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Phytoremediation of metals from fly ash through bacterial augmentation

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Abstract Different combinations of four bacterial strains isolated from fly ash were used by us to study their impact on phytoextraction of metals from fly ash by *Brassica juncea* grown in fly ash amended with farm yard manure (50:50 w/w). Out of 11 bacterial consortia, a combination of two strains i.e. *Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9 (C7) inoculated in the rhizosphere was found to enhance Pb accumulation maximally by 278%, Mn by 75%, Zn by 163%, Cr by 226% and Ni by 414% compared to control. It is possible that these bacteria, known for N_2 fixation, solubilization of phosphorus and uptake of micronutrient, could promote the plant growth resulting in higher accumulation of metals. However, a combination of four bacteria, namely *Micrococcus roseus* NBRFT2 + *Bacillus endophyticus* NBRFT4 + *Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9 (C4) was able to increase Cd uptake maximally by 237%. Further, the translocation of metal was invariably more from root to stem than from stem to leaf which was regulated by plant transport mechanism and metal mobility. Bacteria are known to excrete protons, organic acids, enzymes and siderophores to enhance the mobilization of metals which boosted the phytoextraction of metals from fly ash.

Keywords Fly ash · Bacterial consortia · Metal uptake · *Brassica juncea* · Metal translocation

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Introduction

Phytoremediation of toxic metals is emerging as a self sustaining, cost-effective, eco-friendly alternative technology of today to conventional physicochemical methods (Glass 2000). This technology is now being widely used for decontamination of metal-polluted non-point sources like agricultural fields, wastes disposal sites and polluted waters. As phytoremediation is generally a slow process in extraction of metals because of their low availability in contaminated sites, hence in recent years, microbes have been used to replace the costly and non-biodegradable chelating agents to enhance the process of phytoextraction of metals (Höflich and Metz 1997). Although efficacy of this technology largely depends on the selection of suitable plant species which can accumulate metals quickly and efficiently from the contaminated soils, selected microbes can assist the uptake of metals from the contaminated soils and thus augment the phytoextraction process. 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. Jiang et al. (2008) found a heavy metal-resistant *Burkholderia* sp. to be promoting plant growth as well as heavy metal accumulation. Rhizospheric bacteria of *Typha latifolia* growing on fly ash were found to enhance the mobilization of metals significantly from the fly ash through redox conversion (Tiwari et al. 2008a, b). Thus, phytoextraction of metals may be improved by selecting the high biomass accumulating plants or increasing the plant biomass (PGPR effect) or by making easier the metal uptake through production of

enzymes, siderophores, organic acid or biosurfactants by inoculated microbes (Zhuang et al. 2007).

Besides, there are many other processes which govern metal accumulation in plants e.g. metal mobilization and uptake from soils, compartmentation and sequestration with in the root, efficiency of xylem to transport metal, distribution of metal in the aerial parts, sequestration and storage in leaf cells (Clemens et al. 2002). Plant roots are reported to exude protons to acidify the soil and 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.

Disposal of fly ash (130 m tons) produced by coal-fired thermal power plants is a great environmental concern in India. Despite of several options available for its utilization, we could utilize only 15% of it against the target of 100% utilization of fly ash as per guidelines of Ministry of Environment and Forest (Kalra et al. 1998). Fly ash contains a number of toxic metals like Si, Al, Hg, Pb, Ni etc. as well as essential metals, such as Cu, Zn, Mg, Fe etc. along with As and B as metalloid (Rautaray et al. 2003; Lee et al. 2006). In situ leaching of trace metals has been studied in greater details by Gong et al. (2010). Although presence of essential metals encourages us to use fly ash as a soil amender for better crop yields, metals from fly ash from disposal site continued to contaminate soil and surface and ground water through leaching. In this investigation, our objective is to select a good combination of bacteria for promoting *Brassica juncea* plant to accumulate heavy metals from fly ash to remediate contaminated environment.

Materials and methods

Fly ash collection and its physico–chemical characterization

Fly ash was collected from the fly ash dump site of National Thermal Power Corporation (NTPC) coal-based power plant located at Unnchhar, Rai Barelli, U.P. (India). For the isolation of indigenous fly ash tolerant bacterial strains, fly ash sample from the rhizospheric zone of *Typha latifolia* naturally growing on fly ash dyke was collected in the sterile poly bags.

Fly ash had particle size ranging between 10 and 150 μm, pH 7.5 and the electrical conductivity as high as 389 μS cm^{-1}. As discussed in our earlier publication, after Al and Si, Fe concentration was found to be the highest i.e. 415 μg g^{-1}, followed by B (290 μg g^{-1}), Ni (204 μg g^{-1}) and the least was Zn (82 μg g^{-1}) (Tiwari et al. 2008b). Due to low organic carbon in fly ash, farm yard manure was added to fly ash in ratio of 1:1 w/w to support the growth of *B. juncea* as well as bacteria inoculated.

Isolation and selection of rhizospheric fly ash tolerant bacterial strains

Eleven bacterial strains were isolated from fly ash sample collected from the rhizospheric zone of *Typha* stand by following the serial dilution method on NA plates (Nutrient Agar: 10 g beef extract, 5 g sodium chloride and 12 g agar in 1l). Agar plates were inoculated with fly ash extract in water and incubated at 37°C for 24 h for bacterial growth. Among eleven, four strains were selected based on their potential to mobilize heavy metals from the fly ash and biochemically characterized (Tiwari et al. 2008b).

Selected strains were identified as *Micrococcus roseus* NBRFT2 (MTCC. No. 9018), *Bacillus endophyticus* NBRFT4 (MTCC No. 9021), *Paenibacillus macerans* NBRFT5 (MTCC No. 8912), and *Bacillus pumilus* NBRFT9 (MTCC No. 8913) by Institute of Microbiology Technology, Chandigarh, Punjab (India).

For experimentation, these strains were enriched in nutrient broth (NB: 5 g peptic digest of animal tissue, 1.5 g yeast extract, 1.5 g beef extract and 5 g NaCl in 1l) at 37°C for 24 h in a orbital shaker set at 150 rpm for the preparation of different bacterial consortia in various combinations. After enrichment, CFU values of four strains i.e. *Micrococcus roseus* NBRFT2, *Bacillus endophyticus* NBRFT4, *Paenibacillus macerans* NBRFT5 and *Bacillus pumilus* NBRFT9 were 4.1×10^{12}, 2.5×10^{12}, 6.5×10^{13} and 4.3×10^{13}, respectively in the inocula.

To check the sustainability and growth of the fly ash resistant bacteria in our experiment at different time intervals, the selected four bacteria were previously tagged with rifampicin (100 μg ml^{-1}) before enrichment in NB.

Rifampicin tagging

The spontaneous rifampicin-resistant mutants of the wild strains NBRFT2, NBRFT4, NBRFT5 and NBRFT9 were generated by transferring bacterial culture from TSA (Tryptone Soya Agar) to TSB (Tryptone Soya Broth), containing increasing concentrations of rifampicin (25, 50, 100 μg ml^{-1}) and plating loopful of the culture on TSA plates amended with rifampicin 100 μg ml^{-1} (TSA-rif). NBRFT2, NBRFT4, NBRFT5 and NBRFT9 on TSA-rif plates were selected and checked for stability by transferring it 25 times from TSA-rif to TSA and again to TSA-rif plates.

Experimental set up

Thirty six earthen pots (12 × 3) were filled with fly ash and farm yard manure (FYM) (50:50). Six seeds of

Brassica juncea were sown in each pot in the month of October. After 15 days of sowing when the seedlings were established, thinning was carried out to retain two healthy plants in each pot for their study. Pots were irrigated on regular intervals to maintain adequate moisture level (70%). Thirty three pots with three replicas for each bacterial consortium were labeled as C1 to C11 and three for control without bacteria (Table 1). After one month of sowing, 600 ml inoculum of each bacterial consortium was added separately to the rhizospheric zone of different sets of *Brassica* plants grown in the earthen pots except control in which 600 ml nutrient broth without bacteria was added.

Sample preparation for metal analysis

After the flowering stage, these plants (90 days old) were harvested and washed under running water carefully to remove dust particles adhered to plants body. Excess water was removed by the blotting paper. Plants were partitioned in root, stem and leaves and then roots were kept in EDTA (20 mM) solution for 15 min (Yang et al. 1996) to remove adhered metal on the root surface. Now all samples were kept in oven for drying at 80°C till constant weight was achieved. Samples were powdered in a grinder and sieved with 2 mm sieve. 200 mg of powdered material of each sample was digested in Burgoph microwave digestion unit with 5 ml of HNO_3 (70%) and filtered through Whatman filter paper no. 44. Volume of each digested sample was maintained to 25 ml with the addition of double distilled water. Then after, different metals in sample solutions were analyzed with an Atomic Absorption spectrophotometer (GBC Avanta $\sum$ AAS).

Translocation factor

Translocation factor (TF) was calculated by using the following formulae:

TF (root to stem) = mean accumulation of metal by stem part/mean accumulation of metal by root part.
TF (stem to leaf) = mean accumulation of metal by leaf part/mean accumulation of metal by stem part.

Result

Growth of bacteria

These bacterial strains continued to grow in the rhizospheric zone till 60 days and then showed a declining trend (Table 2). Maximum growth was recorded in the combination of C7 i.e. 7.9×10^{12} CFU g^{-1} of soil and followed by C4 combination i.e. 3.3×10^{12} CFU g^{-1} of soil.

Plant biomass

As evident from Table 3, the plant growth was stimulated in C2, C3, C4, C7 and C11 treated plants as compared to control plants in which no bacterial consortium was added in the rhizospheric zone. However, in other bacterial consortia, the plant biomass was not significantly affected. The maximum biomass of *B. juncea* was recorded in C7 consortium-inoculated plants (2.84 g $plant^{-1}$). This indicated a differential role of various bacterial consortia in promoting the plant growth.

Table 1 Codes represent different combinations of selected four metal resistant bacterial strains isolated from the rhizospheric zone of *Typha latifolia* growing on fly ash dyke

Code	Combination of bacterial strains
C1	*Paenibacillus macerans* NBRFT5 + *Bacillus endophyticus* NBRFT4 + *Bacillus pumilus* NBRFT9
C2	*Bacillus endophyticus* NBRFT4 + *Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9
C3	*Paenibacillus macerans* NBRFT5 + *Micrococcus roseus* NBRFT2 + *Bacillus endophyticus* NBRFT4
C4	*Bacillus endophyticus* NBRFT4 + *Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9 + *Paenibacillus macerans* NBRFT5
C5	*Bacillus endophyticus* NBRFT4 + *Micrococcus roseus* NBRFT2
C6	*Paenibacillus macerans* NBRFT5 + *Bacillus endophyticus* NBRFT4
C7	*Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9
C8	*Paenibacillus macerans* NBRFT5 + *Micrococcus roseus* NBRFT2
C9	*Bacillus endophyticus* NBRFT4 + *Bacillus pumilus* NBRFT9
C10	*Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9
C11	*Paenibacillus macerans* NBRFT5 + *Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9
Control	NIL

Table 2 Colony Forming Unit (CFU) of rifampicin tagged bacterial strains for different incubation periods

Bacterial consortia	Zero day	30 days	60 days	90 days
C1	$2.1 \pm 0.4 \times 10^4$	$2.6 \pm 0.8 \times 10^6$	$4.5 \pm 0.9 \times 10^{10}$	$3.4 \pm 1.1 \times 10^9$
C2	$2.4 \pm 0.9 \times 10^4$	$3.1 \pm 0.7 \times 10^7$	$3.8 \pm 1.2 \times 10^{10}$	$5.2 \pm 0.2 \times 10^9$
C3	$1.6 \pm 0.3 \times 10^5$	$7.5 \pm 1.2 \times 10^7$	$1.5 \pm 0.9 \times 10^{11}$	$2.2 \pm 0.7 \times 10^9$
C4	$2.4 \pm 0.5 \times 10^5$	$6.2 \pm 0.2 \times 10^8$	$3.3 \pm 0.1 \times 10^{12}$	$4.2 \pm 0.5 \times 10^{11}$
C5	$3.2 \pm 0.2 \times 10^4$	$1.4 \pm 0.4 \times 10^6$	$4.5 \pm 1.5 \times 10^{10}$	$2.8 \pm 0.8 \times 10^9$
C6	$1.5 \pm 0.9 \times 10^4$	$4.2 \pm 0.1 \times 10^6$	$5.4 \pm 0.2 \times 10^{10}$	$6.2 \pm 0.9 \times 10^9$
C7	$2.6 \pm 0.7 \times 10^5$	$3.9 \pm 0.4 \times 10^7$	$7.9 \pm 1.9 \times 10^{12}$	$7.5 \pm 0.7 \times 10^{11}$
C8	$6.6 \pm 1.3 \times 10^4$	$1.9 \pm 0.9 \times 10^6$	$5.3 \pm 0.7 \times 10^9$	$3.1 \pm 0.4 \times 10^8$
C9	$2.1 \pm 0.6 \times 10^4$	$2.8 \pm 1.2 \times 10^6$	$4.1 \pm 0.8 \times 10^9$	$1.6 \pm 0.5 \times 10^8$
C10	$2.3 \pm 0.7 \times 10^4$	$1.4 \pm 0.4 \times 10^6$	$3.1 \pm 0.3 \times 10^9$	$3.6 \pm 0.9 \times 10^8$
C11	$3.1 \pm 0.8 \times 10^5$	$5.8 \pm 0.6 \times 10^7$	$8.5 \pm 0.6 \times 10^{11}$	$5.9 \pm 0.5 \times 10^{10}$

Table 3 Biomass of different parts of *Brassica juncea* augmented with various bacterial consortia (g plant^{-1} DW)

S. no.	Root weight	Stem weight	Leaf weight	Total biomass
Control	0.34 ± 0.09	1.12 ± 0.16	0.22 ± 0.04	1.67 ± 0.13
C1	0.51 ± 0.19	1.50 ± 0.41	0.26 ± 0.12	2.27 ± 0.32
C2	0.45 ± 0.13	1.27 ± 0.17	0.32 ± 0.05	2.04 ± 0.11
C3	0.54 ± 0.07	1.18 ± 0.16	0.23 ± 0.06	1.95 ± 0.09
C4	0.53 ± 0.12	1.62 ± 0.36	0.46 ± 0.09	2.61 ± 0.18
C5	0.32 ± 0.08	0.74 ± 0.21	0.09 ± 0.03	1.15 ± 0.08
C6	0.34 ± 0.11	0.76 ± 0.25	0.15 ± 0.01	1.26 ± 0.13
C7	0.70 ± 0.23	1.79 ± 0.18	0.35 ± 0.14	2.84 ± 0.21
C8	0.19 ± 0.08	0.59 ± 0.08	0.07 ± 0.02	0.85 ± 0.07
C9	0.21 ± 0.04	0.45 ± 0.14	0.05 ± 0.02	0.71 ± 0.09
C10	0.23 ± 0.09	0.59 ± 0.12	0.11 ± 0.18	0.93 ± 0.11
C11	0.53 ± 0.17	1.31 ± 0.25	0.16 ± 0.08	2.00 ± 0.19

Metal accumulation

Out of 11 bacterial consortia examined for their ability in boosting metal accumulation, a few of them were found to enhance uptake of different metals by *B. juncea* from fly ash significantly as compared to control.

In case of Pb, it was observed that bacterial consortia, C3, C7 and C11 could push up Pb accumulation in all plant parts i.e. root, stem and leaf considerably as compared to control where no bacterial consortium was inoculated in the rhizospheric zone of the plant (Fig. 1). Among these three consortia, the best was found to be C7 which enhanced Pb accumulation in root up to 51.5 μg plant^{-1}, in stem upto 131.76 μg plant^{-1} and in leaf up to 26.98 μg plant^{-1} which were 3.58 times more in root, 4.13 times in stems and 2.2 times in leaf as compared to control (Fig. 1). However, translocation factor did not follow the same pattern. It was observed that while Pb accumulation was the highest in the plant treated with C7 consortium, but the translocation factor (from root to stem) was observed maximum (3.48) in the C8 combination (Table 4). However, translocation factor (from stem to leaf) was highest (0.68) in C2 combination (Table 5). With this combination of bacteria, Pb accumulation was found 3.5 fold more in root, 4.13 fold in stem, 1.91 fold in leaf.

Similarly, Cr accumulation was induced many times in the plant with C4, C7 and C11 consortia. Its accumulation was found as high as 25.72 μg plant^{-1} in C7, 23.93 μg plant^{-1} in C11 and 20.83 μg plant^{-1} in C4 consortium which were found to be 3.26, 3.03 and 2.64 fold higher in C7, C11 and C4 consortia, respectively than the control. LSD analysis of data also indicates significantly higher accumulation of Cr in different plant parts in the above combinations of bacteria (Fig. 2).

Like Pb and Cr, the Zn accumulation was maximally augmented in the plants inoculated with C7 bacterial consortium, followed by C4 and C11 combinations in the decreasing order (Fig. 3). It was observed that Zn accumulation was maximum in the stem (239.26 μg plant^{-1}), followed by root (45.48 μg plant^{-1}) and the least was in the leaf (42.23 μg plant^{-1}) in C7 combination indicating faster translocation of Zn from root to stem (TF 5.26), but slower transport from stem to leaf (TF 0.18) (Table 5). Zn accumulation in the stem was 3.5 times, in root 2.04 times and in leaf 1.22 times more than control.

Following the same pattern, Ni was also accumulated maximum in the plant with C7 consortium, followed by C4 and C11 consortia in the deceasing order (Fig. 4). Other combinations of bacteria showed no significant impact on Ni uptake by the plant. As far as the partitioning of Ni in the plant body was concerned, it was obviously highest in stem (107.88 μg plant^{-1}), followed by leaf (24.9 μg plant^{-1}) and the least in root (21.42 μg plant^{-1}) in C7 treatment. Maximum accumulation in the stem indicates fast translocation of Ni from root to stem, but very restricted movement to leaf (Table 4). The highest

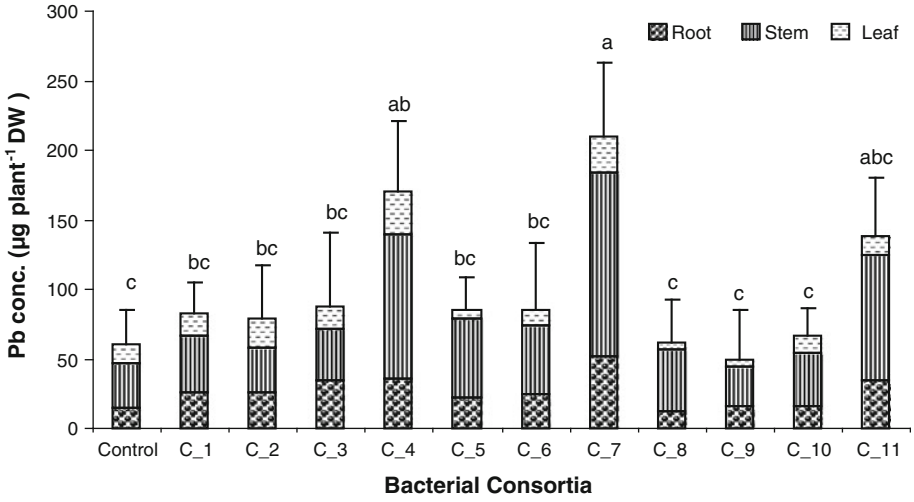

Fig. 1 Pb accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

Table 4 Translocation factor showing translocation of metals from root to stem

Bacterial combinations	Pb	Cr	Zn	Ni	Cd	Fe	Mn
Control	2.22	2.77	3.03	5.83	3.48	0.98	4.61
C1	1.61	1.17	1.48	3.18	2.03	0.45	1.50
C2	1.25	1.94	2.67	4.38	1.45	0.62	2.36
C3	1.08	1.24	0.74	2.28	1.59	1.09	0.98
C4	2.93	9.51	4.98	7.66	5.89	1.78	2.72
C5	2.62	2.74	2.83	4.33	3.68	0.60	1.51
C6	2.03	2.52	3.47	4.40	3.03	0.80	2.31
C7	2.55	5.00	5.26	5.03	2.26	1.21	5.88
C8	3.48	8.63	4.04	6.16	4.35	1.23	2.98
C9	1.82	2.94	2.30	2.71	2.10	0.36	1.42
C10	2.33	5.30	2.54	4.07	3.62	0.47	2.22
C11	2.57	3.32	4.38	3.55	2.58	0.40	2.95

Table 5 Translocation factor showing translocation of metals from stem to leaf

Bacterial combinations	Pb	Cr	Zn	Ni	Cd	Fe	Mn
Control	0.44	1.03	0.51	0.76	0.45	1.36	0.77
C1	0.40	1.09	0.51	0.52	0.33	1.04	0.51
C2	0.68	0.96	0.51	0.77	0.80	1.52	0.65
C3	0.44	0.71	0.37	0.65	0.46	0.32	0.59
C4	0.30	0.50	0.37	0.42	0.31	0.50	0.60
C5	0.11	0.31	0.06	0.16	0.18	0.36	0.20
C6	0.23	0.38	0.18	0.28	0.26	0.76	0.48
C7	0.20	0.34	0.18	0.18	0.36	0.53	0.25
C8	0.12	0.23	0.11	0.14	0.13	0.88	0.27
C9	0.14	0.25	0.17	0.22	0.20	1.36	0.27
C10	0.33	0.34	0.35	0.28	0.30	1.64	0.38
C11	0.14	0.18	0.08	0.16	0.15	1.16	0.20

translocation factor (TF 7.66) was recorded in C4 consortium for Ni transfer from root to stem, although maximum Ni was accumulated in plants with C7 combination. Fast mobility of Ni from the fly ash to plants was reflected by 8.34, 7.2 and 1.69 fold increase in its accumulation in root stem and leaf in C7 combination, respectively, as compared to control.

In contrast to Zn, Ni and Pb, the Cd uptake was enhanced maximally in *B. juncea* with C4 consortium and followed by C11 and C7 consortia in deceasing order. The maximum accumulation of Cd was recorded in stem (16.08 µg plant^{-1}), followed by leaf (4.96 µg plant^{-1}) and the least in root (2.72 µg plant^{-1}) in C4 consortium (Fig. 5) which were 2.33, 3.95 and 2.72 fold higher than control in case of root, stem and leaf, respectively. Like Pb and Ni, the translocation factor of Cd was found to be the highest (TF 5.89) from root to stem, followed by stem to leaf (TF 0.46) with C4 combination. However, the accumulation of Cd, as compared to Ni and Zn, was many folds lower in plants indicating that bacterial strains could not enhance Cd mobility from the fly ash as high as of Ni and Zn.

Among all the metals, the accumulation of Fe was the highest with all the bacteria consortia used (Fig. 6). As compared to control, the uptake of Fe was found maximum in C3 consortium (654 µg plant^{-1}), followed by C7 (486 µg plant^{-1}) and C1 (485 µg plant^{-1}) consortia in decreasing order. However, other bacterial consortia did not enhance Fe uptake by the plants. It is also obvious from the Fig. 6 that Fe was taken up the plants remained largely in the roots and only a part of it was translocated to the above ground parts as reflected by its low translocation factor (1.78) for root to stem in C3 consortium with respect to the translocation of other metals. With the intervention of bacteria, the uptake of Fe was enhanced by 2.32, 2.58 and 0.7 fold in root, stem and leaf of *B. juncea* with the action of C3 bacteria.

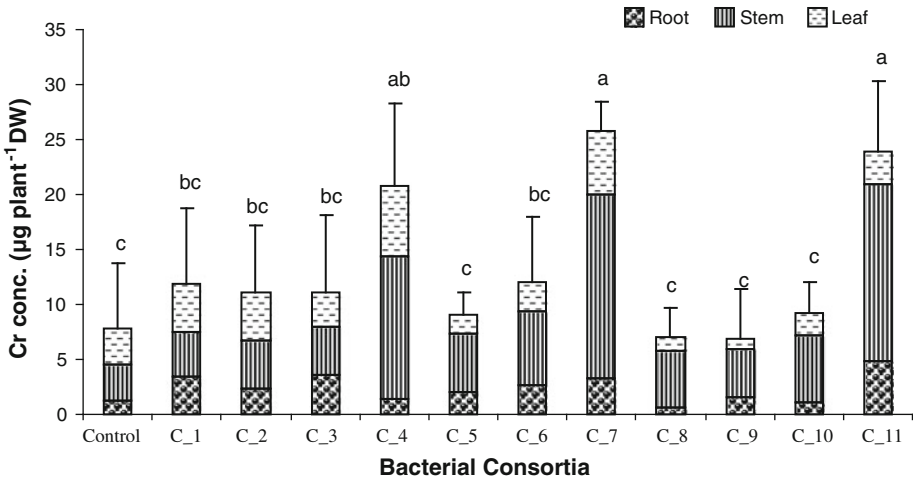

Fig. 2 Cr accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

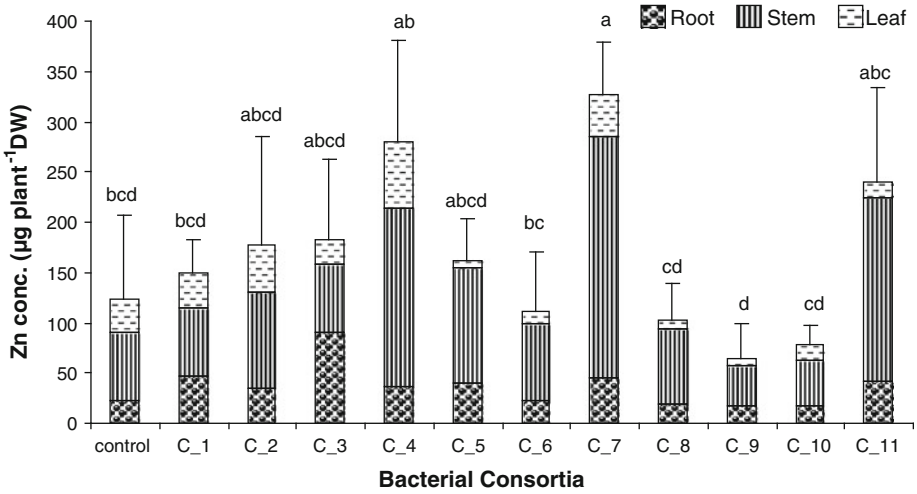

Fig. 3 Zn accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

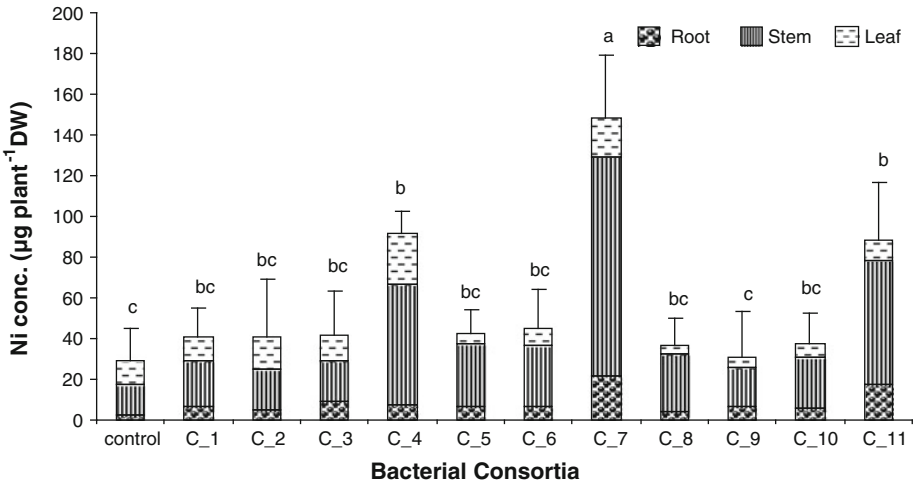

Fig. 4 Ni accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

As observed in Pb, Zn and Ni, Mn accumulation was also pushed up maximum by the intervention of C7 consortium (50.11 µg plant^{-1}), followed by C4 (47.74 µg plant^{-1}) and C2 consortia (35.31 µg plant^{-1}) in the decreasing trend (Fig. 7). It was also observed that Mn accumulation was maximum in the stem (35.27 µg plant^{-1}), followed by leaf (8.84 µg plant^{-1}) and the least in root (5.99 µg plant^{-1}) in the case of C7 combination which clearly indicated that Mn translocation from root to stem was higher (TF 5.88) than from stem to leaf (TF 0.25). This

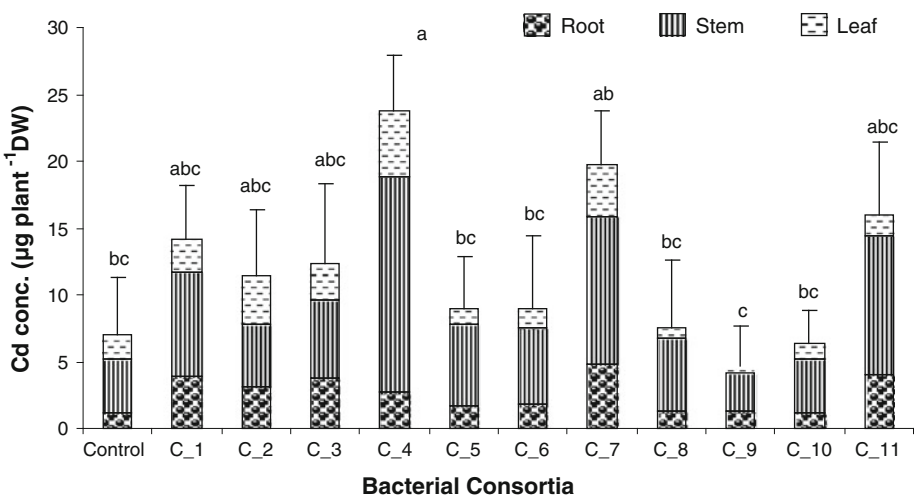

Fig. 5 Cd accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

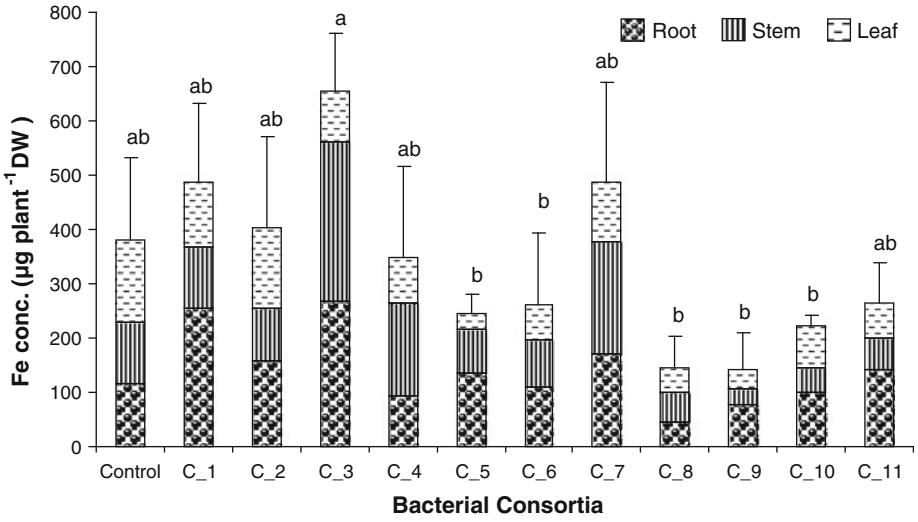

Fig. 6 Fe accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

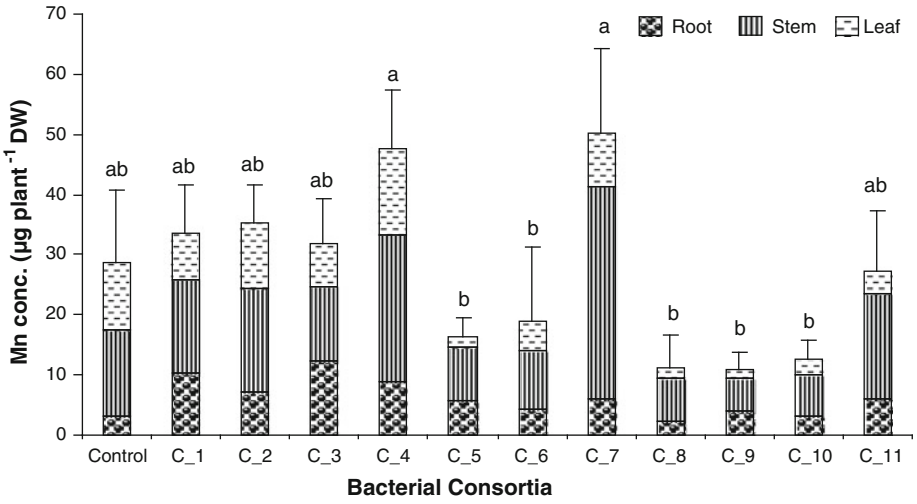

Fig. 7 Mn accumulation by *Brassica juncea* augmented with different bacterial consortia grown in nutrient broth

combination enhanced Mn uptake 1.91, 2.44 and 0.79 times more than control in the region of root, stem and leaf, respectively.

Thus, it is evident from the results that the bacterial combinations C4, C7 and C11 could enhance the growth of *B. juncea* by 56, 69 and 19% as compared to the control

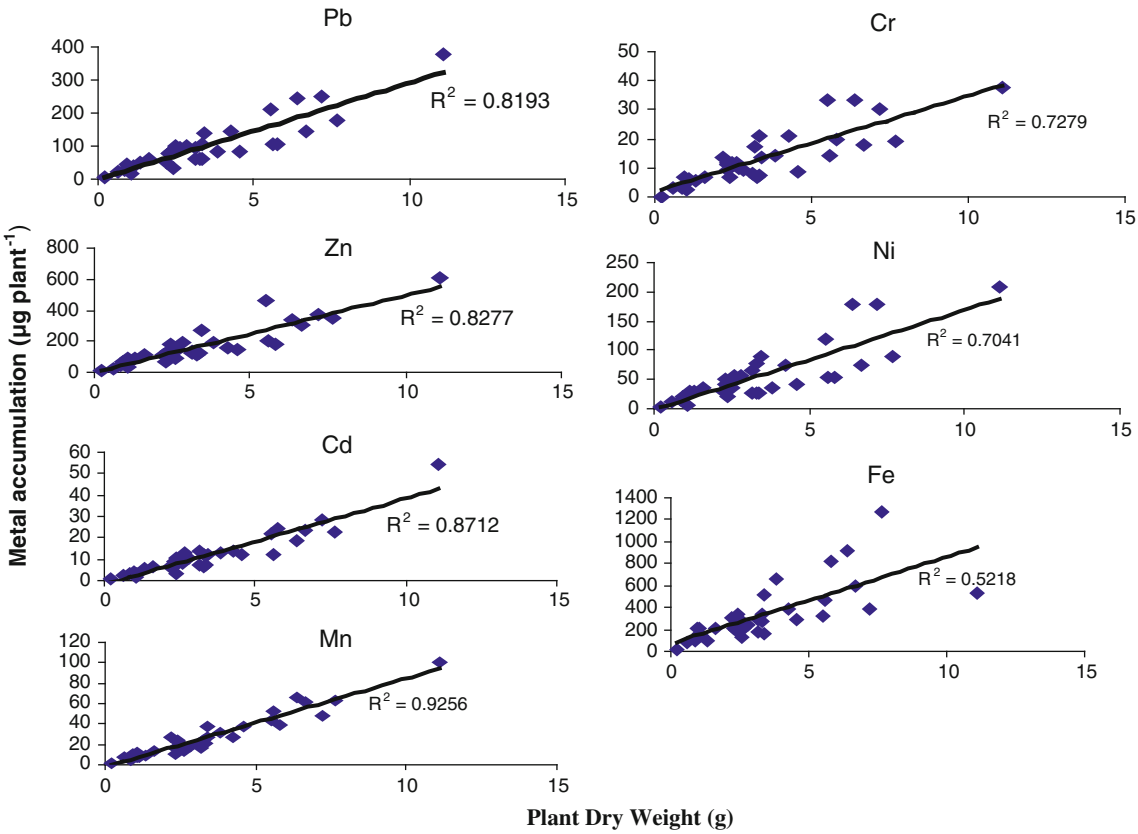

Fig. 8 Correlation between dry biomass of plant and uptake of various metals

plants. Besides, they had also increased the metal uptake leading to its higher concentration in plant parts. Hence, metal accumulation on per plant basis, which is a product of metal concentration and biomass, was significantly higher in these bacterial consortia with respect to all metals except Fe.

The accumulation of heavy metals in *B. juncea* showed the following pattern in the decreasing order: Zn (326 µg plant^{-1}) > Pb (170.79 µg plant^{-1}) > Ni (148.65 µg plant^{-1}) > Mn (50.11 µg plant^{-1}) > Cd (23.77 µg plant^{-1}) > Cr (25.718 µg plant^{-1}).

Correlation between biomass and uptake of different metals

A significant correlation was found in metal accumulation and the plant biomass. Correlation coefficients (R^2) values were found to be 0.82, 0.52, 0.92, 0.83, 0.73, 0.7 and 0.87 for Pb, Fe, Mn, Zn, Cr, Ni and Cd, respectively (Fig. 8).

Discussion

Members of Brassicaceae family have been found by many workers as hyper accumulator plants for a range of heavy metals (Jiang et al. 2004; Ghosh and Singh 2005; Zaidi et al. 2006). Higher accumulation of Zn, As, Pb and Ni has been reported in *Brassica* species (Duquene et al. 2009; Salide et al. 2003; Gupta and Sinha 2006).

Since phytoremediation has been a slow process because of availability of metals in soils and wastes, several efforts were made to boost up the process of metal phytoextraction to make it more effective in metal bioremediation. Saifulla et al. (2009) and Gupta and Sinha (2006) used various metal chelators to enhance the phytoremediation process, but their field application was limited due to high costs of chemical inputs. Hence, in our investigation, bacteria, which are natural agents and also play an important role in mobilization/immobilization of metals, were used in the rhizospheric zone of the plants to speed up the phytoremediation of the metals from fly ash. Bacteria utilize the root exudates as source of carbon and energy for their growth and multiplication in the rhizospheric zone and hence don't require the supply of additional nutrients.

In our investigation, four bacterial strains already tested for their role in metal extractability from fly ash (Tiwari et al. 2008a, b), were inoculated in the rhizospheric zone of the *Brassica juncea* grown in the fly ash with FYM. It was observed that a few consortia had been able to enhance the uptake of metals like Pb, Fe, Mn, Zn, Cr, Ni and Cd by the

plant significantly. However, other consortia either decreased the metal accumulation or played no role in the metal uptake, as evident from the metal accumulation pattern in plants.

Looking at metal accumulation induced by different bacterial consortia, it was noted that C7 consortium had significantly enhanced the accumulation of both essential metals i.e. Mn by (75%) and Zn by (125%) and non-essential metals i.e. Pb by 182%, Cr by 164% and Ni by 413% in *B. juncea*. Perhaps, *Bacillus pumilus* and *Paenibacillus macerans* present in C4, C7 and C11 bacterial consortia were involved in atmospheric N_2 fixation, phosphorus solubilization, micronutrient uptake and production of phytohormones (Govindasamy et al. 2010; de-Bashan et al. 2010) which might have promoted the plant growth to extract more metals from the fly ash. However, Fe accumulation was maximally induced by C3 consortium and Cd by C4 consortium. This indicated that the same combination of the bacteria may not be effective to boost up the phytoextraction of all the metals. It is possible that the same bacterial strains in different combinations play synergistic, antagonistic or neutral role in metal bioavailability as indicated by the trend of metal accumulation in *Brassica juncea*.

Earlier reports also indicate the microbes can accelerate the phytoremediation process as they play an important role in the mobilization of metals (Gadd 1990; Idris et al. 2004). Kalinowski et al. (2000) have also observed that the soil organisms are able to convert unavailable forms of metals into the available forms to be taken up by plants. In the phytoremediation process, the root exudates provide nutrition to rhizospheric bacteria which, in turn, could enhance metal uptake through solubilization of metals (Khan 2005).

Inoculation of *Burkholderia cepacia* enhanced translocation of Cd by 296% and Zn by 135% in *S. alfredii* from root to shoot (Li et al. 2007). In our study, Zn uptake was induced by 156% and Cd by 170% with inoculation of C7 consortium. Other bacteria like *Azotobacter chrococcum* (N-fixing bacteria), *Bacillus megaterium* (P-solubilizer) and *B. mucilaginous* (K-solubilizer) *Bacillus* sp RJ16 (Sheng and Xia 2006) decreased the pH value by excreting low molecular weight organic acids, which enhanced the bioavailability of Cd, Pb and Zn (Chen et al. 2005). Hussein (2008) reported enhanced accumulation of Cd by the inoculation of *B. licheniformis* in rhizosphere of *B. juncea*. In addition, Braud et al. (2009) reported that siderophores produced by rhizospheric bacteria could bind with heavy metals and thus enhanced their bioavailability in the rhizosphere for metal uptake by plants. Braud et al. (2006) also reported that *Pseudomonas aeruginosa* and *P. fluorescens* increased the exchangeable fraction of Pb by 113% and simultaneously decreased the fraction bound to carbonate.

In our experiment also, the Pb accumulation was enhanced by 182% with the inoculation of bacterial consortium C7 as compared to control. Similarly, an increase of extractable Ni with *Microbacterium arabinogalactanolyticum* has been reported to enhance Ni extractability in soil by 15 times as compared to Ni concentration in the soil. In our investigation, Ni uptake by *B. juncea* was induced by 413% with consortium C7, indicating increased Ni extractability from fly ash by the bacterial action. Microbial siderophores were reported to induce Fe III mobility and also various other cations (Diels et al. 2002). In our case, an increase in Fe accumulation by 71.5% was observed in *B. juncea* from fly ash by the action of a consortium C3.

Jiang et al. (2008) analyzed that Indian mustard growing in soil inoculated with J62 (*Bulkholderia*), root Pb was increased from 28 to 67% and Cd from 31% to 170% compared to the uninoculated control. *Pseudomonas* sp RJ10 and *Bacillus* sp RJ16 solubilize Cd and Pb and also promote plant growth. $CaCl_2$ extractable Cd and Pb were increased by 58–104% and 67–93%, respectively. An increase in Cd and Pb contents of above ground tissues varied from 92 to 113% and from 73 to 79% in inoculated plant growing in heavy metal contaminated soil compared to the uninoculated control (He et al. 2009). Braud et al. (2006)have observed a positive correlation between the microorganisms that produce siderophores and the amount of Cr and Pb in the exchangeable fraction.

Soil augmentation with free *P. aeruginosa* cells enhanced significantly Cr and Pb concentration in shoots by a factor of 4.3 and 3.4, respectively. On the contrary, bioaugmentation most often reduced Cr and Pb concentration in roots irrespective of the bacterial species and the inoculants formulation (Braud et al. 2009). Free *R. metallidurans* cells reduced significantly Cr and Pb concentration in root of maize by 2.9 and 4.8 times, respectively. Thus, bacteria are very specific in metal mobilization through siderophore production from metal contaminated sites. Therefore, a suitable consortium may used for the enhanced extraction of targeted metals by plants from fly ash.

Conclusion

This study indicates that bacteria played a significant role in mobilizing the metals from fly ash to be taken up the plants grown in mixture of fly ash and Farm Yard Manure. Out of eleven bacterial consortia, C4 (*Bacillus endophyticus* NBRFT4 + *Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9 + *Paenibacillus macerans* NBRFT5), C7 (*Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9) and C11 (*Paenibacillus macerans*

NBRFT5 + *Micrococcus roseus* NBRFT2 + *Bacillus pumilus* NBRFT9) were found to enhance accumulation of Pb, Cr, Zn, Ni, Mn and Cd many folds, while Fe uptake was enhanced significantly by C1 (*Paenibacillus macerans* NBRFT5 + *Bacillus endophyticus* NBRFT4 + *Bacillus pumilus* NBRFT9), C3 (*Paenibacillus macerans* NBRFT5 + *Micrococcus roseus* NBRFT2 + *Bacillus endophyticus* NBRFT4) and C7 (*Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9) consortia compared to control. Among these bacterial consortia, the best performer was C7 (*Paenibacillus macerans* NBRFT5 + *Bacillus pumilus* NBRFT9) consortium which enhanced accumulation of all the metals. Hence, this combination of bacteria may be recommended for the phytoextraction of toxic and non-toxic metals from the soils and wastes effectively.

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