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Heavy metal tolerant nonendosymbiont plant growth promoting Rhizobacteria associated with the roots of evergreen shrubs *Casuarina equisetifolia*

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ABSTRACT

There is a lack in research related to association of nonendosymbiont bacteria with *Casuarina* spp. Plant Growth Promoting Rhizobacteria (PGPR) nonendosymbiont bacteria such as *Pseudomonas* sp. and *Bacillus* sp. are long associated with their properties of heavy metal accumulation and plant growth promoting activity. This study focuses on heavy metal tolerance and accumulation potential of nonendosymbiont PGPR isolated from rhizosphere of *Casuarina equisetifolia*. The plant growth promoting properties were studied by pot culture studies on fast growing Indian moth bean *Vigna aconitifolia*. In the pot culture study, three bacterial isolates were found to be increasing the root and shoot length by *Pseudomonas* sp. RS-1 (7.4 $\pm$ 0.64 and 21.7 $\pm$ 1.42), *Bacillus* spp. RS-2 (6.5 $\pm$ 0.93 and 21.2$\pm$ 2.09), and *Bacillus* spp. RS-3 (6.4 $\pm$ 0.73, 19.1 $\pm$ 1.83), respectively. The maximum tolerance concentration of *Pseudomonas* spp. RS-1 shows 200 mg/L toward Cr (VI), Pb (II) 150 mg/L, whereas tolerance for Cd (II) and Zn (II) were 100 mg/L. Maximum % removal was shown by *Bacillus* spp. RS-3 towards Cr, Pb, and Zn (42.51%, 26.35%, and 26.65%, respectively), *Pseudomonas* spp. RS-1 toward Cd (36.52%). As *Frankia,* ecological interaction between nonendosymbiont bacteria and phytoremediation ability of *Casuarina* spp. is not fully understood. Further study in this area may lead to better prospect in application of *Casuarina* as a phytoremediation agent.

KEY WORDS: Heavy metal, rhizosphere, tolerance, Pseudomonas spp, Bacillus spp.

INTRODUCTION

Heavy metal in the environment such as agriculture soil and water resources have revealed in many environmental problems (Oztürk, 2007). The increasing industrialization and global economy over the last century has led to dramatically elevated reaction of anthropogenic chemicals into the environment. Discharge of heavy metal by metal processing industries into the soil that in long-term promotes their accumulation (Costa and Duta, 2001). The plantation grown on such land is often contaminated, and their consumption is hazardous (Li *et al.,* 2006).

Although immobilization and soil washing are frequently listed among the best demonstrated available technologies for remediation (He *et al.,* 2005), their high cost is preventing developing countries to benefit from these applications. Alternatives such as phytoremediation, vegetative remediation, uses vegetation and associated microbiota, soil amendments and other agronomic techniques can prove it's economical in developing countries (Helmisaari *et al.,* 2007). *Casuarina* (common ironwood) studied vastly for its phytoremediation of petroleum hydrocarbons and heavy metals in soils (Sun *et al.,* 2004). The endosymbiont of *Casuarina* tree, *Frankia* has been well studied for its ability to bind and sequester several toxic heavy metals and as a potentially bioremediation agent (Gollop *et al.,* 2011). However, the ecological interaction between nonendosymbiont bacteria and phytoremediation ability of *Casuarina* spp. is poorly understood.

PGPR found in the rhizosphere of various crops have been found to assist in root colonization by rhizobia increasing the development of the plant and in suppressing soilborne plant pathogens (Parmar and Dadarwal, 2000; Jeffries *et al.,* 2003). Metal contamination of soil has an important bearing on PGPR functions (Ali and Zulkifli, 2010). Of the various rhizospheric bacteria, *Pseudomonas* spp. and *Bacillus* spp. are aggressive colonizers of the rhizosphere of various crop plants (Schroth and Hancock, 1982). Metal homeostasis resistance in these bacteria is often maintained by sequestration, active efflux, reduced uptake, detoxification, and synthesis of binding protein (Jing *et al.,* 2007). *Pseudomonas* spp. and *Bacillus* spp. are ubiquitous and associated with vast range of vegetation. They are known for mechanisms by which they were able to resist heavy metal and able to carrying out its PGPR function in soil containing high concentration of metals such as Cd, Al, Zn, and Cr (Abou-Shanab *et al.,* 2008). This study focuses on heavy metal accumulation potential of nonendosymbiont bacteria along with their plant growth promoting potential.

MATERIALS AND METHODS

Sample Collection

A month old healthy *Casuarina equisetifolia* seedlings were grown in soil, which was treated with salts of chromium (Cr^{6+}), lead (Pb^{2+}), cadmium (Cd^{2+}), and zinc (Zn^{2+}) at concentration of 100, 100, 50, and 50 mg/kg, respectively, as per the methodology followed by Stuczynski *et al*. (2003). After 1 year rhizosphere soil samples were collected from five different metals treated *C. equisetifolia* trees, respectively, for the study. The samples collected were about 250 g surrounding the root area at a depth of 10-15 cm in separate sterile 1 kg polyethylene bags. The five rhizosphere samples were uniformly mixed to get 1.25 kg of composite sample. From this 500 g of the soil was dried sieved and stored in a sterile polyethylene bag at 4°C for further studies (Bhat and Kaveriappa, 2009).

Isolation of Heavy Metal Tolerant Bacteria

Chromium, lead, cadmium, and zinc resistant bacterial strains were isolated from the rhizosphere soil samples using enrichment media supplemented with heavy metals. 100 ml nutrient broth (NB) was supplemented with Cr (VI), Pb (II), Cd (II), and Zn (II) by addition of $K_2Cr_2O_7$, $PbCl_3$, $CdCl_3$, and $ZnSO_4$ at 100 mg/L concentrated and pH was adjusted to 6; the medium was then inoculated with 1 g of the rhizosphere soil under aseptic condition and incubated at 30°C in the rotary shaker incubator at 120 rpm for 3 days till the medium appeared turbid. The medium was serially diluted by standard spread plate technique onto agar medium supplemented with $K_2Cr_2O_7$, $PbCl_3$, $CdCl_3$, and $ZnSO_4$ each at 100 and 50 mg/L conc. The plates were incubated at 30 ± 2°C for 48 h. After 48 h incubation, larger identical colonies from each plate were isolated.

Seed Germination Bioassay

Seed germination bioassay was carried out for the growth promoting activity of isolates. A total of nine isolates were grown in nutrient medium agar plates at 30 ± 2°C for 24 h. The inoculants for treating *Vigna aconitifolia* seeds were prepared by suspending cells from agar plates in a standard NB as described earlier (Dey *et al*., 2004; Gerhardson *et al.,* 1985; Pal *et al*., 1999). Four pregerminated seeds per boiling tube with three replications for each treatment were used and incubated at 28°C. The length of the each seedling was measured after 7 days and expressed in cm and compared with control on day 7 with sterile NB. A total of three isolates were found to enhance the root length significantly. The isolates were identified by morphological, physiological, and biochemical characteristics following Bergey's Manual of Systematic Bacteriology (8th Edition).

Pot Trials

A total of three cultures were selected to evaluate their effects on the growth in pot trials. The pots containing soil (medium black and calcareous, pH 7.85, organic matter 2.16%, total nitrogen content 287 mg/kg, available phosphorus 200 mg/kg, and potassium exchangeable K 338 mg/kg data shown in Table 1. The sterile soil was used for the experiments. There were a total of four treatments, each having six replications. Each isolate of PGPR was grown overnight in Kings' B broth and NB. The seeds for each treatment were soaked for an hour broth containing the suspension of the PGPR isolates, six to eight seeds (95% germination) were sown at a depth of 5 cm. After germination, five seedlings were maintained in each pot (Dey *et al*., 2004). Dry weight, shoot length, and root length were recorded at 15 days after sowing.

Heavy Metal Solutions

The heavy metal solutions of Cr (VI), Cd (II), Pb (II), and Zn (II) of 1000 mg/L concentration were used as stock solutions, slightly acidified with HNO_3 (2-3 drops of concentrated HNO_3), and were sterilized at 121°C for 15 min. These solutions, in various concentrations according to the metal tested, were kept at 25°C.

Table 1: Physicochemical parameters of the manure soil

Parameters	Manure soil
pH	7.85
Electric conductivity (ms/cm)	0.625
Organic matter (%)	2.16
Phosphorous (mg/kg)	200
Turbidity (NTU)	77
BOD (mg/L)	48
COD (mg/L)	128
TDS (mg/L)	378
Hardness	395
Acidity	650
Alkalinity (mg$CaCO_3$/L)	15
Potassium exchangeable K (mg kg)	338
Calcium exchangeable Ca (mg/kg)	1564
Magnesium exchangeable Mg (mg/kg)	498
Sulfur available S as SO_4^{2} (mg/kg)	29.5
Sodium exchangeable Na (mg/kg)	129
Iron available Fe (mg/kg)	11.56
Available Cu (mg/kg)	0.67
Boron available B (mg/kg)	0.8
Ca saturation (%)	58.37
Mg saturation (%)	30.98
Na saturation (%)	4.19
CEC (by addition) (mEq/100 g soil)	13.40

BOD: Biochemical oxygen demand, COD: Chemical oxygen demand, TDS: Total dissolved solids, NTU: Nephelometric turbidity units

Maximum Tolerance Concentration (MTC)

To check MTC, to each plate of Mueller-Hinton agar medium, 100 µl of the appropriate metal salt solutions were added in four different wells (6 mm diameter). Plates were incubated at 37°C for 24 h to allow diffusion of the metal into the agar, it was supposed by that time, that a concentration gradient of the metal was formed. On each metal plate isolates was swabbed using 24 h broths of RS 1, RS 2, and RS 3. The plates were incubated at 37°C for 48 h, after incubation the plates were observed for the zone of inhibition to determine the tolerance (Hassen *et al.*, 1998).

Heavy Metal Removal Assay

For metal removal studies, 100 ml of sterile media was prepared with 10 mg/L of Cr^{6+} in four different 250 ml conical flasks. Bacterial cultures RS 1, RS 2, and RS 3 were inoculated, respectively. This process was repeated for the other metals, namely, Cd^{2+}, Zn^{2+}, and Pb^{2+}, respectively. The culture was incubated at 35°C in an incubator shaker at 120 rpm, and then, aliquots were taken at regular intervals of 2, 4, 6, 8, 10, and 12 and it were centrifuged at 8000 rpm for 15 min. The residual heavy metal in the supernatant was estimated using Atomic Absorption Spectrophotometer (AAS Varian AA240).

The amount of heavy metal removed by the cell was calculated by the formulae: % Removal= $(C_i - C_f / C_i) \times 100$

Where, C_i = Initial metal concentration (mg/L), C_f = Final metal concentration (mg/L) (Velásquez and Dussan, 2009; Ozdemir *et al.*, 2009).

RESULTS

Isolation of Heavy Metal Tolerant Bacteria

A total of nine types of heavy metal bacteria colonies were isolated, which were able to grow on nutrient agar medium supplemented with $K_2Cr_2O_7$, $PbCl_3$, $CdCl_3$, and $ZnSO_4$ each at 100 and 50 mg/L concentrated.

Seed Germination Bioassay

In germinating seed bioassay, a total of three isolates were found to enhance the length of the seedlings of *V. aconitifolia* significantly. The three isolates were identified based morphological, physiological, and biochemical characteristics and found belonging to the genera *Pseudomonas* spp. and *Bacillus* spp. and further referred as *Pseudomonas* spp. RS-1, *Bacillus* spp. RS-2, and *Bacillus* spp. RS-3. In comparison with the control 5.30 ± 0.76 cm, *Pseudomonas* spp. RS-1, *Bacillus* sp. RS-2 and *Bacillus* sp. RS-3 were found increase the seed length to 8.08 ± 0.6, 7.29 ± 0.39, and 6.37 ± 0.48, respectively. The results show that all the three isolates enhance the length of the seedling, in which *Pseudomonas* spp. RS-1 shows more significant in promotion of growth (Table 2).

Pot Trials

In pot culture study, three isolates when inoculated with the soil the plant showed a significant growth in terms of root and shoot length when compared to the control. The root and shoot length for control are 4.5 ± 0.75 and 16.2 ± 1.02 for pots with PGPR are *Pseudomonas* spp. RS-1 (7.4 ± 0.64 and 21.7 ± 1.42), *Bacillus* spp. RS-2 (6.5 ± 0.93 and 21.2 ± 2.09), and *Bacillus* spp. RS-3 (6.4 ± 0.73 and 19.1 ± 1.83) on 20th day, respectively (Table 3). Comparatively *Pseudomonas* spp. RS-1 showed higher growth (Figure 1).

MTC

The heavy metal tolerance toward Cr (VI), Pb (II), Cd (II), and Zn (II) were determined by well diffusion method. The MTC of *Pseudomonas* sp. RS-1 shows maximum of 200 mg/L toward Cr (VI), Pb (II) 150 mg/L whereas tolerance for Cd (II), and Zn (II) were 100 mg/L (Figure 2).

Heavy Metal Removal Assay

The heavy metal % removal Cr^{6+}, Pb^{2+}, Cd^{2+}, and Zn^{2+} were calculated and derived in the form of graph

Table 2: Germinating seed bioassay

Cultures	Growth of the seedlings
	Length (cm)
Control	5.30±0.76
RS 1	8.08±0.6
RS 2	7.29±0.39
RS 3	6.37±0.48

Length of the seedling plant is reported as mean±standard deviation, (n=12). RS-1: *Pseudomonas* spp., RS-2: *Bacillus* spp., RS-3: *Bacillus* spp.

Table 3: Pot culture assay

Test organisms	Growth of plant (cm)		
	Total length	Root	shoot
Control	20.8±1.64	4.5±0.75	16.2±1.02
RS 1	29.1±1.53	7.4±0.64	21.7±1.42
RS 2	27.2±1.39	6.5±0.93	21.2±2.09
RS 3	25.5±2.20	6.4±0.73	19.1±1.83

Length of the plant is reported as mean±standard deviation (n=24)

(Figure 1), whereas maximum % removal was shown by RS 3 toward Cr, Pb, and Zn (42.51%, 26.35%, and 26.65%, respectively) and RS1 toward Cd (36.52%) shown in Figure 3.

DISCUSSION

Plants are sessile thus releasing an array of chemical signals to interact with other organisms. The rhizosphere interaction is not solely driven by root but are highly integrated and influenced by residing organisms and local edaphic factor (Badri *et al*., 2009). *Casuarina* crop has been recently exploited for it bioremediation properties in heavy metal contaminated sites (Sun *et al*., 2004). Our study highlights, the importance of PGPR's as nonendosymbiont microbes that play an efficient role bioremediation of heavy metal. Previous studies done on *Casuarina* are limited to the endosymbiont, especially *Frankia* in their ability to accumulate metallic ions (Gollop *et al*., 2011).

PGPR are rhizosphere bacteria that exert a positive influence on the plant growth especially under stress condition (Kloepper *et al*., 1980); they can influence plant growth directly either by providing specific compounds that help plant growth or by providing facilitating uptake of nutrients from the soil and indirectly by suppressing the phytopathogenic organisms in the rhizosphere (Glick, 1985). Not all isolates which are found tolerant to heavy metals and able to influence the plant growth only three out of nine were potential growth promoters. *Pseudomonas* spp. RS-1, *Bacillus* spp. RS-2, and *Bacillus* spp. RS-3 in this study showed plant growth promotion in corresponding genera

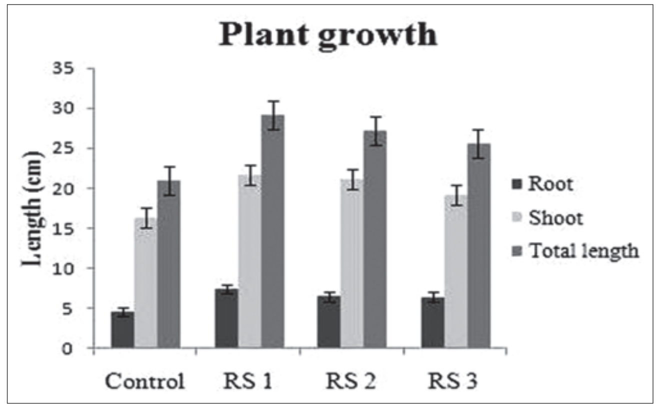

Figure 1: Plant growth in pot culture study by measuring root, shoot, and total lengths of *Vigna aconitifolia* when grown with inoculums of *Pseudomonas* spp. - RS-1, *Bacillus* sp. - RS-2, and *Bacillus* spp. - RS-3, control without any bacterial inoculums

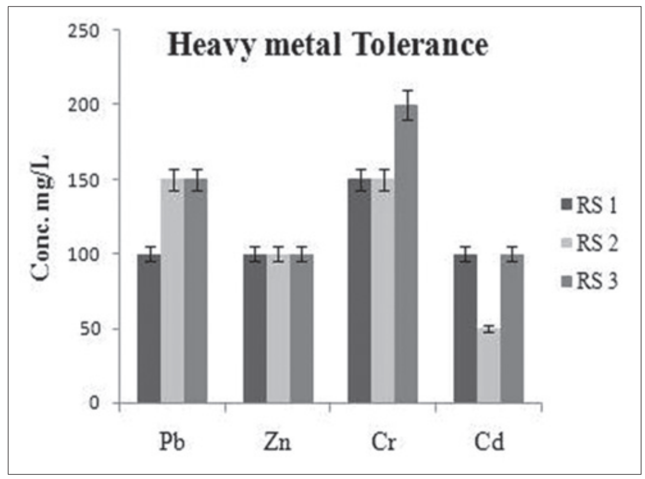

Figure 2: Maximum tolerance concentration, by Mueller-Hinton agar well diffusion method. Heavy metal tolerance of *Pseudomonas* sp. - RS-1, *Bacillus* sp. - RS-2, and *Bacillus* sp. - RS-3 when tested against Pb (II), Zn (II), Cr (VI), and Cd (II)

a number other of PGPR, e.g., *Pseudomonas putida* GR12-2 (Jacobson *et al*., 1994), *Bacillus subtilis* A13 (Turner and Backman, 1991), *B. licheniformis* CECT5106 (Probanza *et al*., 2002), *B. pumilus* CECT5105 (Probanza *et al*., 2002), and others such as *Pseudomonas fluorescens* Pf-5, *P. fluorescens* 2-79, and *P. fluorescens* CHA0 (Wang *et al*., 2000) have been identified to have similar properties. The high resistance of isolates toward Cr, Pb, Cd, and Zn due to the adaptation of microbes to the toxicity of high concentration of these metals in the rhizosphere of the plant (Gadd and Griffiths, 1997) by strategies such as oxidative stress and multiple efflux pump. The metal uptake properties of the isolates explained by Bae *et al*. (2001) as microbes synthesize compounds which bind to metals and reduce toxicity, compounds which accumulate heavy metal by metal binding peptides (Schroth and Hancock, 1982).

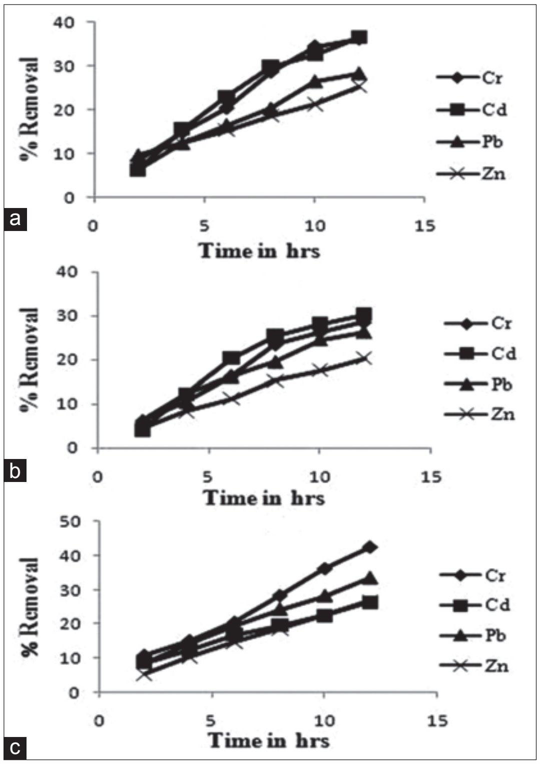

Figure 3: Here metal % metal removal of Cr, Cd, Pb, and Zn are shown for (a) *Pseudomonas* sp. - RS-1, (b) *Bacillus* sp. - RS-2, and (c) *Bacillus* sp. - RS-2

In this study, it was found even under stressed condition, i.e. in the presence of toxic heavy metal, it was observed that all three organisms showed rapid uptake within few hours. The efficiency of absorption slowly increased as the bacterial growth increased, later it was found that the rate of removal was proportional to concentration of the cells (Chatterjee *et al*., 2011). *Pseudomonas* spp. and *Bacillus* spp. complex genomic machineries by are quick to response to the environmental stress (Nelson *et al*., 2002). Although there are different mechanisms for copping different toxic heavy metals (Canovas *et al*., 2001), this study shows they respond with equal efficiency without disturbance in its normal activity PGPR.

In this study, we isolated PGPR from *Casuarina* (common ironwood) and demonstrated their heavy metal removal property. This suggests not only endosymbiotic bacteria such as *Frankia*, the nonendosymbiont bacteria can also help plant to better respond metal contamination stress, further the plant growth-promoting rhizobacteria can be used in the development of phytoremediation strategies to treat plants for better yield and stabilize and remediate metal-contaminated soils. This study must be carried out in field condition to validate the efficacy of these PGPR's in the environment, in this context, the optimization of PGPR inoculums must be tested in the presence of diverse environmental factors. In addition, to maintain the maximum viability and activities of PGPR, an appropriate carrier should be developed.

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REFERENCES

Abou-Shanab R, Ghanem K, Ghanem N, Al-Kolaibe A. The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol 2008;24:253-515.

Ali K, Zulkifli HS. Phytoremediation of heavy metals with several efficiency enhancer methods. Afr J Biotechnol 2010;9:3689-8.

Badri DV, Weir TL, van der Lelie D, Vivanco JM. Rhizosphere chemical dialogues: Plant-microbe interactions. Curr Opin Biotechnol 2009;20:642-50.

Bae W, Mehra RK, Mulchandani A, Chen W. Genetic engineering of *Escherichia coli* for enhanced uptake and bioaccumulation of mercury. Appl Environ Microbiol 2001;67:5335-8.

Bhat R, Kaveriappa KM. Rhizosphere mycoflora of some species of Myristicaceae of the Western Ghats, India. Asian J Microbiol Biotechnol Environ 2009;11:543-57.

Canovas D, Cases I, de Lorenzo V. Heavy metal tolerance and metal homeostasis in *Pseudomonas putida* as revealed by complete genome analysis. Environ Microbiol 2001;5:1242-56.

Chatterjee S, Ghosh I, Mukherje KK. Uptake and removal of toxic Cr (VI) by *Pseudomonas aeruginosa*: Physico-chemical and biological evaluation. Curr Sci 2011;101:645-52.

Costa AC, Duta FP. Bioaccumulation of copper, zinc, cadmium, and lead by *Bacillus* sp., *Bacillus cereus*, *Bacillus sphaericus*, and *Bacillus subtilis*. Braz J Microbiol 2011;2:876-87.

Dey R, Pal KK, Bhatt, DM, Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 2004;159:371-94.

Gadd GM, Griffiths AJ. Microorganisms and heavy metal toxicity. Microb Ecol 1977;4:303-17.

Gerhardson B, Alstrom S, Ramert B. Plant reactions to inoculations of roots with fungi and bacteria. Phytopathol Z 1985;60:1726-33.

Glick BR. The enhancement of plant growth by free living bacteria. Can J Microbiol 1985;41:109-17.

Gollop N, Shoresh M, Guetsky R, Rindner M, Zakin V, Bernstein N. Differential response of *Frankia* strains to heavy metals. Israel J Plant Sci 2011;59:217-21.

Hassen A, Saidi N, Cherifh M, Boudabous A. Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Bioresour Technol 1998;65:73-82.

He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 2005;19:125-40.

Helmisaari HS, Salemaa M, Derome J, Kiikkilä O, Uhlig C, Nieminen TM. Remediation of heavy metal-contaminated forest soil using recycled organic matter and native woody plants. J Environ Qual 2007;36:1145-53.

Jacobson CB, Pasternak JJ, Glick BR. Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from PGPR, *Pseudomonas putida* GR12-2. Can J Microbiol 1994;40:1019-25.

Jing Y-d, He Z-l, Yang X-e. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B. 2007;8:192-207.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 2003;37:1-16.

Kloepper JW, Leang J, Tuntze M, Schroth MN. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 1980;286:885-6.

Li Y, Wang YB, Gou X, Su YB, Wang G. Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, China. J Environ Sci (China) 2006;18:1124-34.

Nelson KE, Weinel C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VA, *et al.* Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. Environ Microbiol 2002;4:799-808.

Ozdemir G, Ceyhan N, Ozturt T, Akirmak F, Cosar T. Biosorption of chromium(VI), cadmium(II) and copper(II) by *Pantoea* sp. TEM18. Chem Eng J 2002;102:249-53.

Oztürk A. Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*. J Hazard Mater 2007;147:518-23.

Pal KK, Dey R, Bhatt DM, Chauhan SM. Enhancement of groundnut growth and yield by plant growth promoting rhizobacteria. Int Arachis Newslett 1999;1:51-3.

Parmar N, Dadarwal KR. Stimulation of plant growth of chickpea by inoculation of fluorescent pseudomonads. J Appl Microbiol 2000;86:36-44.

Probanza A, Garcia JA, Palomino MR, Ramos B, Gutierrez MF. *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). Appl Soil Ecol 2002;20:75-84.

Schroth MN, Hancock JG. Disease-suppressive soil and root-colonizing bacteria. Science 1982;216:1376-81.

Stuczynski TI, McCarty GW, Siebielec G. Response of soil microbiological activities to cadmium, lead, and zinc salt amendments. J Environ Qual 2003: 32(4):1346-55.

Sun WH, Lo JB, Robert FM, Ray C, Tang CS. Phytoremediation of petroleum hydrocarbons in tropical coastal soils. I. Selection of promising woody plants. Environ Sci Pollut Res Int 2004;11:260-6.

Turner JT, Backman PA. Factors relating to peanut yield increases after seed treatments with *Bacillus subtilis*. Plant Dis 1991;75:347-53.

Velásquez L, Dussan J. Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*. J Hazard Mater 2009;167:713-6.

Wang C, Knill E, Glick BR, Defago G. Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHAO and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities. Can J Microbiol 2000;46:898-907.