Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Induced phytoremediation of metals from fly ash mediated by plant growth promoting rhizobacteria

Sadhna Tiwari[1], S.N. Singh[1]* and S.K. Garg[2]

[1]*National Botanical Research Institute, Lucknow-226 001, India*
[2]*DST-FIST Supported Department of Microbiology, Dr. R.M.L. Awadh University, Faizabad-224 001 India*
**Corresponding Author email : drsn06@gmail.com*

Publication Info

Paper received:
06 September 2012

Revised received:
23 January 2013

Re-revised received:
10 February 2013

Accepted:
20 February 2013

Abstract

A study was carried out to observe the impact of a consortium of bacteria isolated from the fly ash on the metal accumulation by *T. latifolia*. When a consortium of bacteria *Bacillus endophyticus* NBRFT4 (MTCC 9021), *Paenibacillus macerans* NBRFT5 (MTCC 8912) and *Bacillus pumilus* NBRFT9 (MTCC 8913) was bioaugmented into the rhizosphere of *T. latifolia*, it enhanced the metal concentration in root, stem and leaves of the plants through increased bioavailability of metals Fe, Cd, Pb, Cr, Ni, Cu and Zn in the fly ash. Besides, these bacteria also promoted the plant growth perhaps due to utilization of ACC, synthesis of phytoharmones and solubilisation of essential metals found in fly ash. As compared to fly ash alone, the accumulation of Fe was maximally enhanced by 164%, 196%, and 251%, followed by Ni by 92%, 44% and 56%, Zn by 82%, 57% and 91%, Cu by 71%, 53% and 60%, Cr by 96%, 80% and 105 %, Pb by 119%, 87% and 140%, Cd by 80%, 109% and 115% in root, stem and leaves, respectively in fly ash with bacteria. Thus, an increased solubilisation of metals coupled with enhanced plant growth stimulated the phytoextraction of metals by *T. latifolia* from fly ash.

Key words

Fly ash, Metal, Phytoremediation, Rhizospheric bacteria, *Typha latifolia*

Introduction

Fly ash, a by-product of coal combustion, is generated in large quantities in coal- based thermal power plants and other industries. Presently, India produces 120 milion tons per year and it will cross the figure of 150 million tons in the coming years (Pandey *et al*., 2010). Besides, being rich in many essential elements, such as B, Fe, Mo, Cu, Zn and P, fly ash contains various toxic metals and metalloids such as Cr, Ni, Pb, Cd, As and Ba etc. Disposal of huge amount of fly ash not only requires more than 30000 ha of agricultural and forest lands, but also becomes a potential source of metal contamination to surface and ground water, threating human health (Kalra *et al*., 1998).

Since conventional remediation methods, such as acid leaching, excavation, land filling processes are very costly and also not eco-friendly, phytoremediation is projected as a cost-efficient and ecologically benign process that uses plants to remove heavy metals from the environment by uptake, accumulation or transformation of these metals in vegetative biomass (McCutcheon and Schnoor, 2003; Cechmankova *et al*., 2011). Bioaugmentation is an aspect of bioremediation processes involving addition of natural microorganisms process which enhances decontamination process (Stephenson and Stephenson, 1992). A combination of the phytoremediation and bioaugmentation techniques may certainly augment bioremediation processes of contaminated soil or water.

Currently, a number of reports are available on metal accumulating plants that are used in removing toxic metals from the soil or fly ash (Zayad *et al*., 1998; Burd *et al*., 2000; Rajkumar *et al*., 2006; Singh *et al*., 2010; Abbaspour *et al*., 2012). In phytoremediation, plants are used to extract, sequester, and/or detoxify pollutants through physical, chemical, and biological processes (Cunningham and Ow, 1996; Saxena *et al*., 1999; Wenzel *et al*., 1999). *Brassica juncea* (Indian mustard) is one of such plant species which may serve as potential useful bioagent for phytoremediation of metals. Braud *et al*. (2009) have reported enhanced phytoextraction of Cr and Pb from contaminated agricultural

soils through bioaugmentation of siderophore producing bacteria. Kuffner *et al*. (2008) also observed that rhizospheric bacteria could contribute immensely to the metal extraction process of the plants, although mechanism of microbe and metal interaction is not yet well understood. Phytoextraction can be enhanced by increasing the plant biomass, plant growth promoting rhizobacteria (PGPR) effect) or by facilitating metal uptake through production of enzymes, siderophores, organic acids or biosurfactants by the inoculated microorganisms (Jing *et al*., 2007; Lugtenberg and Kamilova, 2009). Besides, other processes like metal accumulation in plants compartmentation and sequestration within the root, the efficiency of xylem to transport metal, distribution of metal in the aerial parts, sequestration and storage in the leaves cells also regulate the phytoremediation process (Clemens *et al*., 2002). Plant roots are reported to exude protons to acidify the soil to mobilize the metals. This phenomenon was clearly observed for Fe mobilization in Fe- deficient dicot plants (Crowley *et al*., 1991). Lebeau *et al*.(2008) have reviewed the performance of bioaugmentation- assisted phytoextraction applied to metal contaminated soils. Sarma (2011) has recently reviewed hyperaccumulation of metals with a focus on plants for phytoextraction technology.

In light of the above, the present investigation was aimed to develop microbe-based phytoremediation process to remove toxic metals from fly ash and soil amended with fly ash.

Materials and Methods

Collection of soil and fly ash: Field experiment was conducted at the Botanical garden, NBRI, Lucknow, India from March to May 2009. The soil used in this experiment was collected from NBRI garden, Lucknow and fly ash (FA) from NTPC Thermal Power Plant, Unchahar (U.P.) and then air-dried for use in experimentation.

Isolation, characterization and enrichment of bacterial strains: Eleven bacterial strains were isolated from the rhizospheric zone of *T. latifolia*, naturally growing on fly ash dumps located at Unchahar, by serial dilution method using NA plates. These strains were tested for metal extractability from fly ash using metal chelating agents like DTPA and EDTA after incubation with bacteria for various periods (Tiwari *et al*., 2008 a, b). Based on the extractability of metals from fly ash, four bacterial strains were selected out of the eleven for further experimentation. Different combinations of selected four bacterial strains were prepared to test their role in enhancing the phytoextraction of metals from fly ash by a well known metal hyper accumulator plant *B. juncea* (Tiwari *et al*., 2012). The best combination of bacteria ST3 (*Paenibacillus macerans* NBRFT5 + *Bacillus endophyticus* NBRFT4 + *Bacillus pumilus* NBRFT9) which enhanced phytoextraction of metals from fly ash was selected to carry out an experiment on phytoremediation of metals from fly ash by a wild plant: *T. latifolia*.

Typha latifolia, also known as Cattail, belongs to family Typhaceae. It is an aquatic or semi aquatic perennial herbaceous plant that grows in marshy areas and boggy places, and also along borders of lakes and water reservoirs. The plant is 1.5 to 3 m high and it has 2-4cm broad leaves.

These bacterial strains were inoculated separately in nutrient broth in a glass conical flask and then incubated at 37°C for 2 days in an orbital shaker (180 rpm) for enrichment. Then after, they were mixed together to make a consortium of *Paenibacillus macerans* NBRFT5 + *Bacillus endophyticus* NBRFT4 + *Bacillus pumilus* NBRFT9 to attain a CFU value in the range of 4.13×10^{11} to 5.64×10^{11} CFU in the stock cultures.

Experimental design : Field experiment with *T. latifolia* was set up separately in 100% FA, 50% FA + 50% soil + bacterial consortium (BC) and 100% FA + BC. In this experiment, control was set up with fly ash without BC. For raising *T. latifolia* for experimentation, 9 plots of size (1x1 m), were prepared by ploughing of soil to the depth of 1 ft. Experimental plots were separated by 0.5m from each other. Out of 9 plots, 3 plots were dug to 1 ft depth and soil was completely replaced by fly ash without BC, in another three plots the soil was mixed with 50% FA and BC and then filled and in remaining 3plots, 100% FA was mixed with BC and then filled as mentioned below. The plots were irrigated and then left for fifteen days for multiplication of bacteria. Small plants of *T. latifolia* were escavated and planted in 1 x 1 m plots at distance of 30 cm from plant to plant.

In all plots, moisture level was maintained between 50-60% to facilitate plant growth and bacterial multiplication. The plants were raised in all plots for a period of three months and harvested at monthly interval for biomass interval and for analysis of metal concentration. Thirty plants of *T. latifolia* were randomly harvested every month.

The spontaneous rifampicin-resistant mutants of wild bacterial strains such as NBRFT4, NBRFT5 and NBRFT9 were generated by inoculating bacterial culture from Tryptone soya agar (TSA) to Tryptone soya broth (TSB) containing increasing concentrations of rifampicin (25, 50, 100 $\mu g\ ml^{-1}$) and plating loopful of the culture on TSA plates amended with 100 μg of rifampicin ml^{-1} (TSA-rif). NBRFT4, NBRFT5 and NBRFT9 on TSA-rif plates were checked for stability by transferring them twenty five times from TSA-rif to TSA and TSA-rif plates. The stability of rif-tagged bacteria was found to be 60 days in field application

Physico-chemical analysis of fly ash and fly ash amended soil: Fly ash and fly ash amended soil samples were air

dried, crushed, passed through a sieve of 2 mm mesh size and then subjected to physico-chemical analyses. The pH of fly ash, soil and fly ash amended soil was measured in the soil suspension of 1:5 (w/v) using a pH meter (Model EA940, Orion pH meter, USA) previously calibrated with pH 4.0, 7.0 and 9.2 reference buffers. The EC of fly ash and soil was determined by Orion electrical conductivity meter, while water holding capacity and bulk density were measured according to Black (1965). The organic carbon was determined according to Walkley and Black (1934) and total nitrogen by Kjeldahl (Allison, 1973) method. The available phosphorus was quantified by $NaHCO_3$ extraction method given by Olsen and Sommers (1982). Cation exchange capacity was measured according to Kalra and Maynard (1991).

Heavy metal analysis of fly ash and fly ash amended soil: One gram air dried soil/fly ash sample was digested in a Microwave Digestion System (MDS 2000) at 630 W, 40 psi with 20 ml of tri acid mixture (HNO_3: H_2SO_4: $HClO_4$: : 5:1:1) for 20 min till transparent colour appeared (Allen *et al*., 1986). The digested samples were filtered through Whatman No 42 filter paper and the volume was made up to 50 ml in volumetric flask with deionised water. Heavy metal concentration in samples was determined using an atomic absorption spectrophotometer (GBC Avanta, precision accuracy 0.01).

Heavy metal analysis of plant samples: The plants were gently uprooted and washed thoroughly with deionized water to remove soil particles clinging to the roots. Again washed by rinsing with 10 mM $HgCl_2$ solution for surface sterilization of the tissues. The plants were separated into root, shoot and leaves and subsequently oven-dried at 80°C for 7 days to a constant weight. After homogenization, plant samples (0.3-0.6 g) were digested with 5 ml of 70% HNO_3 in Microwave Digestion System (MDS 2000) at 630 W, 40 psi for 20 min. Digested samples were filtered through Whatman filter paper (No. 44) prior to metal analysis with (GBC Avanta) atomic absorption spectrophotometer.

Translocation factor (TF): Translocation factor of metals was calculated by using the following formula:

TF (root to stem) = metal accumulation in stem / metal accumulation in root

TF (stem to leaves) = metal accumulation in leaves / metal accumulation in stem

To manage accuracy and precision, all the experiments were carried out in the triplicates and the mean of triplicate analysis was used in results. Certified research materials for mono metals provided by NPL, New Delhi were used for the calibration of AAS for analysis of different metals.

Statistical analysis: All data were subjected to two-way ANOVA (analysis of variance) to determine the significant difference between bacterial treatments and control plants (without consortium) with respect to metal accumulation in different plant parts. Mean and standard deviation (SD) were also worked as per standard procedure (Gomez and Gomez, 1984).

Results and Discussion

Soil characteristics: Soil of the experimental site in the Botanical Garden, NBRI was sandy loam in texture (sand 57%, silt 20% and clay 20%), alkaline in nature with EC 236 ± 11 µS cm^{-1}, organic carbon 1.5%, total nitrogen 1.5%, total phosphorus 0.1% and cation exchange capacity 9.6 meq 100 g^{-1}. Soil contained maximum concentration of Fe and Ni. Where as other metals (µg g^{-1} soil) present in the soil were in the following order : B>Zn>Pb>Cd>Mo>Cr>Cu>Mn. Al and Si were not present in the garden soil used in this study (Table 1).

Physico-chemical properties of fly ash and soil amended: The results of metal analysis in 100% FA showed that the level of heavy metals (Zn, Pb, Ni, Cu, Cr and Cd) was significantly higher than FA + GS, except Fe. Fly ash had a high EC of 389 µS cm^{-1} and cation exchange capacity of 3.9 meq, but contained low organic carbon (Table 2).

In fly ash, Si and Al were present at the maximum concentration of 5600 and 4615 µg g^{-1} as compared to other metals. Among other metals in fly ash, Fe concentration

Table 1 : Physico-chemical properties of garden soil of NBRI, Lucknow used in the experiment

Physical parameters	Garden Soil
pH	7.43-7.62
EC (µS cm^{-1})	236 ± 11
Water holding capacity (%)	42 ± 2.1
Cation exchange capacity [meq (100 g)$^{-1}$]	9.6 ± 0.1
Total nitrogen (%)	1.5 ± 0.1
Total phosphorus (%)	0.1 ± 0.0
Organic carbon (%)	1.5 ± 0.1
Metals (µg g^{-1})	
Fe	708.56 ± 29.1
Zn	69.43 ± 2.16
Cu	12.61 ± 2.13
Cr	15.89 ± 2.12
Cd	27.45 ± 2.12
Pb	28.12 ± 2.10
Ni	168 ± 21.10
Mn	11.11 ± 3.20
B	115 ± 12.0
Al	0.0
Si	0.0
Mo	26 ± 1.20

Values are mean of three replicates ± SD

(416 μg g^{-1}) was maximum followed by B (290 μg g^{-1}), Ni (204 μg g^{-1}) and Mn (12 μg g^{-1}). Other metals present in fly ash were present in the following decreasing order of their concentration: Zn>Cd>Pb>Mo>Cu>Cr. After amendment with soil, the metal concentration decreased considerably (B, 184 μg g^{-1}, Ni 178 μg g^{-1}, Mn 8 μg g^{-1}). However, the Fe content was significantly higher in fly ash amended soil as compared to fly ash alone. The garden soil mixed with fly ash used in this study had slightly alkaline pH 7.43-7.62, which was not significantly different from pH of fly ash (7.54-7.58). Besides, N and P were higher in fly ash amended soil than fly ash alone for supporting plant growth.

Growth of bacteria: It was observed that bacterial strains continued to grow in the rhizospheric zone till 60 days and then showed a declining trend (Table 3). Maximum growth of tagged bacterial consortium was recorded as 4.5 x 10^{12} cfu g^{-1} in 50% FA + 50% GS + BC, followed by 100% FA + BC (3.4 x 10^{11} cfu g^{-1}) after 60 days of incubation.

Plant biomass: As evident from Fig. 1, the plant biomass was maximally enhanced in 50% FA + 50% GS treated with BC, followed by 100% FA treated with BC and the least was in plants raised in 100% FA. A maximum biomass (25.03 μg $plant^{-1}$) of *T. latifolia* was found in 50% FA + 50% GS + BC, followed by 100% FA + BC (21.5 μg $plant^{-1}$) and the least (17.1 μg $plant^{-1}$) was observed in 100% FA without BC.

Metal accumulation in *T. latifolia*: Metal accumulation in different plant parts indicated a product of metal concentration and biomass. All the metals were accumulated maximum in the stem, followed by root and minimum in leaves of *T. latifolia*. It is evident from Fig. 2 that although Pb accumulation continued to increase with the plant growth (biomass), but there was differentiated accumulation of Pb in different plant parts. The highest accumulation of Pb was observed in stem, followed by root and minimum was in leaves in all the treatments. As compared to FA alone, the Pb accumulation was evidently higher in other treatments

Table 2 : Physico-chemical properties of fly ash alone and in combination with the soil and bacterial consortium used in the experimentation

Physical parameters	100% FA without BC	50% FA + 50% GS + BC	100%FA + BC
pH	7.5-7.54	7.45-7.67	7.5-7.58
EC (μS cm^{-1})	389 ± 120	306 ± 11.0	388 ± 130
Water holding capacity (%)	80 ± 4.30	59 ± 3.20	79 ± 3.30
Cation exchange capacity [meq (100 g)$^{-1}$]	3.9 ± 0.10	6.4 ± 0.20	3.8 ± 0.10
Total nitrogen (%)	0.02 ± 0.10	0.06 ± 0.02	0.02 ± 0.01
Total phosphorus (%)	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.00
Organic carbon (%)	0.4 ± 0.10	1.0 ± 0.30	0.5 ± 0.10
Metals (μg g^{-1})			
Fe	415.56 ± 21	548 ± 20.0	415 ± 19.0
Zn	82.43 ± 2.16	62.35 ± 2.16	82 ± 3.00
Cu	24.0 ± 2.13	20.67 ± 2.12	24.0 ± 2.00
Cr	23.89 ± 2.12	17.55 ± 2.11	23.4 ± 1.31
Cd	42.45 ± 2.12	32.98 ± 2.11	42.3 ± 1.55
Pb	40.12 ± 2.1	30 ± 1.10	40.1 ± 2.10
Ni	203 ± 11.0	178 ± 6.20	204 ± 9.53
Mn	12.11 ± 3.2	8.00 ± 1.20	12.14 ± 1.15
B	290 ± 13.0	184 ± 10.01	290 ± 14.0
Al	4615 ± 132	2223 ± 111.2	4615 ± 133
Si	5600 ± 180	2711 ± 110.2	5600 ± 181
Mo	26.0 ± 1.20	25.0 ± 5.33	24.0 ± 1.20

Values are mean of three replicates ± SD

Table 3 : Colony forming unit (CFU) of rifampicin tagged bacterial strains for different incubation periods (A) 50% FA + 50% GS + BC (B) 100% FA + BC

Bacterial consortium		cfu g^{-1}			
		Zero day	30 days	60 days	90 days
(A)	*Bacillus endophyticus*+*Paenibacillus macerans*+ *Bacillus pumilus*	2.5 ± 0.4 x 10^{5}	2.2 ± 0.3 x 10^{7}	4.5 ± 0.7 x 10^{12}	2.1 ± 0.4 x 10^{11}
(B)	*Bacillus endophyticus*+*Paenibacillus macerans*+ *Bacillus pumilus*	1.6 ± 0.2 x 10^{4}	7.5 ± 0.4 x 10^{6}	3.4 ± 0.3 x 10^{11}	2.2 ± 0.5 x 10^{9}

Values are mean of three replicates ± SD

Table 4 : Translocation factor showing translocation of metals from root to stem in *T. latifolia*

Days	Treatments	Pb	Cd	Cr	Cu	Ni	Zn	Fe
	100% FA - BC	1.06	1.19	1.29	1.44	1.15	2.07	0.91
30	100% FA + BC	1.15	1.30	1.49	1.44	1.52	2.22	1.40
	50% FA + 50% GS + BC	1.08	1.06	1.39	1.43	1.29	1.87	0.54
	100% FA - BC	1.11	1.28	1.27	1.43	1.19	2.15	1.09
60	100% FA + BC	1.13	1.21	1.47	1.45	1.50	2.12	1.38
	50% FA + 50% GS + BC	1.03	1.03	1.23	1.24	1.30	1.81	1.12
	100% FA - BC	1.26	1.12	1.38	1.41	2.60	2.08	1.03
90	100% FA + BC	1.07	1.16	1.27	1.26	1.95	1.7	1.23
	50% FA + 50% GS + BC	1.05	0.97	1.21	1.26	1.91	1.80	1.15

Table 5 : Translocation factor showing translocation of metals from stem to leaves in *T. latifolia*

Days	Treatments	Pb	Cd	Cr	Cu	Ni	Zn	Fe
	100% FA - BC	0.04	0.09	0.07	0.15	0.18	0.09	0.17
30	100% FA + BC	0.09	0.15	0.13	0.17	0.21	0.11	0.19
	50% FA + 50% GS + BC	0.09	0.14	0.10	0.17	0.23	0.12	0.47
	100% FA - BC	0.10	0.13	0.12	0.19	0.22	0.12	0.22
60	100% FA + BC	0.14	0.19	0.17	0.22	0.26	0.15	0.25
	50% FA + 50% GS + BC	0.13	0.18	0.14	0.20	0.25	0.13	0.22
	100% FA - BC	0.12	0.17	0.16	0.22	0.23	0.14	0.25
90	100% FA + BC	0.17	0.20	0.18	0.23	0.24	0.19	0.23
	50% FA + 50% GS + BC	0.21	0.22	0.18	0.25	0.31	0.16	0.30

with BC, indicating the role of bacteria in pushing up metal accumulation ($p>0.05$). Sheng *et al*. (2008) noted that *Brassica napus* grown in soil inoculated with *P. fluorescens* G10, accumulated 59 to 80% more Pb in root and 20 to 25% more in shoot while *Microbacterium* sp. G16 induced 76 to 131% more accumulation in root and 29 to 33% more in shoot as compared to the dead bacterial-inoculation control.

In case of Cd, the BC with FA and FA with GS could push up Cd accumulation from fly ash in all plant parts significantly ($p>0.05$) as compared to FA alone Among three treatments, the highest accumulation of Cd was found in FA + BC, which was 80.4% more in root, 109.5% more in stem and 115% more in leaves at 90 days of plant growth, as compared to FA alone (Fig. 3). Li *et al.* (2007) reported that inoculation of *Burkholderia cepacia* enhanced translocation of Cd by 296% and Zn by 135% in *S. alfredii* from root to shoot. Hussein (2008) also reported enhanced accumulation of Cd by the inoculation of *B. licheniformis* in the rhizosphere of *B. juncea*. Other bacteria like *Azotobacter chrococcum* (N-fixing bacteria), *Bacillus megaterium* (P-solubilizer), *Pseudomonas* sp. and *B. mucilaginous* (K-solubilizer) and *Bacillus* sp. RJ16 decreased the pH value by excreting low molecular weight organic acids, which enhanced the bioavailability of Cd, Pb and Zn to facilitate plant uptake (Chen *et al*., 2005 ; Sheng and Xia, 2006; Li and Ramakrishna, 2011).

Similarly, Cr accumulation was also observed more in FA with BC like other metals in all plant parts as compared to FA alone (Fig. 4). Among three treatments, the highest accumulation of Cr was found in FA with BC which was 96% more in root, 80% more in stem and 105 % more in leaves at 90 days as compared to FA alone ($p>0.05$). Abou-Shanab *et al*. (2008) observed that *Sorghum bicolor* inoculated with *B. subtillus* accumulated Cr in plant shoots by 11-fold higher than the control.

Following the same pattern, Cu was also accumulated 71% more in root, 53% more in stem and 60% more in leaves at 90 days in the plants raised in the FA with BC as compared to FA alone ($p>0.01$). However, Cu accumulation in this treatment was less than FA and GS with BC, but more than FA alone (Fig. 5)

Like Cu, Ni accumulation was also maximum in the FA and GS with BC among all the treatments. It was observed that Ni accumulation was enhanced by 92% in root, by 44% in stem and by 56% in leaves in the FA with BC at 90 days as compared to FA alone ($p>0.01$). However, in FA with BC, the Ni accumulation was less than FA and GS with BC but more than FA alone (Fig. 6). Abou-Shanab *et al*. (2003) observed that inoculation of *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *Microbacterium arabinogalactanolyticum* in the rhizosphere significantly

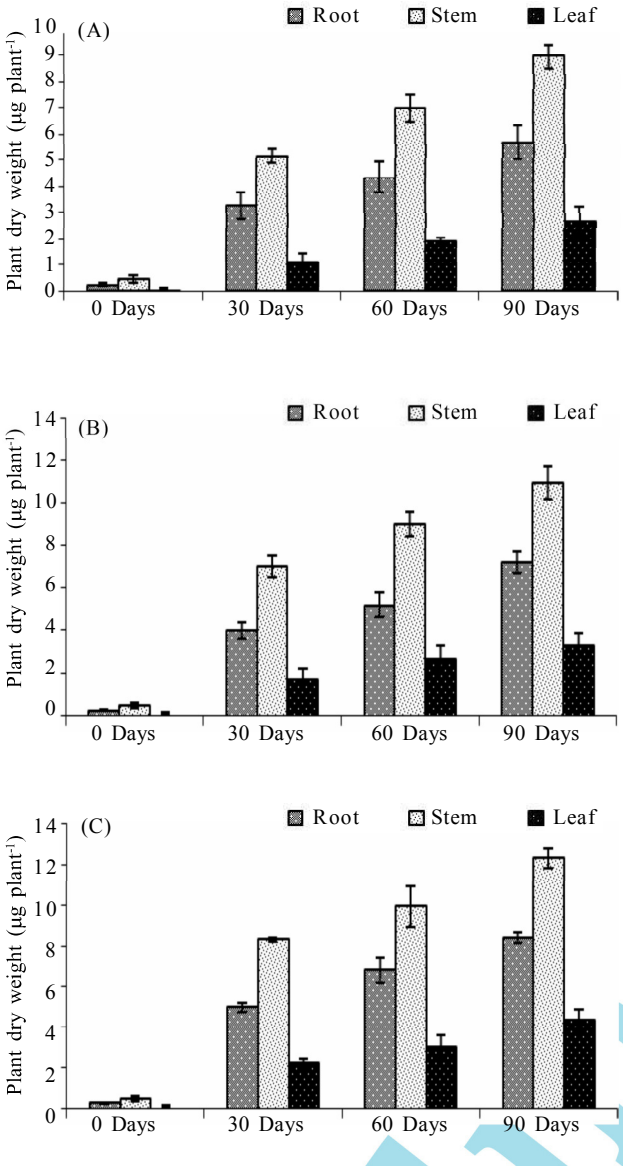

Fig. 1 : Biomass of root, stem and leaf of *T. latifolia* grown in (A) 100% FA without BC; (B) 100% FA+BC and (C) 50% FA + 50% GS +BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.05

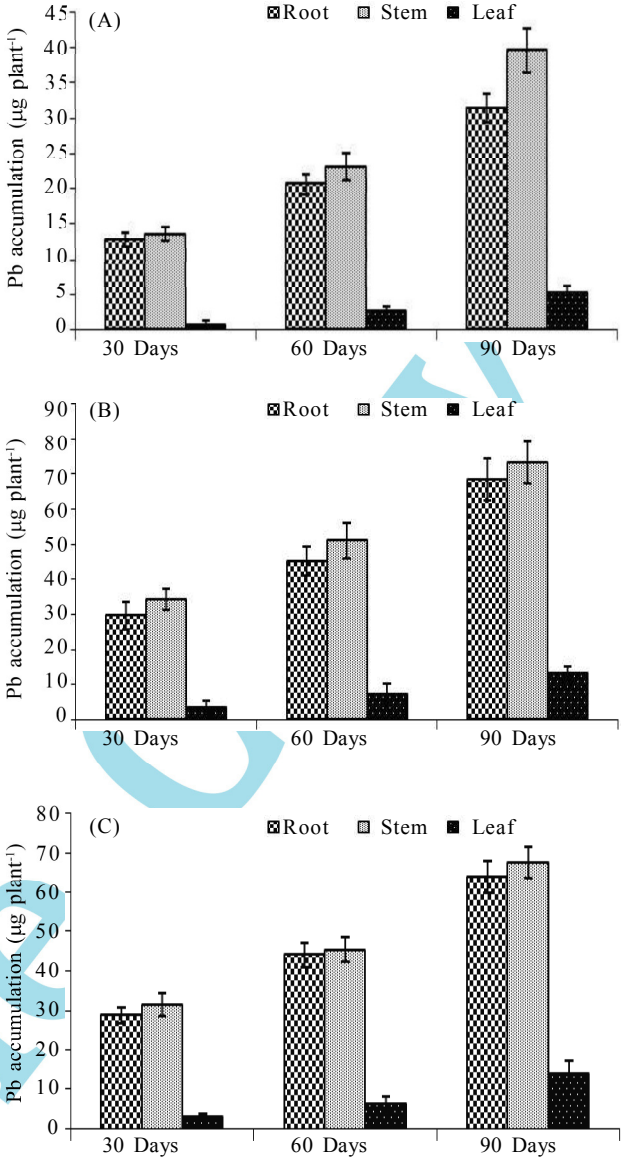

Fig. 2 : Lead accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.05

increased the uptake of Ni by *Alyssum murale* grown in serpentine soil when compared to the un-inoculated soil as a result of soil pH reduction.

Like Cu and Ni, accumulation of Fe and Zn was observed highest in *T. latifolia* raised in FA and GS with BC instead of FA with BC. As far as the partitioning of Fe and Zn in the plant body was concerned, it was found highest in stem, followed by root and the least was observed in leaves. At 90 days, Fe accumulation was increased by 164% in root, by 196% in stem and by 251% in leaves (Fig.7) and Zn accumulation by 82% in root, 57% in stem and 91% in leaves in FA and GS with BC as compared to FA without BC (Fig. 8) (p>0.01). Whiting *et al*. (2001) reported that an addition of a mixed inoculum of *M. saperdae, P. monteilii* and *E. cancerogenes* to surface sterilized seeds of *Thalaspi caerulescens* sown in autoclaved soil increased the Zn concentration in shoots by 2-fold as compared to non-inoculated controls while the total accumulation of Zn was enhanced 4-fold. Tripathi *et al.* (2004) and Sinha and Gupta (2005) reported significantly high accumulation of metals (Fe, Mn, Zn, Cu) in different parts of plants *Cassia siamea* and *Sesbania cannabina*, respectively from fly ash.

Translocation factor: The translocation factors (TF) of metal transport from root to stem and stem to leaves have been

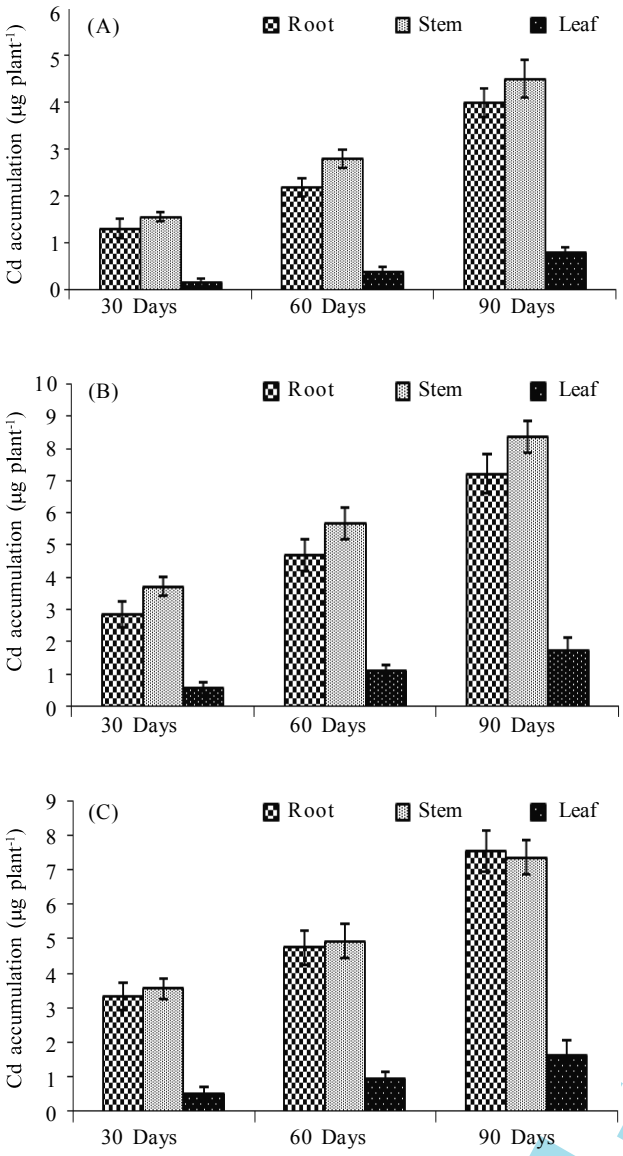

Fig. 3 : Cadmium accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.05

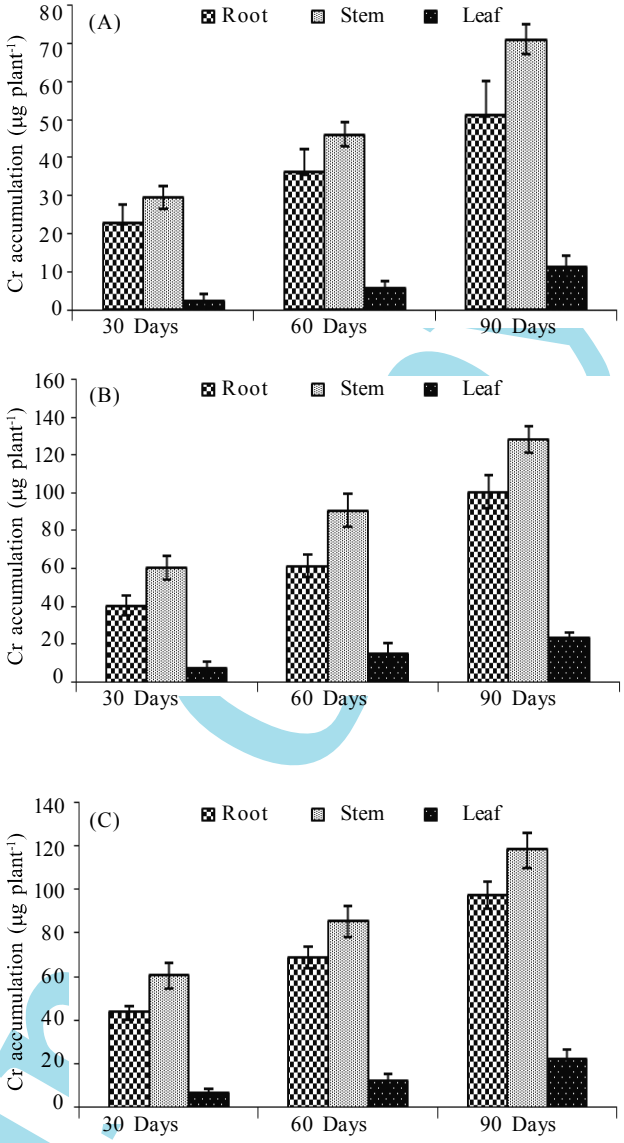

Fig. 4 : Chromium accumulation in different plant parts of T. latifolia raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.05

worked out as reflected in Table 4 and 5. The TF of metal transfer from root to stem was found in the following order : Ni (1.91)> Zn (1.80), Cu (1.26), Cr (1.21), Fe (1.15), Pb (1.05) and Cd (0.97) in 50% FA + 50% GS + BC, respectively. However, in FA + BC, the pattern of TF was in the order of Ni (1.95)> Zn (1.70)> Cr (1.27)> Cu (1.26)> Fe (1.23)> Cd (1.16)> Pb (1.07) after 90 days. Similarly, in 100 % FA without BC, the TF for different metals was in the order of Ni (2.60)> Zn (2.08)> Cu (1.41)> Cr (1.38)> Pb (1.26)> Cd (1.12)> Fe (1.03), respectively.

In case of metal transfer from stem to leaves, TF was found in the decreasing order of Ni (0.31)>Fe (0.30)>Cu (0.25)>Cd (0.22)>Pb (0.21)>Cu (0.18)>Zn (0.16) in 50% FA+50% GS+BC and as Ni (0.24)>Fe (0.23)>Cu (0.23)>Cd (0.20)>Zn (0.19) Cr (0.18)>Pb (0.17) in FA with BC at 90 days which were invariably higher than those found in FA without BC.

In the present study, it was observed that bacterial consortium had been able to enhance concentration of both essential and non-essential metals like Pb, Fe, Cu, Zn, Cr, Ni and Cd in different plant parts and also promoted plant growth in FA + BC and FA + GS + BC treatments. Among these metals, Fe was accumulated more in all plant parts than the other metals.

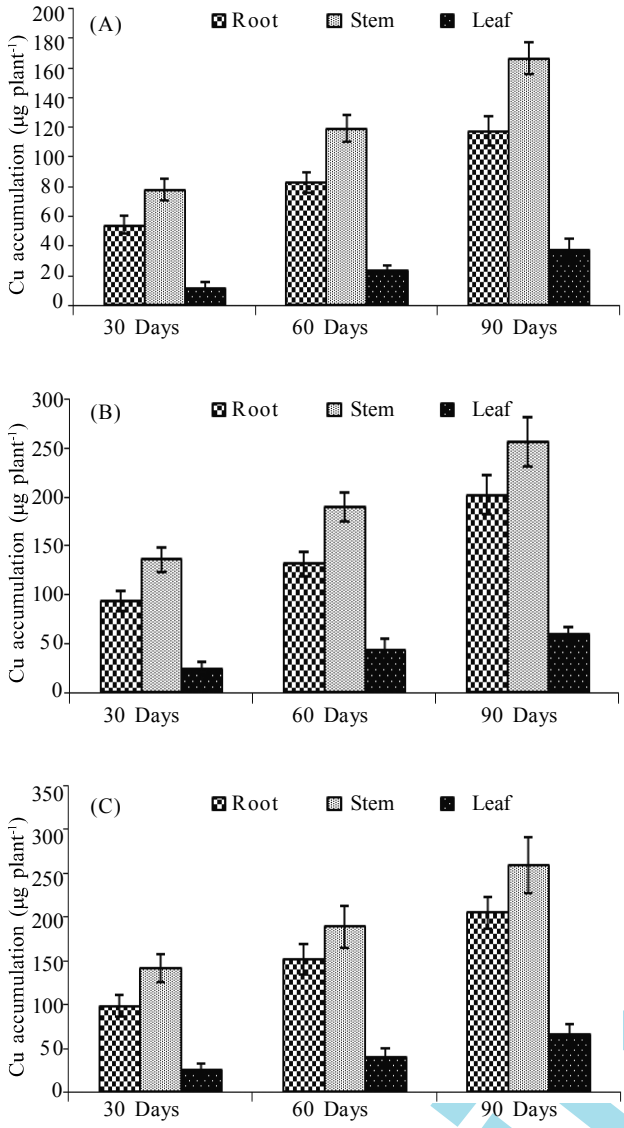

Fig. 5 : Copper accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.01

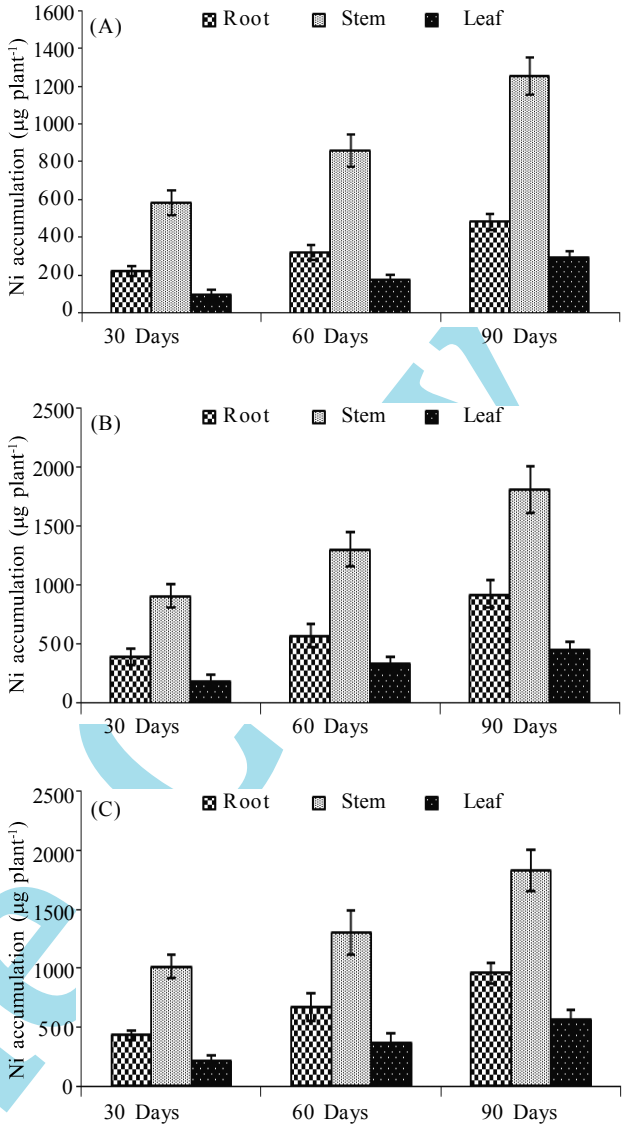

Fig. 6 : Nickel accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.01

The availability, non-availability, and bioaccumulation of metal depend on several environmental factors such as pH, solubility, salinity, soil mineralogy, texture, chemical speciation of the metal, presence of humic substances, other organic chelators, presence of other metals, and amorphous Fe and Al content and microbes. The observed differences in the metal concentration in the various parts of the plant suggest different cellular mechanisms of bioaccumulation of metals that may control their translocation and partitioning in the plant systems (Sinha *et al*., 2007). As roots act as a barrier against heavy metal translocation, maximum metal accumulation was observed in the root systems (Ernst *et al*., 1992). Low accumulation of metals in the shoots as compared to root could be due to sequestration of most of the metals in the vacuoles of the root cells which render it non-toxic (Shanker *et al*., 2005). In general, addition of bacterial consortium caused differentiated accumulation of metals (Fe, Zn, Cu, Cr, Ni, Cd and Pb) in the roots, shoots, and leaves of *T. latifolia*. For differentiated accumulation of metals in plant parts, Baker and Walker (1990) suggested that uptake, translocation and accumulation mechanisms of the plant species differed widely for different heavy metals. In the present study, *T. latifolia* showed higher potential to accumulate heavy metals. This may be attributed to well-developed root-shoot system in *T. latifolia*. Burd *et al*. (2000) have also recorded similar observations upon inoculation with *K. ascorbata* under Ni, Pb and Zn stress. Similarly,

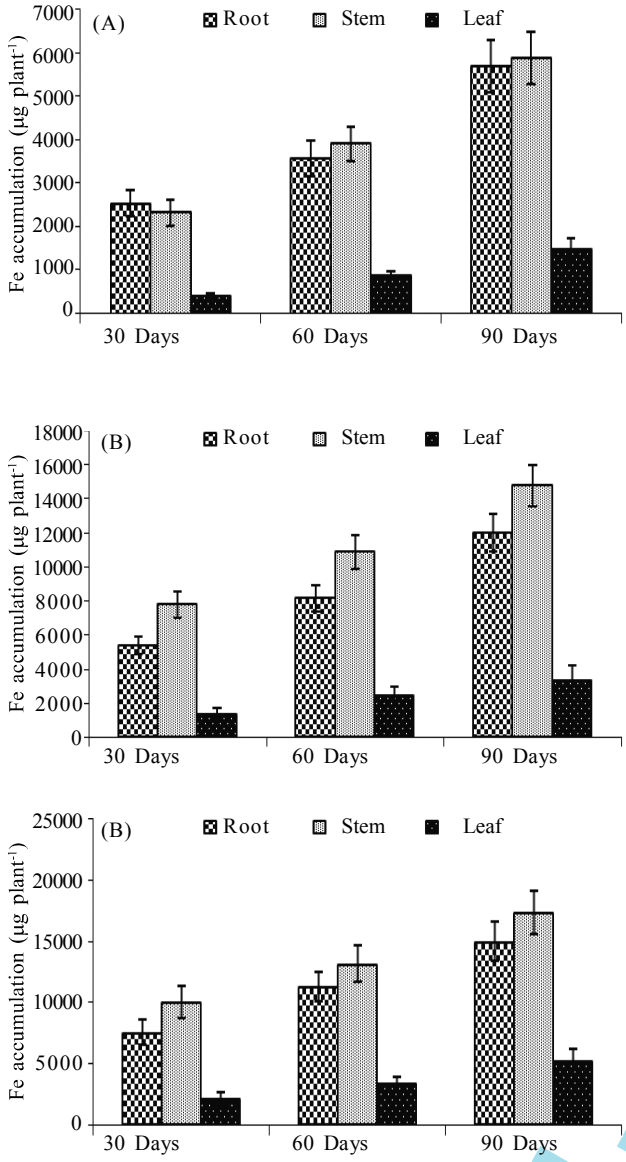

Fig. 7 : Iron accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.05

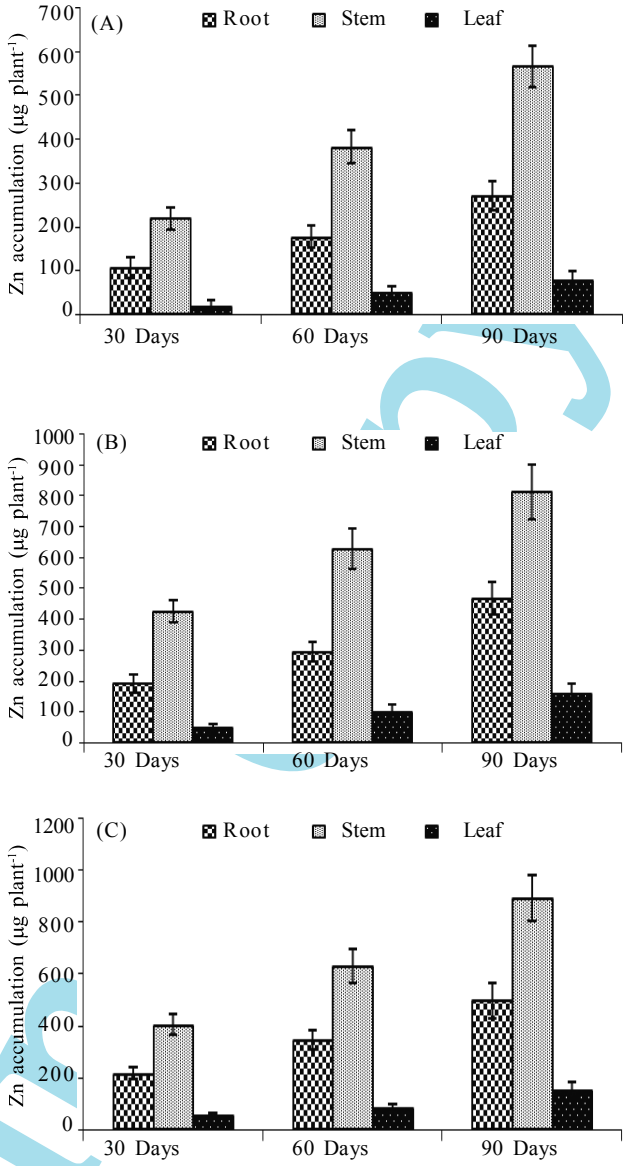

Fig. 8 : Zinc accumulation in different plant parts of *T. latifolia* raised in (A) 100% FA without BC; (B) 100% FA + BC and (C) 50% FA + 50% GS + BC at different stages of growth. Values are average of three replicates ± SD. Data are significant at level of p>0.01

appreciable amounts of Cr and Zn were accumulated in roots as compared to shoot as reported earlier by Rajkumar *et al*. (2006).

Rhizobacteria have been shown to possess several traits that can induce heavy metal bioavailability (McGrath *et al*., 2001; Whiting *et al*., 2001; Lasat, 2002; Turgay *et al*., 2012) through the release of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential (Smith and Read, 1997).

Some of the microorganisms, which are closely associated with roots, are also found to be plant growth promoting rhizobacteria (PGPR) (Glick, 1995). Gupta *et al*. (2002) attributed the enhanced plant growth to utilization of ACC, synthesis of phytoharmone and solubilization of minerals. Plant growth-promoting rhizobacteria include a diverse group of free-living soil bacteria that can improve host plant growth and development in heavy metal contaminated soils by mitigating toxic effects of heavy metals on the plants (Belimov *et al*., 2004). Induced plant growth by PGPR can further boost up metal accumulation by plants, as observed in our experimentation. Induced plant growth by PGPR can further boost up metal accumulation by enhanced plant growth, as observed in our experimentation. Hence, bioaugmentation of bacteria in the rhizosphere may be used as a tool to push up phytoextraction process by suitable plants from the fly ash.

Acknowledgment

The authors are thankful to the Director, National Botanical Research Institute, Lucknow for providing laboratory facilities and to CSIR for financial support to networked project COR008.

References

Abbaspour, A., J.M. Arocena and M. Kalbasi: Uptake of phosphorous and lead by *Brassica juncea* and *Medicago sativa* from chloromorphite. *Int. J. Phyto*., **14**, 531-542 (2012).

Abou-Shanab, R.A., J.S. Angle, T.A. Delorme, R.L. Chaney, P. Van Berkum, H. Moawad, K. Ghanem and H.A. Ghozlan: Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. *New Phytol.,* **158**, 219-224 (2003).

Abou-Shanab, R.A., K. Ghanem, N. Ghanem and A. Al-Kolaibe: The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. *World J. Microbiol. Biotechnol.*, **24**, 253-262 (2008).

Allen, S.E., H.M. Grimshaw and A.P. Rowland: Chemical analysis. In: Methods in Plant Ecology (Eds. P.D. Moore and S.B. Chapman). Blackwell Scientific Publication, Oxford, London, p. 285-344 (1986).

Allison, F.E.: Soil organic matter and its role in crop production. Developments in Soil Science No. 3. P. 637. Elsevier Scientific Publishing, London, New York (1973).

Baker, A.J.M. and P.L. Walker: Ecophysiology of metal uptake by tolerant plants. In: Heavy Metal Tolerance in Plants (Ed.: A.J. Shaw). Evolutionary Astteets, CRC Press, Florida, p. 155-177 (1990).

Belimov, A.A., A.M. Kunakova, V.I. Safronova, V.V. Stepanok, L.Y. Yudkin, Y.V. Alekseev and A.P. Kozhemyakov: Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. *Microbiol.* (Moscow), **73**, 99-106 (2004).

Black, C.A: Methods of soil analysis. Part 2. 2nd Edn., Agronomy Monog., ASA, Madison, WI ((1965).

Burd, G.I., D.G. Dixon and B.R. Glick: Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol*., **46**, 237-245 (2000).

Braud, A., K.M. Jezequel, S. Bazot and T. Lebeau: Enhanced phytoextraction of an agricultural Cr and Pb contaminated soil by bioaugmentation with siderophore producing bacteria. *Chemosphere*, **74**, 280-286 (2009).

Cechmankova, J., R. Vacha, J. Sakala and M. Havelkova: Heavy metals phytoextraction from heavily and moderately contaminated soil by field crops grown in monoculture and crop rotation. *Soil Water Res.*, **6**, 120-130 (2011).

Chen, Y.X., Y.P. Wang, Q. Lin and Y.M. Luo: Effect of copper-tolerant rhizosphere bacteria on mobility of copper in soil and copper accumulation by *Elsholtzia splendens*. *Environ. Int.*, **31**, 861-866 (2005).

Clemens, S., M.G. Palmgren and U. Kramer: A long way ahead: Understanding and engineering plant metal accumulation. *TRENDS Plant Sci.*, **7**, 309-315 (2002).

Crowley, D.E., Y.C. Wang, C.P.P. Reid and P.J. Szaniszlo: Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil*, **130**, 179-198 (1991).

Cunningham, S.D. and D.W. Ow: Promises and prospects of phytoremediation. *Plant Physiol.*, **110**, 715-719 (1996).

Ernst, W.H.O., J.A.C. Verkleij and H. Schat: Metal tolerance in plants. *Acta Bot. Neerl.*, **41**, 229-248 (1992).

Glick, B.R.: The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.*, **41**, 109-117 (1995).

Gomez, K.A. and A.A. Gomez: Statistical procedure for agricultural research. John Wiley and Sons, New York, (1984).

Gupta, D.K., U.N. Rai, R.D. Tripathi and M. Inouhe: Impacts of fly ash on soil and plant responses. *J. Plant Res.*, **115**, 401-409 (2002).

Hussein, H.S.: Optimization of plant-bacteria complex for phytoremediation of contaminated soils. *Int. J. Bot.*, **4**, 437-443 (2008).

Jing, Y.D., Z.L. He and X.E. Yang: Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Phytoremediation*, **3**, 192-207 (2007).

Kalra, N., M.C. Jain, H.C. Joshi, R. Choudhary, R.C. Harit, V.K. Vasta, S.K. Sharma and V. Kumar: Fly ash as a soil conditioner and fertilizer. *Biores. Technol.*, **64,** 163-167 (1998).

Kalra, Y.P. and D.G. Maynard: Methods: Manual for forest soil and plant analysis. Forest Cannadian. Northwest Region., North. For. Cent., Edmonton, Alberta (1991).

Kuffner, M., M. Puschenreiter, G. Wieshammer, M. Gorfer and A. Sessitsch: Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating *willows*. *Plant Soil*, **304**, 35-44 (2008).

Lasat, H.A.: Phytoextraction of toxic metals: A review of biological mechanisms. *J. Environ. Qual.*, **31**, 109-120 (2002).

Lebeau, T., A. Braud and K. Jezequel: Performance of bioaugmentation -assisted phytoextraction applied to metal contaminated soil: A review. *Environ. Pollut.*, **153**, 497-522 (2008).

Li, W.C., Z.H. Ye and M.H. Wong: Effects of bacteria on enhanced metal uptake of CD/Zn-hyperaccumulating plant *Sedum alfredii*. *J. Exp. Bot.*, **58**, 4173-4182 (2007).

Li, K. and W. Ramakrishna: Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth. *J. Hazard. Mater.*, **189**, 531-539 (2011).

Lugtenberg, B. and F. Kamilova: Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.*, **63**, 541-556 (2009).

McCutcheon, S.C. and J.E. Schnoor: Phytoremediation: Transformation and control of contaminants. John Willey & Sons (2003).

McGrath, S.P., F.J. Zhao and E. Lombi: Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. *Plant Soil*, **232**, 207-214 (2001).

Olsen, S.R. and L.E. Sommers: Phosphorus. In: Methods of Soil Analysis (Eds.: A.L. Page, R.H. Miller and D.R. Keeney). Part 2. 2nd Edn. ASA Monograph No 9, Madison, WI, pp. 403-430 (1982).

Pandey, V.C., J.S. Singh, A. Kumar and D.D. Tewari: Accumulation of heavy metals by chickpea grown in fly ash treated soil: Effect on antioxidants. *Clean- Soil Air Water*, **38**, 1116-1123 (2010).

Rajkumar, M., K.J. Lee, W.H. Lee, R. Nagendran and S.Z. Kim: Influence of plant growth promoting bacteria and Cr^{6+}on the growth of Indian mustard. *Chemosphere*, **62**, 741-748 (2006).

Sarma, H.: Metal hyperaccumulation in plants: A review focusing on phytoremediation technology. *J. Environ. Technolol.*, **4**, 118-138 (2011).

Saxena, P.K., S. Krishnaraj and T. Dan: Phytoremediation of heavy metal contaminated and polluted soils. In: Heavy Metal Stress in Plants: from Molecules to Ecosystems (Eds.: M.N.V. Prasad and J. Hagemeyer) Berlin, Springer. p. 305-329 (1999).

Shanker, A.K., C. Carlos, H. Loza-Tavera and S. Avudainayagam: Chromium toxicity in plants. *Environ. Int.*, **31**, 739-753 (2005).

Sheng, X.F. and J.J. Xia: Improvement of rape (*Brassica juncea*) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere*, **64**, 1036-1042 (2006).

Sheng, X.F., J.J. Xia, C.Y. Jiang, L.Y. He and M. Qian: Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ. Pollut.*, **156**, 1164-1170 (2008).

Singh, R., D.P. Singh, N. Kumar, S.K. Bhargava and S.C. Barman: Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. *J. Environ. Biol.*, **31**, 421-430 (2010).

Sinha, S. and A.K. Gupta: Translocation of metals from fly ash amended soil in the plant of *Sesbania cannabina* L. Ritz: Effect on antioxidants. *Chemosphere*, **61**, 1204-1214 (2005).

Sinha, S., A. Gupta and K. Bhatt: Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: Involvement of antioxidants. *Ecotoxicol. Environ. Saf.*, **67**, 267-277 (2007).

Smith, S.E. and D.J. Read: Mycorrhizal Symbiosis. San Diego Academic Press Inc. (1997).

Stephenson, D. and T. Stephenson: Biaugmentation for enhancing biological wastewater treatment. *Biotechnol. Adv.*, **10**, 549-559 (1992).

Tiwari, S., B. Kumari and S.N. Singh: Evaluation of metal mobility/immobility in fly ash induced by bacterial strains isolated from rhizospheric zone of *Typha latifolia* growing on fly ash dumps. *Biores. Technol.*, **99**, 1305-1310 (2008a).

Tiwari, S., B. Kumari and S.N. Singh: Microbe-induced changes in metal extractability from fly ash. *Chemosphere*, **71**, 1284-94 (2008b).

Tiwari, S., S. N. Singh and S.K. Garg: Stimulated phytoextraction of metals from fly ash by microbial interventions. *Environ. Technol.*, **33**, 2405-2413 (2012).

Tripathi, R.D., P. Vajpayee, N. Singh, U.N. Rai, A. Kumar, M.B. Ali, B. Kumar and M. Yunus: Efficacy of various amendments for amelioration of fly ash toxicity: Growth performance and metal composition of *Cassia siamea* Lamk. *Chemosphere*, **54,** 1581-1588 (2004).

Turgay, O.C., A. Gormez and S. Bilen: Isolation and characterization of metal resistant-tolerant rhizosphere bacteria from the serpentine soils in Turkey. *Environ. Monit. Assess.*, **184**, 515-526 (2012).

Wenzel, W.W., E. Lombi and D.C. Adriano: Biogeochemical processes in the rhizosphere: Role in phytoremediation of metal-polluted soils. In: Heavy Metal Stress in Plants (Eds.: M.N.V. Prasad and J. Hagemeyer). Berlin, Springer, p. 273-303 (1999).

Whiting, S.N., M.P. De-Souza and N. Terry: Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. *Environ. Sci. Technol.*, **35**, 3144-3150 (2001).

Walkley, A. and I.A. Black: An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.*, **63**, 251-263 (1934).

Zayad, A., C.M. Lytle, J.H. Qian and N. Terry: Chromium accumulation, translation and chemical speciation in vegetable crops. *Planta*, **206**, 293-299 (1998).