (M)				
Control (without inoculation)	17.32b	5.06a	96.91b	6.16b
Inoculation with mycorrhiza	20.45a	4.49a	111.77a	6.48a
Metal (C)×Mycorrhiza (M)				
C0×M0	19.99a	5.06a	103.3	6.53a
C0×M1	19.18b	5.44a	111/4a	6.42a
C1×M0	19.19a	5.45a	99.0ab	6.40a
C1×M1	18.24a	5.21a	105.2ab	6.28a
C2×M0	16.43a	4.45a	96.1b	6.05a
C2×M1	14.5b	4.58a	86.5c	6.01a

The rates in each column with different letters have significant difference (P<0.05).

Table 6. Results of mean comparisons of the effect of Cd concentration and biological factors on nutrients uptake by eucalyptus

	Shoot K (mg/plant)	Root K (mg/plant)	Shoot P (mg/plant)	Root P (mg/plant)	Shoot Cd (µg/plant)	Root Cd (µg/plant)
Heavy metal tal concentration						
Control	229.4b	318.3a	17.2a	5.74a	6.76c	1.81c
30 mg/kg Cd	271.7a	297.4a	15.1b	4.67a	241.1b	131.2b
60 mg/kg Cd	283.1a	303.0a	14.8b	3.07b	332.2a	181.9a
Bacteria (B)						
Control (without inoculation)	245.7b	242.3a	7.86c	2.79c	153.8b	71.1c
Bacillus 105 (Ba105)	287.4a	322.7a	16.17b	4.49b	200.4a	108.4b
Pseudomonas 448 (Ps448)	260.7ab	353.7a	23.07a	3.07b	225.9a	135.4a
Fungi (M)						
Control (without inoculation)	277.6a	309.8a	14.92a	4.14a	196.48a	93.74b
Inoculation with mycorrhiza	245.1b	307.3a	16.47a	4.84a	190.25a	116.21a

The rates in each column with different letters have significant differences.

CONCLUSION

Plants' inoculation with microbial compounds, like arbuscular mycorrhiza (AM) and PGPR, have improved plant establishment. Using selected woody species, with such characteristics as heavy metal resistance, rapid growth, deep root system, and growth capability in infertile soils, is an appropriate alternative to remediate metal-contaminated soils. Additionally, use of soil microorganisms, as a safe, inexpensive, and natural source, could have several effects (including secretion of chelating agents, growth regulating factors, and increased plant biomass) in phytoremediation process and could be considered a management strategy under environmental stress conditions. Moreover, tree species with deep rooting systems, high biomass, and rapid growth, such as eucalyptus, can be used to prevent heavy metal distribution in the environment by rhizo-filtration and contaminant fixation processes. It can be concluded that the eucalyptus seedlings, considering planting density of 2500 plant per hectare, are able to absorb and extract at least 1.62 gram Cd per hectare.

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