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Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*

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ABSTRACT

In this study, we have shown that the plant growth promoting bacterial strain NBRI K24 and strain NBRI K3 from fly ash (FA) contaminated soil reduce the toxicity of Ni and Cr in *Brassica juncea* (Indian mustard) and promote plant growth under pot culture experiments. Isolated strains NBRI K24 and NBRI K3 were characterized based on the 16S rDNA sequencing and identified as *Enterobacter aerogenes* and *Rahnella aquatilis* respectively. Both the strains were siderophore producing and found capable of stimulating plant biomass and enhance phytoextraction of metals (Ni and Cr) from FA by metal accumulating plant i.e. *B. juncea*. Concurrent production of siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA) and phosphate solubilization revealed their plant growth promotion potential.

1. Introduction

Despite of other energy sources, coal still continues to be a major source of energy in India. More than 70% of energy is generated today by coal-based thermal power plants. Since Indian coal contains around 40% ash, these power plants generate enormous amounts of fly ash which is dumped in the near by areas. As per available estimate, the production of coal ash in India, including both fly ash and bottom ash, is likely to touch 140 million tons per year by 2020 [1]. The disposal of such a huge amount of FA is one of the major problems of developing countries and is usually disposed in basins or landfills near the power plants. The use of fly ash to vegetate the landfill areas is an alternative for FA management, which will serve for both the stabilization and providing a pleasant landscape [2,3].

Fly ash is largely alkaline in nature and contains many essential elements like S, B, Ca, Mg, Fe, Cu, Zn, Mn and P along with toxic metals, such as Cr, Pb, Hg, Ni, As, Cd etc. [4–6].

FA application to soil at a low rate has been reported to promote the growth of the plants through improvement of soil conductivity, organic carbon, microbial activity, soil porosity and water holding capacity [7,8]. This raises the possibility of using phytoremediation as an applicable technology to remediate the FA contaminated areas as well as to restore these for beneficial purposes [9,10]. Currently there are number of reports available on metal accumulating plants that are used in removing toxic metals from the soil [11–13]. Amongst the metal accumulators, *B. juncea* is one of the promising and widely studied plants for extraction of heavy metals from contaminated sites [14].

The efficiency of phytoaccumulation may not only depend on the plant itself but also on the interaction of the plant roots with microbes and the concentrations of bio-available metals in soil [15]. Certain rhizosphere bacteria have exceptional ability to promote the growth of the host plant by various mechanisms, namely fixation of atmospheric nitrogen, utilization of 1-aminocyclopropane-1-carboxylic acid (ACC) as a sole N source, production of siderophores, or production of plant growth regulators (hormones) [16]. In addition, many microorganisms in the soil are able to solubilize “unavailable” forms of heavy metal-bearing minerals by excreting organic acids [17]. Therefore, improvement of the interactions between plants and beneficial rhizosphere microbes can enhance biomass production and tolerance of the plants to heavy metals, and are considered to be an important component of phytoremediation technology [11].

Although a lot of works have been done on the use of fly ash as a soil amender to boost up crop production due to presence of some essential element in fly ash [18], only a few reports are available on the microbe-assisted remediation of metals from wastes to check surface and ground water metal contamination. Hence, this study was planned to investigate the effect of metal tolerant, plant growth promoting bacteria *Enterobacter aerogenes* strain NBRI

Abbreviations: FA, fly ash; ACC, 1-aminocyclopropane-1-carboxylic acid; IAA, indole acetic acid; NTPP, National Thermal Power Plant; LB, Luria-Bertani agar; BLAST, basic local alignment and search tool; GS, Garden soil; PGPB, plant growth promoting bacteria.

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K24 and *Rahnella aquatilis* strain NBRI K3 on *B. juncea* grown in FA amended soil.

2. Materials and methods

2.1. Isolation of chromium and nickel resistant bacteria from FA

E. aerogenes strain NBRI K24 and *R. aquatilis* strain NBRI K3 were isolated from the soil samples collected from FA contaminated region near National Thermal Power Plant (NTPP), Raibarielly district, Uttar Pradesh, India. For isolation of resistant bacterial strains, serially diluted samples were plated on Luria-Bertani (LB) agar medium supplemented with 50 mg L^{-1} level of heavy metals as $K_2Cr_2O_7$ and $NiCl_2{\cdot}6H_2O$. Plates were incubated at 35 °C for 48 h to screen metal resistant colonies. Chromium and nickel resistant colonies were subsequently purified on the same media and stored at 4 °C. To check the extent of resistance, selected strains were grown in LB agar media containing different concentrations of Cr and Ni ranging from 100 to 800 mg L^{-1} [19]. In addition to being resistant to nickel and chromium, both the strains possesses ACC deaminase and siderophore producing activity. The strains resistant for 50 μg mL^{-1} of rifampicin, were used as selection marker.

2.2. DNA isolation and PCR amplification of 16S rDNA for genetic characterization of bacterial strains

The strains were identified by determination of 16S rDNA sequences. Total genomic DNA was extracted by the phenol/chloroform method described by Sambrook et al. [20] and amplified using polymerase chain reaction (PCR) amplification of the 16S ribosomal DNA (16S rDNA). Universal eubacterial primer set fD1 and rP2 [21] were used. The amplification reaction was cycled at 94 °C for 1 min; 50 °C for 1 min and 72 °C for 1 min for 35 cycles. After amplification the reaction product was analyzed on 1% agarose gel using minielute™ PCR purification kit (Qiagen, USA). The sequence data were then compared to similar sequences in the database using BLAST analysis (Basic local alignment and search tool, BLAST at NCBI).

2.3. Analysis of FA and GS

FA used in the present study was collected randomly from dumping sites of NTPP, Raibarielly, Uttar Pradesh, India. The Garden soil (GS) was collected from National Botanical Research Institute (NBRI), Lucknow (India). Before making various amendments, FA and soil were air dried and sieved with 2 mm mesh size. Physico–chemical analysis of FA and GS were done by procedures described by Kalra and Maynard [22]. For metal analysis oven-dried samples of FA and soil (1 g) were digested with a mixture of nitric, sulphuric and perchloric acid (6:1:2 by volume) at 100 °C. Digested material was diluted with double distilled water and Fe, Zn, Mn, Cr and Ni contents were analyzed using a Perkin Elmer (8500) atomic absorption spectrophotometer.

2.4. Phosphate solubilization, IAA and siderophore production

2.4.1. Phosphate solubilization

The bacterial cultures were grown in NBRIP medium [23] with 0.5% of tri calcium phosphate at 30 °C for 144 h at 200 rpm. The culture supernatants were collected by centrifugation at 8000 rpm for 20 min. Soluble phosphate in the culture supernatants were determined by using the vanadomolybdophosphoric acid colorimetric method [24].

2.4.2. IAA

IAA production was determined according to the method of Bric et al. [25]. Briefly, an aliquot of 2 mL supernatant obtained from bacterial cultures grown in Luria-Bertani's medium supplemented with tryptophan (500 μg mL^{-1}), was mixed with 100 μL of 10 mM orthophosphoric acid and 4 mL of Salkowski's reagent. The absorbance of pink color developed after 25 min incubation was read at 530 nm. The IAA concentration in cultures was determined using a calibration curve of pure IAA as a standard following the linear regression analysis.

2.4.3. Siderophore

Bacterial siderophore production was determined by using a chrome azurol S shuttle solution as described by Schwyn and Neilands [26]. The assay was calibrated by generating standard curve for samples containing 1–100 μM deferoxamine messylate.

2.5. ACC deaminase determination

Lysates in four replicates of bacterial cultures were prepared as described by Saleh and Glick [27]. Briefly, 25 μL of toluene was added to a 200 μL aliquot of the washed bacterial suspensions and was vortexed vigorously for 30 s. Then, 20 μL of 0.5 M ACC was added and after an incubation period of 15 min at 30 °C, 1 mL of 0.56N HCl was added. The bacterial lysates were centrifuged (10,000 *g*, 10 min) and 1 mL of supernatant was mixed with 800 μL of 0.56N HCl and 300 μL of 2,4-dinitrophenylhydrazine (0.2 g in 100 mL of 2N HCl). The mixtures were incubated for 30 min at 30 °C after which 2 mL of 2N NaOH was added. The absorbance was measured at 540 nm. The ACC deaminase activity of *E. aerogenes* strain, NBRI K24 and *R. aquatilis*, strain NBRI K3 was evaluated quantitatively by measuring the amount of α-ketobutyrate produced by deamination of ACC. ACC deaminase activity was expressed in μmol of α-ketobutyrate mg $protein^{-1}$ h^{-1}.

2.6. Biosorption of metal by bacterial isolates

Biosorption of metal by bacterial isolates was done according to the procedure of Hernandez et al. [28] with some modifications. Bacteria were cultured overnight on LB agar plates without metals. Confluent bacterial lawns were collected and suspended in phosphate-buffered saline, and aliquots were divided into 1 mL of samples; and pelleted by centrifugation at 8000 rpm for 20 min. The samples were re-suspended in Eppendorff's tubes containing 100, 200 and 400 μg mL^{-1} of nickel chloride and potassium dichromate per mL dissolved in sterile Milli-Q water. The samples were incubated at room temperature for 2, 4 and 6 h, and the cells were harvested again by centrifugation under the same experimental condition. The amount of residual metal present in the supernatant was measured by atomic absorption spectrophotometer.

2.7. Chlorophyll & protein assay

The chlorophyll content of *B. juncea* leaves were measured by the method of Hiscox and Israelstam [29].

Leaves were ground in a porcelain mortar and then extracted with 1 mL of 0.05 M K–Na phosphate pH 7.2, 40 μg mL^{-1} phenylmethyl sulphonyl fluoride, and 0.01% sodium dodecyl sulphate. The protein content of supernatant was estimated by the method of Bradford [30] using bovine serum albumin as standard.

2.8. Bacterial suspension preparation

For inoculation, the selected bacterial strains were grown overnight in 500 mL Erlenmeyer flasks containing 200 mL of sterilized Nutrient broth on a shaker at 150 rpm at 32 °C. Bacterial cells in

the exponential phase were harvested by centrifugation at (8000 *g*, 15 min, 4 °C), and the pellets were washed twice with sterile distilled water, and recentrifuged. Bacterial suspensions in distilled water were adjusted to an absorbance of 0.5 at 600 nm (equivalent to approximately 5.7×10^7 cfu mL^{-1}).

2.9. Influence of plant growth promoting bacteria (PGPB) on B. juncea growth

Growth promotion potential of NBRI K24 and NBRI K3 was checked against Indian mustard (*B. juncea*). Surface sterilized seeds were sown in plastic pots filled with GS amended with 25% and 50% FA collected from NTPP, Raibarielly. The experimental design was a randomized complete block design with five replicates for each treatment. The amendments were denoted as 25% FA (25% FA + 75% soil), 50% FA (50% FA + 50% soil) and GS alone served as control. Five surface-sterilized seeds were placed in each pot at a 2 cm depth. After germination (7 d), plants were thinned to three plants per pot. Each plant represented a sampling unit. After 15 d of seedling emergence, bacterial suspensions (50 mL pot^{-1}) were sprayed on the soil surface. Control plants received 50 mL of sterile distilled water. Inoculated and non-inoculated plants were reared in pots under typical greenhouse conditions [temperatures of 19 °C (night) and 25 °C (day), 80% relative humidity]. The soil was moistened with water and maintained at 60% of its holding capacity. Growth parameters like plant height, root length, wet and dry weight were recorded after 25 d of germination.

Leaves for protein and chlorophyll assays were stored frozen at −20 °C until analysis were performed.

2.10. Analysis of plant material

After harvesting the plants were sampled for quantifying metal accumulation in *B. juncea*. Roots were separated and washed extensively three times with 10 mM EDTA and then distilled water in order to remove surface adsorbed metal ions. Shoots and roots were oven-dried at 100 °C for 48 h. The oven-dried samples were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v) [31,32]. After digestion, the volume of each sample was adjusted to 20 mL using double deionised water. The content of nickel and chromium in the digested samples was determined by atomic absorption spectrophotometer.

3. Result and discussion

3.1. Analysis of FA and soil

Physico–chemical properties of FA and GS are presented in Table 1. The result of FA analysis showed an alkaline pH (8.6–9.4), low nitrogen and phosphorus and high metal (Fe, Zn, Mn, Ni and Cr) contents. FA had a high electro chemical conductivity of 7.34 d Sm^{-1} and cation exchange capacity of 1.15 mol_c, however it contained low organic matter. High alkalinity of FA may be due to the presence of oxides of Ca and Mg [33]. In contrast the ameliorant GS used in this study had slightly alkaline pH (7.5–7.8), which was significantly less than that of FA. It has significant amount of N and P for supporting plant growth and comparatively lower concentrations of potentially toxic metals.

Table 1
Physico–chemical properties of FA and GS used in this study.

Parameters	FA	GS
pH	8.68–9.44	7.53–7.62
Electrical conductivity (dS m^{-1})	7.4 ± 0.3	1.3 ± 0.3
Cation exchange capacity [meq (100 g)$^{-1}$]	1.2 ± 0.1	1.6 ± 0.1
Total nitrogen (%)	0.02 ± 0.0	1.5 ± 0.1
Total phosphorus (%)	0.05 ± 0.0	0.1 ± 0.0
Organic carbon (%)	1.2 ± 0.1	1.5 ± 0.1
Metals (μg g^{-1} dw)		
Fe	4210 ± 198	637 ± 28
Zn	83 ± 4	113 ± 4
Mn	68 ± 2	209 ± 3
Cr	41 ± 2	35 ± 1
Ni	212 ± 11	23 ± 1

All the values are mean of three replicates ± SD.

3.2. Isolation of chromium and nickel resistant bacteria

Chromium is a highly toxic non-essential element for microorganisms and plants. At higher concentration, chromium inhibits the growth of most wild type bacteria and is tolerated by only a minority of organisms [34,35]. During initial screening, we have isolated 32 bacterial strains from FA contaminated soil. Out of 32 isolates, only six strains showed a high degree of metal resistance and were selected for further studies. In order to isolate plant growth promoting bacteria, metal resistant strains were tested for siderophore production and ACC deaminase ability. Among the six isolates tested, NBRI K24 and NBRI K3 showed good plant growth promoting potential.

3.3. Characterization of Cr^{2+} and Ni^{2+} resistant bacteria

3.3.1. Molecular characterization

The Cr and Ni tolerant bacterial strains NBRI K24 and NBRI K3, isolated from metalliferous soil, contaminated with FA emission and effluent of NTPP, Raibareilly district, U.P., India were characterized and identified as *E. aerogenes* and *R. aquatilis* based on its 16S rDNA sequence homology and phylogenetic analysis (results not shown).

3.3.2. Functional characterization

The use of PGPB in phytoremediation technologies is now being considered to play an important role as adding PGPB can aid plant growth on contaminated sites, and enhance detoxification of soil [36]. Free-living as well as symbiotic PGPB can enhance plant growth directly by providing bio-available phosphorus for plant uptake, fixing nitrogen for plant use, sequestering trace elements like iron for plants by siderophores, producing plant hormones like auxins, cytokinins and gibberellins, and lowering of plant ethylene levels [37].

The results shown in Table 2 clearly indicate production of substantial amount of IAA during stationary phase of culture in LB medium supplemented with L-tryptophan. The growth and IAA production increased simultaneously and maximum IAA production (30.1 μg mg^{-1}) was observed by NBRI K24 followed by NBRI K3 (28.2 μg mg^{-1}) after 48 h of incubation. These results concur with the earlier observations indicating induction of IAA in stationary phase culture [38,19]. The production of IAA was found dependent

Table 2
Growth promotion properties of NBRI K24 and NBRI K3.

Parameters	Strain	
	NBRI K3	NBRI K24
P solubilization (μg mL^{-1})	206.6[a] ± 2.4	245.8 ± 3.1
IAA production (μg mg^{-1})	28.2 ± 1.6	30.1 ± 1.5
Siderophore Production (μM mg^{-1})	25.7 ± 1.4	62.7 ± 2.7
ACC deaminase (μM αKB mg protien^{-1} h^{-1})	7.5 ± 0.5	7.5 ± 0.5
Metal tolerance level (mg L^{-1})		
Ni	650	700
Cr	600	600

[a] Values represent average of five replicates ± SD except the metal tolerance levels.

upon bacterial isolate and concentration of tryptophan. Such findings may have direct practical application, although intrinsic ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant [39]. The growth of both the strains NBRI K24 and NBRI K3 in NBRIP medium demonstrates the potential for phosphate solubilization (Table 2). Growth of bacterial strains NBRI K24 and NBRI K3 was observed up to 144 h at 30 °C. The maximum solubilization of phosphate (245.8 μg mL^{-1}) by NBRI K24, followed by NBRI K3 (206.6 μg mL^{-1}) was achieved after 120 h of incubation. Further incubation did not improve the extent of solubilization. Final pH of the growth medium was recorded at regular interval of 24 h to find out if solubilization was accompanied by acid production. The isolate NBRI K24 reduced the pH of medium from neutral to 2.5–3.5 after 96 h of incubation, further decrease in pH was not observed. The data showed time dependent increase in the amount of phosphate solubilized and also exhibited inverse relationship with pH of medium. Earlier studies have demonstrated that Ni in soil tends to reduce the amount of phosphorus in plants, which adversely affects the growth [40,41]. This deficiency can be compensated by the inorganic phosphate solubilizing ability of selected strain, reducing the pH of the medium [41]. They also suggested that the process of solubilizing inorganic phosphates helps in uptake of the metals from soil.

The siderophore is another important metabolite released by the plant growth promoting bacteria that directly alleviate heavy metal toxicity by increasing the supply of iron to the plant [42–47]. In the present study, both *E. aerogenes* strain, NBRI K24 and *R. aquatilis*, strain NBRI K3 were considered to be putative siderophore producing bacteria as they were able to grow in the presence of 8-hydroxy quinoline. Approximately 10 μL of the saline suspension of bacterial colonies were spread on blue agar plate containing chromeazurol S/Iron (III), hexadecyl trimethyl ammonium bromide, which served as indicator of siderophore production [26]. Single colony with the largest and clear orange halo was selected for quantitative analysis of siderophore estimation. Increased siderophore production was observed in NBRI K24 strain as compared to NBRI K3.

Heavy metal contamination of soil is often associated with iron deficiency in a range of different plant species [48,49]. The low iron content of plants that are grown in the presence of high levels of heavy metals generally results in these plants becoming chlorotic, since iron deficiency inhibits both chloroplast development and biosynthesis [50,51]. The best way to prevent plants from becoming chlorotic in the presence of high levels of heavy metals was to provide them with an associated siderophore producing bacterium that could supply a sufficient amount of iron to the plant. Therefore we studied the influence of *E. aerogenes* NBRI K24 and *R. aquatilis* NBRI K3 on Ni and Cr toxicity of *B. juncea*.

Several strategies have been successfully applied to generate plants able to grow in adverse environmental conditions and accumulate or transfer number of metals [52]. However, the elevated levels of heavy metals in soil interfere with uptake of nutrients as Fe and P and lead to plant growth retardation [53]. In the present study strain NBRI K24 and NBRI K3 both protect the plants against the inhibitory effects of Ni and Cr. It is likely that the siderophore producing and phosphate solubilizing isolates might have helped plant root proliferation and enhanced the uptake of soil minerals as Fe and P by the host plants [54,4]. Data revealed that besides siderophore, ACC deaminase and P solubilization there was not much change in IAA level of both the strains. Approximately similar level of IAA was found in *E. aerogenes* strain, NBRI K24 and *R. aquatilis*, strain NBRI K3. *E. aerogenes* strain, NBRI K24 had a high level of ACC deaminase activity, whereas *R. aquatilis*, strain NBRI K3 exhibited only a low level of activity. Earlier studies have confirmed the potential of ACC utilizing bacteria to promote the growth of *Brassica campestris*, *Brassica napus*, *B. juncea*, *Lycopersicon esculentum*, Zea Mays and *Triticum aestivum* plants. [55–61]. Jiang et al. [60] also investigated that a heavy metal resistant *Burkholderia* sp J62 isolated from heavy metal-contaminated soils prompted plant growth by the synthesis of ACC deaminase. Similarly, Ma et al. [61] isolated Ni-resistant *Pseudomonas* sp. Ps29C and *Bacillus megaterium* Bm4C from serpentine soil for promoting *B. juncea* growth in Ni contaminated soils.

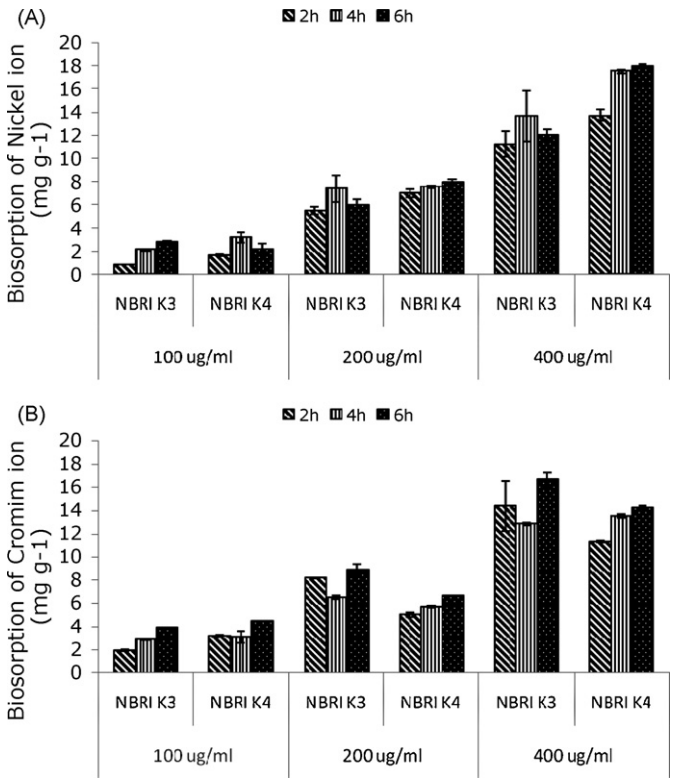

Fig. 1. Biosorption of (A) Nickel and (B) Chromium (mg g^{-1} dry weight of cell) by NBRI K3 and NBRI K24 at different time intervals. All the values are mean of three replicates ± SD.

3.3.3. Biosorption of metals by bacterial strains

The data expressing the capabilities of NBRI K3 and NBRI K24 to uptake Ni and Cr are given in Fig. 1(A) and (B). The biosorption of Ni and Cr by bacterial strains showed an increase in biosorption with increasing concentration of metal. These results were in accordance with those of Watanabe et al.[62]. Pradhan and Rai [63] also demonstrated that the biosorption of Cu, Zn and Cd increased with increasing metal concentrations. The maximum sorption was achieved after 6 h of incubation. Further incubation did not improve the extent of biosorption. The present study suggested that each concentration had specific equilibrium, after which there was no significant effect on biosorption by increasing the time of incubation. The maximum biosorption capacity for Ni and Cr was observed in NBRI K24 followed by NBRI K3.

3.4. Influence of PGPB on growth of B. juncea

A number of heavy metals are required by plants as micronutrient to act as cofactors as part of prosthetic groups of enzymes which are involved in a wide variety of metabolic pathways. However, when they are present in high levels most heavy metals are toxic to plants [64]. Thus significant phytoremediation of heavy metal contaminated soils can only succeed if this normal phytotoxic effect can be overcome.

Ni is known to inhibit enzymatic activity [65,66], biosynthesis of chlorophyll and protein [67] and henceforth the growth of

Table 3
Effect of NBRI K3 and NBRI K24 on the growth of *Brassica juncea* in FA amendments.

Treatments	Root lengths (cm)	Shoot lengths (cm)	Wet weight (mg plant^{-1})	Dry weight (mg plant^{-1})	Chlorophyll (mg g^{-1})	Protein (mg g^{-1})
Control	5.6 ± 0.2^{e}	8.4 ± 0.2^{b}	23.3 ± 0.5^{e}	4.9 ± 0.2^{d}	8.3 ± 0.2^{c}	0.6 ± 0.0^{bc}
NBRI K3	8.1 ± 0.3^{b}	9.2 ± 0.3^{a}	31.2 ± 0.4^{b}	6.6 ± 0.3^{ab}	9.5 ± 0.2^{ab}	0.7 ± 0.0^{ab}
NBRI K24	9.3 ± 0.2^{a}	9.6 ± 0.4^{a}	32.3 ± 0.5^{a}	6.9 ± 0.3^{a}	9.8 ± 0.2^{a}	0.8 ± 0.1^{a}
FA 25%	4.6 ± 0.3^{f}	5.3 ± 0.4^{e}	21.2 ± 0.3^{f}	4.7 ± 0.2^{d}	6.4 ± 0.5^{d}	0.6 ± 0.1^{bc}
FA 25% + NBRI K3	7.1 ± 0.3^{c}	7.6 ± 0.3^{c}	28.3 ± 0.3^{d}	6.4 ± 0.3^{bc}	8.2 ± 0.4^{c}	0.7 ± 0.0^{ab}
FA 25% + NBRI K24	8.0 ± 0.2^{b}	9.2 ± 0.4^{a}	29.5 ± 0.3^{c}	6.1 ± 0.4^{c}	9.1 ± 0.5^{b}	0.6 ± 0.1^{bc}
FA 50%	4.2 ± 0.2^{f}	6.3 ± 0.3^{d}	16.8 ± 0.2^{g}	3.3 ± 0.1^{e}	5.7 ± 0.5^{e}	0.5 ± 0.0^{c}
FA 50% + NBRI K3	6.7 ± 0.1^{d}	7.4 ± 0.4^{c}	23.6 ± 0.3^{e}	5.0 ± 0.3^{d}	6.4 ± 0.5^{d}	0.5 ± 0.0^{c}
FA 50% + NBRI K24	7.3 ± 0.1^{c}	8.9 ± 0.4^{a}	24.8 ± 0.4^{e}	5.2 ± 0.2^{d}	8.3 ± 0.6^{c}	0.6 ± 0.0^{bc}

All the values are mean of five replicates ± SD. Different letters above bars denote significant differences ($p = 0.05$) between different treatments in plant part according to DMRT (Duncan's multiple range test).

plants. In this study, the presence of Ni and Cr in the soil for 25 d decreased growth, as well as the protein and chlorophyll content of *B. juncea* plants. The different amendments of FA with soil and bacterial strains i.e. NBRI K24 and NBRI K3 have shown significant effects on the growth of *B. juncea*. Under control conditions, NBRI K24 showed maximum increase in root length (66.07%), shoot length (14.28%), fresh weight (38.62%) and dry weight (40.81%) of plants. Similarly NBRI K3 also enhanced plant biomass as root length (44.64%); shoot length (9.52%), wet and dry weight (33.90% and 34.69%, respectively).

Data showed that the strains, significantly enhanced plant biomass, protein and chlorophyll content, more than overcoming the inhibition attributable to the presence of nickel and chromium in the soil. The result is in agreement with a previous report describing increased biomass production of *B. juncea* inoculated with ACC utilizing strains and grown in Ni-supplemented soil [68].

In the present study both plant growth promoting bacteria protected the plant against inhibitory effect of heavy metals and helped the plant to grow. Plants inoculated with NBRI K24, grown in amendment of 25% FA showed increase in root length (73.91%), shoot length (67.92%), fresh and dry weight (39.15% and 29.78%) respectively. Similarly in amendment of 50% FA with soil, highest effect was found for NBRI K24 followed by NBRI K3. Statistically the root and shoot growth, dry as well as wet weight of the plant are significantly higher in NBRI K 24 than NBRI K 3 according to Duncan's multiple range test (DMRT) at $p = 0.05$ (Table 3). The trends observed indicated that siderophore producing and phosphate solubilizing strain increased plant root proliferation of the host plant as also reported by Glick et al. and Lamrecht et al. [37,54]. In presence of heavy metals significant increase in chlorophyll content (42.18% in 25% FA and 45.61% in 50% FA), was also observed when *E. aerogenes* strain, NBRI K24 was added to plant as bioinoculant.

3.5. Uptake of metals (Ni and Cr) in B. juncea

Studies have evidenced that heavy metal-resistant bacteria can enhance metal uptake by hyper accumulator plants [69,70]. Similar results were also obtained in our experiment that the Ni and Cr uptake by *B. juncea* was enhanced by the metal resistant PGPB. The accumulation of Ni and Cr in root and shoot tissues of the plant parts after 25 d of treatment in different amendments of FA and with bacterial inoculations is shown in Table 4. The accumulation of Ni is higher than Cr in root system of the plant. However, a different trend as Cr > Ni was observed in shoot tissues of *B. juncea* plants.

The plants grown in FA amendment have accumulated appreciable amount of metals (Ni and Cr) maximum being in roots. The concentration of metal in plant tissues increased with increase in concentration of FA in soil. Inoculated plants of *B. juncea* showed significant high accumulation of metals in roots than shoots. Singh et al. [8] also reported that the concentrations of Zn, Cr, Ni, Cu, Cd and Pb in root and shoots of *Beta vulgaris* plants showed strong positive correlation with concentrations of FA. Growth retardation in plants is due to the presence of excess chromium in soil, a common feature, and has been observed in many plant species [71,72]. Higher concentration of chromium exerts severe effect on root growth and function resulting in root damage, reduction in fresh and dry weight and diminish the uptake of water and nutrients [73]. The present observations indicate that both the inoculated and non-inoculated root systems accumulated considerably more chromium as compared to shoot system.

The accumulation and distribution of metals in the plants tissue are important aspects to evaluate the role of plants in remediation of contamination sites [74]. The process of metal uptake and accumulation by different plants depend on the concentration of available metals in the soil, solubility sequence and plant species [75]. The data presented in Table 4 showed that *B. juncea* plants grown in FA amended soil accumulated only small amount of metals in root and shoot tissues. However the addition of NBRI K24 and NBRI K3 to *B. juncea* increased the level of metal accumulation. Though statistically the difference is substantial between NBRI K24 and NBRI K3 in accumulating the metals (Ni and Cr) yet NBRI K24 exhibited better accumulation than NBRI K3 alone as well as when added with FA (25% and 50%). Statistical analysis was performed by using DMRT at $p = 0.05$. Similar observations were also recorded upon inoculation with *Kluyvera ascorbata* under Ni, Pb and Zn stress [42]. Like wise Zaidi et al. [41] suggested the protective effect of the strain *Bacillus subtilis* SJ-101 against Ni phytotoxicity in plants grown in soil treated with $NiCl_2$. Previously Indian mustard plants grown in Ni amended soil were found to be able to accumulate significant amount of nickel in their shoots, however in this work, it was found that the inoculated and non-inoculated root systems accumulated considerably more nickel as compared to shoot systems. This can be attributed to poor translocation of nickel from root to shoot system [42]. Similarly appreciable amount of chromium was accumulated in roots as compared to shoot system as reported earlier by Burd et

Table 4
Accumulation of Cr and Ni (μg g^{-1} dw) in *Brassica juncea* plants inoculated with NBRI K3 and NBRI K24 grown in FA amendments.

Treatments	Shoot (μg g^{-1} dw)		Root (μg g^{-1} dw)	
	Nickel	Chromium	Nickel	Chromium
Control	2.4 ± 0.5^{e}	8.9 ± 0.4^{d}	14.8 ± 0.6^{b}	7.0 ± 0.4^{e}
NBRI K3	4.8 ± 0.5^{d}	9.2 ± 0.9^{d}	15.2 ± 0.4^{b}	7.3 ± 0.2^{e}
NBRI K24	5.5 ± 0.7^{d}	9.8 ± 0.4^{d}	16.2 ± 0.6^{b}	7.8 ± 0.3^{e}
FA 25%	35.0 ± 1.9^{c}	45.7 ± 2.0^{c}	190.9 ± 10.3^{a}	132.8 ± 0.7^{d}
FA 25% + NBRI K3	36.3 ± 1.4^{bc}	47.2 ± 2.5^{c}	193.2 ± 10.8^{a}	135.1 ± 0.5^{c}
FA 25% + NBRI K24	37.9 ± 1.9^{ab}	48.5 ± 3.2^{c}	195.6 ± 12.5^{a}	137.0 ± 0.4^{b}
FA 50%	37.8 ± 1.1^{ab}	58.4 ± 2.4^{b}	197.2 ± 14.4^{a}	157.2 ± 1.9^{a}
FA 50% + NBRI K3	38.0 ± 1.8^{ab}	60.2 ± 3.6^{ab}	200.2 ± 15.3^{a}	157.9 ± 1.0^{a}
FA 50% + NBRI K24	39.5 ± 1.9^{a}	62.1 ± 3.7^{a}	204.0 ± 10.5^{a}	158.2 ± 0.4^{d}

All the values are mean of five replicates ± SD; dw = dry weight; Different letters above bars denote significant differences ($p = 0.05$) between different treatments in plant part according to DMRT (Duncan's multiple range test).

al. and Rajkumar et al. [42,35]. The higher accumulation of heavy metals in roots may be ascribed to formation of complex between heavy metals and sulphydryl groups that results less transport of heavy metals to shoots [12]. The accumulation of tested metals was more in the roots than shoots in all the amendments showing less translocation of metals from the underground part to the aerial part of the plant. With this study it can be said that the use of these metal accumulating bacteria can be considered as a great biotechnological tool for ecological and economical significance.

4. Conclusions

The present observations indicate that both *E. aerogenes* strain, NBRI K24 and *R. aquatilis*, strain NBRI K3 protect the plant against inhibitory effect of Cr^{2+} and Ni^{2+}.

On over-all comparison plants grown in inoculated soil amended FA showed maximum increase in all growth parameters as compared to control. It is likely that PGPB might increase the tolerance of host plants to the toxic effects of heavy metals by producing IAA, ACC deaminase, siderophore and solubilizing the phosphate.

Maximum accumulation of both tested metals was found in the plants grown in NBRI K24 inoculated treatment followed by NBRI K3. This study clearly indicates that both NBRI K24 and NBRI K3 are potent plant growth promoters and their mutant can be exploited in the FA contaminated soil due to their stability and persistence in the presence of heavy metals.

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References

[1] S. Tiwari, B. Kumari, S.N. Singh, Evaluation of metal mobility/immobility in fly ash induced by bacterial strains isolated from the rhizospheric zone of *Typha latifolia* growing on fly ash dumps, Bioresour. Technol. 99 (2008) 1305–1310.
[2] K.K.C. Cheung, J.P.K. Wong, Z.Q. Zhang, J.W.C. Wong, M.H. Wong, Revegetation of lagoon ash using the legume species *Acacia auriculiformis* and *Leucaena leucocephala*, Environ. Pollut. 109 (2000) 75–82.
[3] P. Vajpayee, U.N. Rai, R.D. Choudhary, R.D. Tripathi, S.N. Singh, Management of fly ash landfills with *Cassia surattensis* burn: a case study, Bull. Environ. Contam. Toxicol. 65 (2000) 675–682.
[4] D.K. Gupta, U.N. Rai, R.D. Tripathi, M. Inouhe, Impacts of fly ash on soil and plant responses, J. Plant. Res. 115 (2002) 401–409.
[5] S.K. Rautaray, B.C. Ghosh, B.N. Mitra, Effect of fly ash, organic waste and chemical fertilizers on yield, nutrient uptake and heavy metal content and residual fertility in a rice–mustard cropping sequence under acid lateritic soils, Bioresour. Technol. 90 (2003) 275–283.
[6] H. Lee, H.S. Ha, C.S. Lee, Y.B. Lee, P.J. Kim, Fly ash effect on improving soil properties and rice productivity in Korean paddy soil, Bioresour. Technol. 97 (2006) 1490–1497.
[7] B.N. Mittra, S. Karmakar, D.K. Swain, B.C. Ghosh, Fly ash—a potential source of soil amendment and a component of integrated plant nutrient supply system, Fuel 84 (2005) 1447–1451.
[8] A. Singh, R.K. Sharma, S.B. Agrawal, Effects of fly ash incorporation on heavy metal accumulation, growth and yield responses of *Beta vulgaris* plants, Bioresour. Technol. 99 (2008) 7200–7207.
[9] S. Eapen, S.F. D'Souza, Prospects of genetic engineering of plants for phytoremediation of toxic metals, Biotechnol. Adv. 23 (2005) 97–112.
[10] S. Dwivedi, R.D. Tripathi, S. Srivastava, S. Mishra, M.K. Shukla, K.K. Tiwari, R. Singh, U.N. Rai, Growth performance and biochemical responses of three rice (*Oryza sativa* L.) cultivars grown in fly ash amended soil, Chemosphere 67 (2007) 140–151.
[11] B.R. Glick, Phytoremediation: synergistic use of plants and bacteria to clean up the environment, Biotechnol. Adv. 21 (2003) 383–393.
[12] S. Singh, S. Sinha, R. Saxena, K. Pandey, K. Bhatt, Translocation of metals and its effects in the tomato plants grown on various amendment of tannery wastes: evidence for involvement of antioxidants, Chemosphere 57 (2004) 91–99.
[13] X.F. Sheng, J.J. Xia, Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria, Chemosphere 64 (2006) 1036–1042.
[14] M.N.V. Prasad, H. Freitas, Metal hyperaccumulation in plants–biodiversity prospecting for phytoremediation technology, Electron. J. Biotechnol. 6 (2003) 285–321.
[15] P.C. Wang, T. Mori, K. Komori, M. Sasatsu, K. Toda, H. Ohtake, Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions, Appl. Environ. Microbiol. 55 (1989) 1665–1669.
[16] B.R. Glick, C.L. Patten, G. Holguin, G.M. Penrose, Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria, Imperial College Press, London, 1999.
[17] R.A. Abou-Shanab, J.S. Angle, T.A. Delorme, R.L. Chaney, P. van Berkum, H. Moawad, K. Ghanem, H.A. Ghozlan, Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*, New Phytol. 158 (2003) 219–224.
[18] S. Jala, D. Goyal, Fly ash as a soil ameliorant for improving crop production-a review, Bioresour. Technol. 97 (2006) 1136–1147.
[19] M. Rajkumar, R. Nagendran, K.J. Lee, W.H. Lee, Characterization of a novel Cr^{6+} reducing *Pseudomonas* sp. with plant growth–promoting potential, Curr. Microbiol. 50 (2005) 266–271.
[20] J. Sambrook, F.E. Fritsch, T.A. Maniatis, Molecular cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.
[21] W.G. Wiesburg, S.M. Barns, D.A. Pelletier, D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study, J. Bacterial. 173 (1991) 697–703.
[22] Y.P. Kalra, D.G. Maynard, Methods manual for forest soil and plant analysis, Forestry Canada, Northwest Region, Northern Forest Centre, Edmonton, Alberta. Information Report NOR-X-319, 1991.
[23] C.S. Nautiyal, An efficient microbiological growth medium for screening phosphate solubilizing microorganisms, FEMS Microbiol. Lett. 170 (1999) 265–270.
[24] L.S. Clesscerl, A.E. Greenberg, A.D. Eaton, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA-AWWA-WEF, Washington, DC, 1998.
[25] J.M. Bric, R.M. Bostock, S.E. Silversone, Rapid in situ assay for indole acetic acid production by bacteria immobilization on a nitrocellulose membrane, Appl. Environ. Microbiol. 57 (1991) 535–538.
[26] B. Schwyn, J.B. Neilands, Universal chemical assay for detection and determination of siderophores, Anal. Biochem. 160 (1987) 47–56.
[27] S.S. Saleh, B.R. Glick, Involvement of *gacS* and *rpoS* in enhancement of the plant growth-promoting capabilities of *Enterobacter cloacae* CAL2 and UW4, Can. J. Microbiol. 47 (2001) 698–705.
[28] A. Hernandez, R.P. Mellado, J.L. Martinez, Metal accumulation and vanadium-induced multidrug resistance by environmental isolates of *Escherichia hermannii* and *Enterobacter cloacae*, Appl. Environ. Microbiol. 64 (1998) 4317J.D–4320J.D.
[29] J.D. Hiscox, G.F. Israelstam, A method for the extraction of chlorophyll from leaf tissues with out maceration, Can. J. Bot. 57 (1979) 1332–1334.
[30] M. Bradford, A rapid and sensitive method for the quantitation and microgram quantitiaties of protein utilizing the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–258.
[31] Y.H. Chen, Z.G. Shen, X.D. Li, The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals, Appl. Geochem. 19 (2004) 1553–1565.
[32] Y.X. Chen, J.Y. Shi, W.D. Zhang, Q. Lin, G.M. Tian, EDTA and industrial waste water improving the bioavailability of different Cu forms in contaminated soil, Plant Soil 261 (2004) 117–125.
[33] K. Furr, G.S. Stoewsand, C.A. Bache, W.H. Gutenman, D.J. Lisk, Multielement residues in tissues of guinea pigs feds weed clover grown on fly ash, Arch. Environ. Health 30 (1975) 244–248.
[34] U.N. Rai, K. Pandey, S. Sinha, A. Singh, R. Saxena, D.K. Gupta, Revegetating fly ash landfills with *Prosopis juliflora* L.: impact of different amendments and *Rhizobium* inoculation, Environ. International 30 (2004) 293–300.
[35] M. Rajkumar, K.J. Lee, W.H. Lee, R. Nagendran, S.Z. Kim, Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard, Chemosphere 62 (2006) 741–748.
[36] S. Mayak, S. Tirosh, B.R. Glick, Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers, Plant Physiol. 166 (2004) 525–530.
[37] B.R. Glick, C.L. Patten, G. Holguin, D.M. Penrose, Biochemical and genetic mechanisms used by plant growth-promoting bacteria, J. Theor. Biol. 190 (1999) 63–68.
[38] I.E. Garcia de Salamone, R.K. Hynes, L.N. Nelson, Cytokinin production by plant growth promoting rhizo-bacteria and selected mutants, Can. J. Microbiol. 47 (2001) 103–113.
[39] S. Arshad Jr.W.T., Frankenberger, Microbial production of plant growth regulators, in: F. Blaine Jr. (Ed.), Metting, Soil Microbial Ecology, Marcel and Dekker, Inc, NewYork, 1993, pp. 307–347.
[40] R.L. Halstead, B.J. Finn, A.J. Maclean, Extractability of nickel added to soils and its concentration in plants, Can. J. Soil Sci. 49 (1969) 335–342.
[41] S. Zaidi, S. Usmani, B.R. Singh, J. Musarrat, Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*, Chemosphere 64 (2006) 991–997.
[42] G.I. Burd, D.G. Dixon, B.R. Glick, Plant growth promoting bacteria that decrease heavy metal toxicity in plants, Can. J. Microbiol. 46 (2000) 237–245.
[43] A.K. Gupta, S. Sinha, Chemical fractionation and heavy metals accumulation in the plants of *Sesamum indicum* (L.) var T55 grown on soil amended with tannery sludge: selection of single extractants, Chemosphere 64 (2006) 161–173.
[44] R.A.I. Abou-Shanab, J.S. Angle, R.L. Chaney, Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils, Soil Biol. Biochem. 38 (2006) 2882–2889.
[45] X.F. Sheng, J.J. Xia, Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria, Chemosphere. 64 (2006) 1036–1042.

[46] M. Madhaiyan, S. Poonguzhali, T.M. Sa, Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.), Chemosphere 69 (2007) 220–228.
[47] P.B.A.N. Kumar, V. Dushenkov, H. Motto, I. Raskin, Phytoextraction: the use of plants to remove heavy metals, Environ. Sci. Technol. 29 (1995) 1232–1238.
[48] D. Mishra, M. Kar, Nickel in plant growth and metabolism, Bot. Rev. 40 (1974) 395–452.
[49] F. Ma, K. Nomoto, Inhibition of mugieneic acid-ferric complex in barley by copper, zinc and cobalt, Physiol. Plant. 89 (1993) 331–334.
[50] J. Imsande, Iron, sulfur and chlorophyll deficiencies: a need for an integrative approach in plant physiology, Physiol. Plant. 103 (1998) 139–144.
[51] K.V. Kumar, N. Singh, H.M. Behl, S. Srivastava, Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil, Chemosphere 72 (2008) 678–683.
[52] S.D. Cunningham, D.W. Ow, Promises and prospects of phytoremediation, Plant Physiol. 110 (1996) (1996) 715–719.
[53] A. Zayad, C.M. Lytle, J.H. Qian, N. Terry, Chromium accumulation, translation and chemical speciation in vegetable crops, Planta 206 (1998) 293–299.
[54] M. Lamrecht, Y. Okon, A.V. Brock, J. Vanderleyden, Indole-3-acetic acid: a reciprocal signaling molecule in bacteria- plant interaction, Trends Microbiol. 8 (2000) 298–300.
[55] M. Madhaiyan, S. Poonguzhali, J.H. Ryu, T.M. Sa, Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*, Planta 224 (2006) 268–278.
[56] V. Gravel, H. Antoun, R.J. Tweddell, Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA), Soil Biol. Biochem. 39 (2007) 1968–1977.
[57] B. Shaharoona, G.M. Jamro, Z.A. Zahir, M. Arshad, K.S. Memon, Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC deaminase for improving growth and yield of wheat (*Triticum aestivum* L.), J. Microbiol. Biotechnol. 17 (8) (2007) 1300–1307.
[58] C. Contesto, G. Desbrosses, C. Lefoulon, G. Béna, F. Borel, M. Galland, L. Gamet, F. Varoquaux, B. Touraine, Effects of rhizobacterial ACC deaminase activity on *Arabidopsis* indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria, Plant Sci. PSL-7826 (2008) 12.
[59] E. Dell'Amico, L. Cavalca, V. Andreoni, Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria, Soil Biol. Biochem. 40 (2008) 74–84.
[60] C.Y. Jiang, X.F. Sheng, M. Qian, Q.Y. Wang, Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. From heavy metal-contaminated paddy field soil and it's potential in promoting plant growth and heavy metal accumulation in metal-polluted soil, Chemosphere 72 (2008) 157–164.
[61] Y. Ma, M. Rajkumar, H. Freitas, Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*, J. Environ. Manage. 90 (2009) 831–837.
[62] M. Watanabe, K. Kawahara, K. Sasaki, N. Noparatnaraporn, Biosorption of cadmium ions using a photosynthetic bacterium, *Rhodobacter sphaeroides* and a marine photosynthetic bacterium, *Rhodovulum* sp. and their biosorption kinetics, J. Biosci. Bioeng. 95 (2003) 374–378.
[63] S. Pradhan, L.C. Rai, Biotechnological potential of *Microcystis* sp. in Cu, Zn and Cd biosorption from single and multimetallic systems, Biometals 14 (2001) 67–74.
[64] F. van Assche, H. Clijsters, Effect of metals on enzyme activity in plants, Plant Cell Environ. 13 (1990) 195–206.
[65] C. Mattioni, R. Gabbrielli, J. Van Gronsveld, H. Clijsters, Nickel and cadmium toxicity and enzymatic activity in Ni-tolerant and non-tolerant populations of *Silene italica* Pers, J. Plant Physiol. 150 (1997) 173–177.
[66] M.M. Alam, S. Hayat, B. Ali, A. Ahmad, Effect of 28- homobrassinolide treatment on nickel toxicity in *Brassica juncea*, Photosynthetica 45 (2007) 139–142.
[67] A.K. Sen, M. Bhattacharyya, Studies of uptake and toxic effects of Ni (II) on *Salvinia natans*, Water Air Soil Pollut. 78 (2004) 141–152.
[68] M. Rajkumar, H. Freitas, Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard, Bioresour. Technol. 99 (2008) 3491–3498.
[69] M.P. de Souza, D. Chu, M. Zhao, A.M. Zayed, S.E. Ruzin, D. Schichnes, N. Terry, Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard, Plant Physiol. 119 (1999) 565–574.
[70] S.N. Whiting, M.P. de Souza, N. Terry, Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*, Environ. Sci. Technol. 15 (2001) 3144–3150.
[71] N.R. Bishnoi, A. Dua, V.K. Gupta, S.K. Sawhney, Effect of chromium on seed germination, seedling growth and yield of peas, Agric. Ecosyst. Environ. 47 (1996) 47–57.
[72] D.C. Sharma, C.P. Sharma, Chromium uptake and toxicity effects on growth and metabolic activities in wheat: *Triticum aestivum* CVUP2003, Indian J. Exp. Biol. 34 (1996) 684–691.
[73] N. Terry, An analysis of the growth responses of *Beta vulgaris* L. to phytotoxic trace elements. II. Chromium, Final Report to the Kearney Foundation of Soil Sciences, July 1975–June 1980, 1981.
[74] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol. Plant Mol. Biol. 13 (1998) 468–474.
[75] A.K. Gupta, S. Sinha, Chemical fractionation and heavy metals accumulation in the plants of *Sesamum indicum* L. var. T55 grown on soil amended with tannery sludge: selection of single extractants, Chemosphere 64 (2006) 161–173.