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RESEARCH ARTICLE

Augmentation with potential endophytes enhances phytostabilization of Cr in contaminated soil

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Abstract

The contamination of soil with heavy metals is a major environmental problem worldwide. The combined use of plants and their associated microbes has gained popularity in recent years for their potential to remediate heavy metal-contaminated soil. In the current study, the effect that augmentation of soil with plant growth-promoting endophytes has on the phytostabilization of chromium (Cr)-contaminated soil was investigated. Three potential endophytic bacterial strains (*Enterobacter* sp. HU38, *Microbacterium arborescens* HU33, and *Pantoea stewartii* ASI11) were inoculated individually as well as in combination to *Leptochloa fusca* and *Brachiaria mutica* vegetated in Cr-contaminated soil. The accumulation of Cr in the root and shoot of the plants was determined. Moreover, bacterial persistence in the rhizosphere and endosphere was determined. Augmentation with potential endophytes significantly increased root length (24–45%), shoot height (39–64%), chlorophyll content (20–55%), and the overall biomass (32–61%) of the plants. Although *L. fusca and B. mutica* showed potential to accumulate Cr in their root and shoot, endophytic augmentation increased uptake, translocation, and accumulation of Cr in the roots and shoots of both plant species. However, *L. fusca* showed more potential to phytostabilize Cr as compared to *B. mutica*. Furthermore, the potential endophytes showed more survival and persistence within the roots than in the rhizosphere and shoot interior. This study provides useful evidence of endophyte-assisted phytoremediation to be the most sustainable and affordable approach for in situ remediation of Cr-contaminated soil.

Keywords Heavy metals · Phytoremediation · *Brachiaria mutica* · *Leptochloa fusca* · Plant-endophyte partnership

Introduction

Heavy metal (HM) pollution of soil and water has become a serious environmental problem worldwide. Environmental pollution due to heavy metals accelerated dramatically since the beginning of the industrial revolution. Heavy metals are non-biodegradable, and thus, persist indefinitely in the soil and water (Sessitsch et al. 2013, Khan et al. 2015, Ashraf et al. 2017). Among HMs, Cr is one of the most toxic elements in the environment arising from discharged effluents of industrial operations such as leather tanning, electroplating, alloy preparation, fabric printing, and crude oil extraction and processing (Ali et al. 2013, Afzal et al. 2014a, Shehzadi et al. 2016). Chromium is the second most common HM contaminating ground water and soil; hence, it poses a serious environmental concern (Lotfy and Mostafa 2014, Nordberg et al. 2014, Song et al. 2017).

To remediate HM-contaminated soil and water, microbe-assisted phytoremediation has proven to be an excellent strategy (Rajkumar et al. 2009, Sessitsch et al. 2013, Khan et al. 2015, Ijaz et al. 2016a). It is an economical and environment friendly approach as compared to conventional remediation techniques (Shanker et al. 2005, Yousaf et al. 2014, Ijaz et al. 2016b, Arslan et al. 2017). Phytostabilization is a mode of action for phytoremediation in which plants immobilize metals in order to minimize the transportation and leaching of contaminants while allowing metal accumulation in their roots with only small amounts of HM being translocated to the

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shoots (Dary et al. 2010, Korzeniowska et al. 2011, Karczewska et al. 2013). Microbial population is known to affect HM mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, redox changes, and N_2 fixation (Abou-Shanab et al. 2008, Khan et al. 2013a, Ahemad 2015, Ijaz et al. 2015, Fatima et al. 2016).

Despite the effectiveness of phytoremediation, Cr is a phytotoxic element and may reduce plant growth, induce chlorosis, harm the roots, disrupt photosynthesis, and eventually lead to plant death (Gill et al. 2015, Ashraf et al. 2017). Bacterial endophytes have considerable potential to reverse such adverse effects on plants, thus ensuring unhindered and effective phytoremediation of HM-polluted soils (Wu et al. 2006, Dell'Amico et al. 2008, Lebeau et al. 2008, Sessitsch et al. 2013, Khan et al. 2015). Endophytic bacteria make metals bioavailable and provide protection against toxic effects of HMs using a variety of processes including biotransformation, biosorption, and bioaccumulation (Sessitsch et al. 2013, Wang et al. 2013, Khan et al. 2015). In addition, endophytic bacteria have the potential to fix atmospheric nitrogen, solubilize inorganic phosphate, and release plant growth regulators like gibberellic acid, 1-aminocyclopropane-1-carboxylic acid, siderophores, cytokinin, and indole acetic acid which stimulate plant growth processes and enhance metal accumulation in plants (Lugtenberg and Kamilova 2009, Afzal et al. 2014b, Vejan et al. 2016). Recently, the augmentation of plant growth-promoting endophytes in constructed wetlands improved plant growth and enhanced the removal of Cr and other heavy metals from tannery wastewater (Ashraf et al. 2017). *Leptochloa fusca* (L.) Kunth (*L. fusca*) and *Brachiaria mutica* (Forssk) Stapf (*B. mutica*) are well-known plant species having well-developed fibrous root systems, large biomass, and long-term growth cycles; they have been used extensively around the world for remediation of stressed soils (Akhter et al. 2004, Mohanty and Patra 2012, Fatima et al. 2016). However, the potential of these plants has not been evaluated for phytoremediation of Cr-contaminated soil. Moreover, the effects of endophytic augmentation on the growth and phytoremediation potential of the plants have not been observed. Therefore, the present investigation aimed to study the effect of endophytic augmentation on phytoremediation of Cr-contaminated soil as well as the survival and persistence of inoculated endophytes were observed in the rhizosphere and endosphere of plants.

Materials and methods

Preparation of Cr-contaminated soil

The soil used in this experiment was an agricultural soil collected from the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan (soil properties are shown in Table 1). The soil was air dried, milled, and passed through a 2-mm mesh. The sieved soil was mixed with compost (10% *w*/*w*) and sieved again for homogeneous mixing.

The soil was artificially contaminated using salt solutions of K_2CrO_4 in varying concentrations of 50, 100, 200, 400, and 600 mg Cr kg^{-1}. Plastic pots were then filled with the contaminated soil (1.5 kg/pot), whereas one set of pots with uncontaminated soil was kept as control.

Preparation of bacterial strains for soil augmentation

Three bacterial strains, *Enterobacter* sp. HU38 (NCBI accession number KJ933404), *Microbacterium arborescens* HU33 (NCBI accession number KJ933403), and *Pantoea stewartii* ASI11 (NCBI accession number KJ933399) that were isolated from the shoots of *Prosopis juliflora* (Khan et al. 2015), were used in the present investigation. Based on metal-resistance and plant growth-promoting activities, these bacteria were selected to exploit their potential for phytoremediation of Cr-contaminated soil. All these strains have the ability to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA), and siderophores along with the ability to solubilize inorganic phosphate (Khan et al. 2015). The bacterial strains were grown in Luria Bertani (LB) broth for 24 h at 28 °C and 150 rpm. Following incubation, bacterial cells were harvested by centrifugation, washed with 0.9% NaCl solution, and re-suspended in the same solution.

Experimental design

Pot experiments were conducted in plastic containers with each treatment and control in triplicate. The experimental design consisted of two plant species used to test five levels of Cr contamination (50, 100, 200, 400, and 600 mg Cr kg^{-1} in soil) with and without bacterial augmentation and a control. Surface-

Table 1 Physical and chemical properties of the agricultural soil used in the study

Parameter	Value
pH (soil/water = 1:2.5)	7.62
Conductivity (μS cm^{-1})	560 ± 7
Organic matter (%)	3.87
Cr (mg kg^{-1})	13 ± 0.5
Cd (mg kg^{-1})	0.4 ± 0.03
Co (mg kg^{-1})	15 ± 0.8
Cu (mg kg^{-1})	20 ± 2
Mg (mg kg^{-1})	525 ± 43
Mn (mg kg^{-1})	475 ± 41
Ni (mg kg^{-1})	25 ± 3
Pb (mg kg^{-1})	10 ± 0.4
Zn (mg kg^{-1})	65 ± 7

Values represent means ± SD (n = 3)

sterilized cuttings of *L. fusca* and *B. mutica* with equal size, length, and weight were planted in agricultural and contaminated soil as per the treatments/control (10 cuttings per pot). Following this, 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of each strain was used to augment the soil as described earlier (Khan et al. 2013b). The bacterial strains were applied separately and in combination (16.66 ml of each strain) to check their individual as well as combined effects on plant growth and metal accumulation. In the control, 50 ml of 0.9% NaCl solution was added instead of the cell suspension. Distilled water was used for watering the plants on alternate days. Once grown for a week, the plants were thinned to six plants per pot. The pots were placed randomly in the premises of NIBGE, Faisalabad (31° 25′ 45″ N 73° 4′ 44″ E), Pakistan, for a period of 3 months (March–May, 2016) under natural climatic conditions (the average day/night temperatures were 43/33 °C).

Plant and soil analyses

Plants were harvested after 90 days of sowing. Different physiological parameters, such as root and shoot length and biomass, were determined. The harvested plants were brought to the laboratory, washed thoroughly with tap water to remove soil particles, and then rinsed thrice with double distilled water (Qiu et al. 2014, Adediran et al. 2015, Cheng et al. 2015). Plant biomass was dried in an oven at 70 °C for 48 h and dry weight was determined.

Chlorophyll content was estimated in leaves by following Arnon's method (Arnon 1949) using double beam UV/visible spectrophotometer (Hitachi 557, Hitachi Ltd. Tokyo, Japan). The plant and soil samples were dried in an oven, ground to obtain homogenous mixture, passed through a sieve of 0.5 mm, and digested in a microwave digestion system (Multiwave3000, Anton Paar GmbH Graz, Austria) as described earlier (Brunetti et al. 2012). The metal concentration was quantified by inductively coupled plasma optical emission spectrometer (iCAP6500 ICP-OES, Thermo Scientific, UK). Electrical conductivity and pH of soil were measured using conductivity meter (XL 30, Fisher Scientific Pte Ltd. Singapore) and digital pH meter (781 pH/ion meter, Metrohm Herisau, Switzerland), respectively.

All chemicals used for sample preparation and standard dilutions were of analytical reagent grade. Reagent blank and analytical duplicates were used to ensure precision in the analysis (Jiang et al. 2008). Laboratory NIST certified standards and spikes were used in each batch for validation of ICP-OES results as described earlier (Brunetti et al. 2012).

Enumeration of bacteria

The bacterial population in the endosphere and rhizosphere of *L. fusca* and *B. mutica* was estimated by plate count method on LB agar medium having 50 mg L^{-1} Cr (Afzal et al. 2012). Briefly, the roots and shoots of different treatments were surface sterilized using freshly prepared 70% ethanol and 2% sodium hypochlorite solutions. Surface-sterilized roots and shoots as well as rhizosphere soil were homogenized in 0.9% NaCl solution and shaken at 180 rpm for 30 min. Serial dilutions were prepared after settlement of plant and soil particles, and aliquots (100 μl) were spread on LB agar medium containing 50 mg L^{-1} Cr. The plates were incubated at 37 °C for 2 days to determine CFU g^{-1} rhizosphere soil or plant material. Bacterial colonies were picked randomly from all treatments and identity of the isolates with inoculant strains was confirmed by restriction fragment length polymorphism (RFLP) analysis (Andria et al. 2009).

Statistical analysis

For analyzing experimental data, OriginPro 2016 (OriginLab, Northampton, MA) software was used. The ANOVA was applied after conducting *Shapiro-Wilk* test. The statistical significance was determined using 2-way analysis of variance (ANOVA) and the Bonferroni post hoc test for multiple comparisons between treatments with $p \leq 0.05$ was considered significant.

Results

Effect of Cr contamination and bacterial augmentation on plant growth

Plant growth parameters (root and shoot length), chlorophyll content, and dry weight were determined to evaluate the effect of Cr contamination and the effectiveness of bacterial augmentation on the growth and development of *L. fusca* and *B. mutica* (Table 2; Figs. 1, 2, 3, 4). As compared to the plants grown in uncontaminated soil, a gradual decrease in growth and development as well as chlorophyll content of both plant species was observed with the increase in Cr concentration in soil (Table 2). Bacterial augmentation increased plant growth and chlorophyll content with maximum plant growth obtained with the application of all three bacterial strains together. In comparison to uninoculated controls, augmentation with bacterial consortium (mixture of *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38) increased root length by 34–41% and 24– 45%; shoot height by 42–64% and 39–61%; chlorophyll content by 20–31% and 43–55%; and plant dry weight by 45–61% and 32–49% in *L. fusca* and *B. mutica* grown in soil contaminated with 50, 100, and 200 mg Cr kg^{-1}, respectively (Figs. 3 and 4). Both, *L. fusca* and *B. mutica*, showed no growth at 400 and 600 mg Cr kg^{-1}.

Table 2 Effect of bacterial augmentation on shoot length (cm), root length (cm), and chlorophyll (mg g^{-1} fresh weight) of *Leptochloa fusca* and *Brachiaria mutica* grown in Cr-contaminated soil at different metal contamination levels

Treatment	SL	RL	Chl (a + b)	SL	RL	Chl (a + b)	SL	RL	Chl (a + b)
Leptochloa fusca									
Control (without Cr)	168 ± 9	55.2 ± 2.8	4.43 ± 0.21						
	50 mg Cr kg^{-1} soil			100 mg Cr kg^{-1} soil			200 mg Cr kg^{-1} soil		
Uninoculated	89^{def} ± 7	33.4^{bcde} ± 2.3	3.04^{bc} ± 0.14	83^{ef} ± 6	28.6^{def} ± 2.3	2.85^{bcd} ± 0.12	76^{f} ± 7	25.6^{f} ± 2.1	2.67^{cd} ± 0.16
Pantoea stewartii ASI11	117^{bc} ± 11	35.7^{bcd} ± 3.1	3.11^{bc} ± 0.16	105^{cde} ± 7	31.9^{cdef} ± 1.8	2.91^{bcd} ± 0.14	97^{cdef} ± 5	28.2^{ef} ± 1.4	2.70^{cd} ± 0.14
Microbacterium arborescens HU33	115^{bc} ± 8	35.3^{bcd} ± 2.9	3.13^{bc} ± 0.17	106^{bcde} ± 9	31.4^{cdef} ± 2.4	2.99^{bcd} ± 0.17	95^{cdef} ± 6	27.5^{ef} ± 1.5	2.73^{cd} ± 0.15
Enterobacter sp. HU38	114^{bcd} ± 5	36.5^{bc} ± 1.6	3.10^{bc} ± 0.12	104^{cde} ± 9	32.1^{cdef} ± 1.7	2.93^{bcd} ± 0.15	99^{cdef} ± 4	27.9^{ef} ± 2.3	2.74^{cd} ± 0.13
Consortium*	146^{a} ± 10	47.2^{a} ± 3.2	3.97^{a} ± 0.19	131^{ab} ± 12	39.6^{b} ± 2.6	3.52^{b} ± 0.12	108^{bcde} ± 9	34.2^{bcde} ± 1.7	3.21^{bc} ± 0.17
Brachiaria mutica									
Control (without Cr)	137 ± 7.4	62.2 ± 3.1	2.61 ± 0.17						
Uninoculated	76^{defg} ± 7.6	31.3^{bcd} ± 2.9	1.43^{bc} ± 0.15	69efg ± 5.5	29.6^{cde} ± 3.6	1.13^{de} ± 0.09	59^{g} ± 6.4	23.7^{e} ± 1.9	0.68^{f} ± 0.07
Pantoea stewartii ASI11	99^{bc} ± 6.4	36.5^{bc} ± 1.8	1.54^{b} ± 0.06	86^{bcde} ± 7.3	31.9^{bcd} ± 2.3	1.22^{cd} ± 0.07	67^{efg} ± 3.7	25.6^{de} ± 2.1	0.79^{f} ± 0.04
Microbacterium arborescens HU33	95^{bc} ± 5.6	37.2^{bc} ± 2.1	1.51^{b} ± 0.05	84^{cdef} ± 6.2	32.4^{bcd} ± 3.1	1.23^{cd} ± 0.06	65^{fg} ± 2.9	25.23^{e} ± 1.3	0.75^{f} ± 0.06
Enterobacter sp. HU38	96^{bc} ± 7.3	36.8^{bc} ± 1.6	1.55^{b} ± 0.02	83^{cdef} ± 4.8	31.7^{bcd} ± 2.6	1.20^{cd} ± 0.04	68^{efg} ± 3.8	24.8^{de} ± 1.7	0.74^{f} ± 0.05
Consortium*	122^{a} ± 6.8	45.3^{a} ± 3.3	2.21^{a} ± 0.13	104^{ab} ± 7.3	39.8^{b} ± 3.1	1.67^{b} ± 0.13	82^{cdef} ± 5.9	29.4^{cde} ± 2.1	0.97^{e} ± 0.05

The values presented are the means ± SD of at least three measurements. Significant differences ($p < 0.05$) were determined using a two-way ANOVA followed by a Bonferroni post hoc test. Treatment groups with at least one common letter are not significantly different from each other. Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of each strain

SL shoot length, *RL* root length, *Chl* (*a* + *b*) chlorophyll (a + b)

*Mixture of *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38

Effect of endophytic augmentation on Cr accumulation in plant tissues

The concentration of Cr in plant tissues (roots, shoots, and leaves) was determined to evaluate the effect of inoculated plant growth-promoting endophytes on Cr uptake, translocation, and accumulation in plant tissues (Table 3). Both plant species showed the potential to take up and accumulate Cr in their roots and shoots. Augmentation with potential endophytes further enhanced metal uptake, translocation, and accumulation in the plants. Among different augmentation treatments, bacterial consortium was found more effective than the application of a single bacterial strain. In contrast to uninoculated plants, plants inoculated with bacterial consortium had increased Cr concentrations in different plant tissues; amounts increased by 34–54% and 30–43% in roots; amounts increased by 31–48% and 26–38% in shoot and by 23–43% and 33–56% in leaves of *L. fusca* and *B. mutica* grown in soil containing 50, 100, and 200 mg Cr kg^{-1} soil, respectively. Plants grown at a higher contamination level of 200 mg Cr kg^{-1} showed maximum accumulation of Cr 493 and 325 mg kg^{-1} in roots, 47 and 54 mg kg^{-1} in shoots, and 27 and 3.2 mg kg^{-1} in leaves of *L. fusca* and *B. mutica*, respectively. The overall accumulation of Cr in plant biomass was found in the order of roots > shoots > leaves.

Bioconcentration and translocation factor

Bioconcentration factor (BCF) and translocation factor (TF) were calculated to estimate Cr accumulation in plant tissues. Although bacterial augmentation increased BCF and TF values, most pronounced effect was observed when endophytes were used in consortium (Table 4). *L. fusca* and *B. mutica* augmented with bacterial consortium showed significant ($p < 0.05$) increase in root BCF values from 3.68 to 5.66 and 1.12 to 1.60; 2.26 to 3.17 and 0.78 to 1.13; and 1.84 to 2.47 and 0.77 to 1.07 at Cr contamination levels of 50, 100, and 200 mg kg^{-1}, respectively. The shoot BCF and TF values

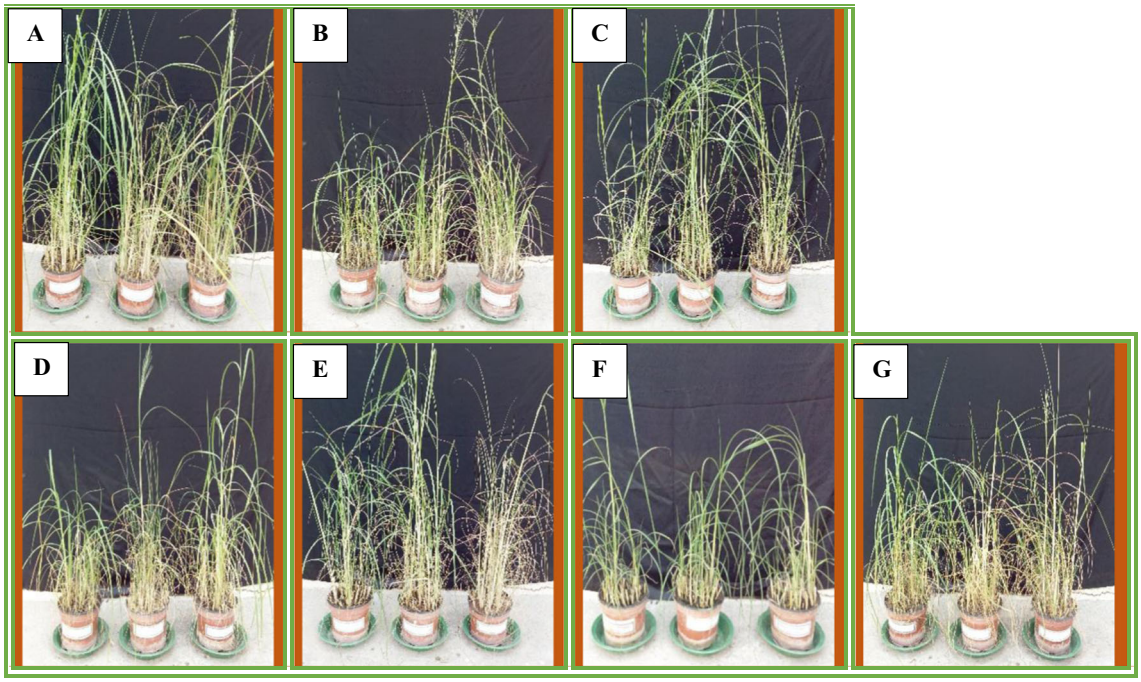

Fig. 1 Effect of chromium (Cr) contamination and endophytes augmentation on growth of *Leptochloa fusca* vegetated in Cr-contaminated soil. **a** uncontaminated soil; **b** soil contaminated with Cr (50 mg kg^{-1} soil); **c** soil contaminated with Cr (50 mg kg^{-1} soil) and augmented with bacterial consortium; **d** soil contaminated with Cr (100 mg kg^{-1} soil); **e** soil contaminated with Cr (100 mg kg^{-1} soil) and augmented with bacterial consortium; **f** soil contaminated with Cr (200 mg kg^{-1} soil); and **g** soil contaminated with Cr (200 mg kg^{-1} soil) and augmented with bacterial consortium

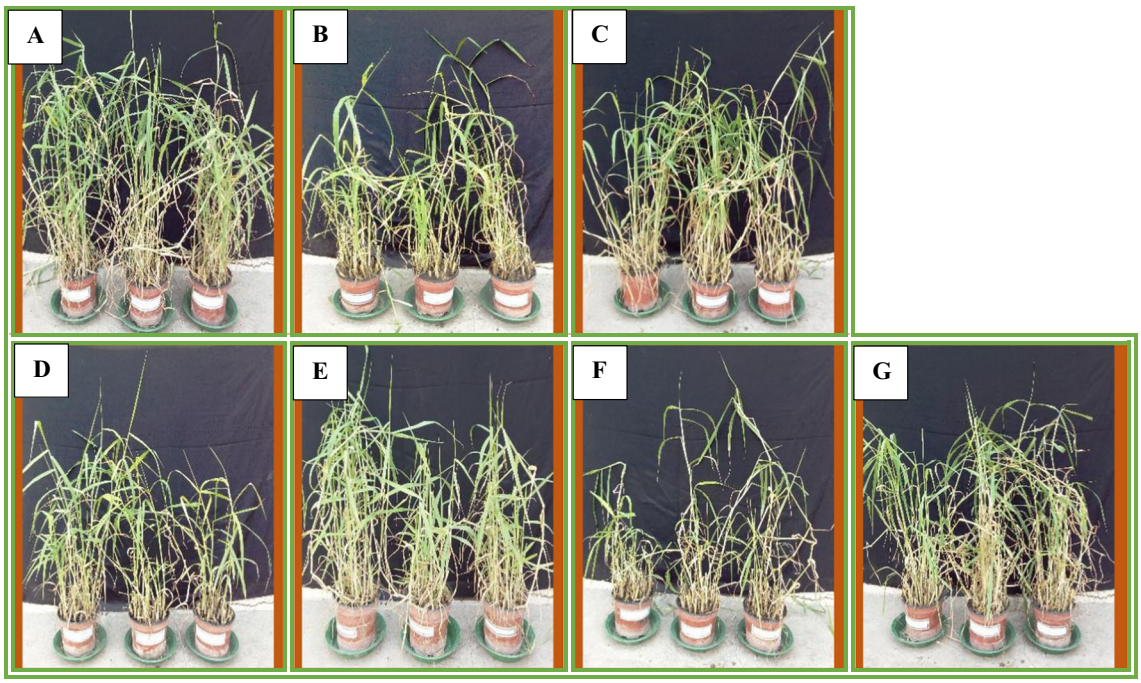

Fig. 2 Effect of chromium (Cr) contamination and endophytes augmentation on growth of *Brachiaria mutica* vegetated in Cr-contaminated soil. **a** uncontaminated soil; **b** soil contaminated with Cr (50 mg kg^{-1} soil); **c** soil contaminated with Cr (50 mg kg^{-1} soil) and augmented with bacterial consortium; **d** soil contaminated with Cr (100 mg kg^{-1} soil); **e** soil contaminated with Cr (100 mg kg^{-1} soil) and augmented with bacterial consortium; **f** soil contaminated with Cr (200 mg kg^{-1} soil); and **g** soil contaminated with Cr (200 mg kg^{-1} soil) and augmented with bacterial consortium

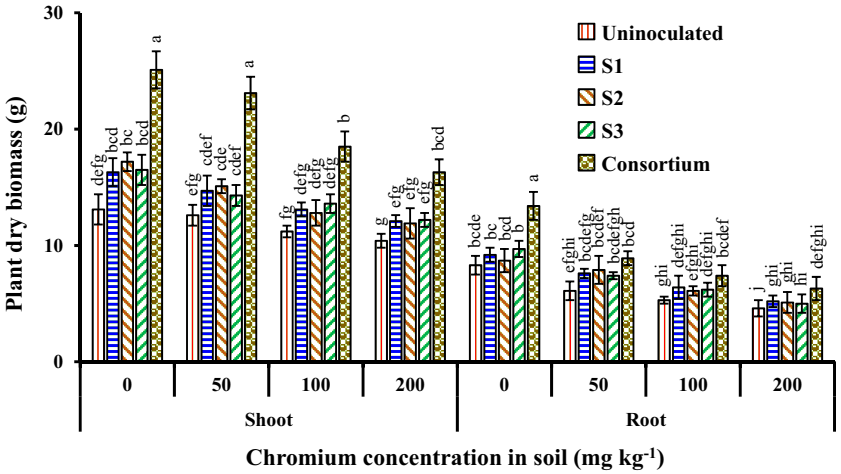

Fig. 3 Effect of bacterial augmentation on shoot and root biomass of *Leptochloa fusca* grown in Cr-amended soils at different contamination level. Significant differences ($p < 0.05$) were determined using a two-way ANOVA followed by a Bonferroni post hoc test. Treatment groups with at least one common letter are not significantly different from each other. Each error bar represents the standard deviation (SD) of the measurements. Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of strains S1 (*Pantoea stewartii* ASI11), S2 (*Microbacterium arborescens* HU33), and S3 (*Enterobacter* sp. HU38), and consortium (mixture of these three strains)

were found to be < 1 mg kg^{-1} in all treatments even after bacterial inoculation. Both plants showed high values for root BCF (> 1), low shoot BCF (< 1), and low TF with least value for TF being < 0.5.

Effect of Cr on bacterial survival and persistence

The survival and persistence of inoculated strains were observed in the rhizosphere and endosphere of *L. fusca* and *B. mutica* (Table 5). The inoculated bacterial strains showed persistence in the rhizosphere as well as roots and shoots of both plants; higher numbers of bacteria were observed in cases where bacterial consortium was applied as compared to application of a single bacterial strain. Bacterial survival and persistence decreased with the increase in Cr contamination levels. Maximum number of CFUs was found in the roots of treatment with 50 mg Cr kg^{-1} soil where *L. fusca* yielded $894 \pm 37 \times 10^4$ CFU g^{-1} dry biomass and *B. mutica* yielded $462 \pm 19 \times 10^4$ CFU g^{-1} dry biomass. More bacterial population was observed within the roots than in shoot and rhizosphere of plants.

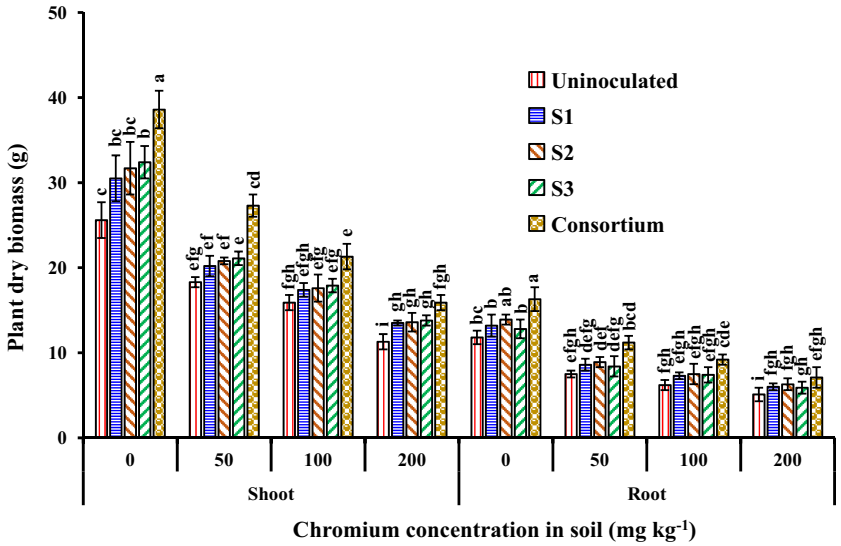

Fig. 4 Effect of bacterial augmentation on shoot and root biomass of *Brachiaria mutica* grown in Cr-amended soils at different contamination level. Significant differences ($p < 0.05$) were determined using a two-way ANOVA followed by a Bonferroni post hoc test. Treatment groups with at least one common letter are not significantly different from each other. Each error bar represents the standard deviation (SD) of the measurements. Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of strains S1 (*Pantoea stewartii* ASI11), S2 (*Microbacterium arborescens* HU33), and S3 (*Enterobacter* sp. HU38), and consortium (mixture of these three strains)

Table 3 Effect of bacterial augmentation on accumulation of chromium (mg kg^{-1}) in the root, shoot, and leaves of *Leptochloa fusca* and *Brachiaria mutica* grown in Cr-contaminated soil at different metal contamination levels

Treatment	50 mg Cr kg^{-1} soil			100 mg Cr kg^{-1} soil			200 mg Cr kg^{-1} soil		
	Root	Shoot	Leave	Root	Soot	Leave	Root	Soot	Leave
Leptochloa fusca									
Uninoculated	$184^{g} \pm 12$	$23^{e} \pm 1.2$	$14^{g} \pm 1.1$	$226^{fg} \pm 11$	$32^{cd} \pm 1.3$	$16^{fg} \pm 1.3$	$367^{bc} \pm 22$	$36^{bc} \pm 2.1$	$22^{bcd} \pm 1.6$
Pantoea stewartii ASI11	$209^{fg} \pm 15$	$25^{e} \pm 1.8$	$16^{fg} \pm 1.3$	$248^{ef} \pm 12$	$34^{bc} \pm 1.7$	$17^{efg} \pm 1.2$	$379^{b} \pm 28$	$38^{bc} \pm 2.5$	$25^{ab} \pm 2.1$
Microbacterium arborescens HU33	$216^{fg} \pm 11$	$27^{de} \pm 1.3$	$17^{efg} \pm 1.2$	$236^{ef} \pm 15$	$37^{bc} \pm 1.8$	$16^{fg} \pm 1.1$	$387^{b} \pm 21$	$41^{b} \pm 1.8$	$23^{abc} \pm 1.5$
Enterobacter sp. HU38	$205^{fg} \pm 16$	$25^{de} \pm 1.1$	$15^{g} \pm 1.2$	$253^{ef} \pm 18$	$35^{bc} \pm 1.4$	$18^{defg} \pm 1.2$	$396^{b} \pm 19$	$39^{b} \pm 2.3$	$24^{abc} \pm 1.3$
Consortium*	$283^{de} \pm 13$	$34^{bc} \pm 2.1$	$20^{cdef} \pm 1.8$	$317^{cd} \pm 19$	$46^{a} \pm 3.1$	$21^{bcde} \pm 2.1$	$493^{a} \pm 23$	$47^{a} \pm 3.1$	$27^{a} \pm 1.8$
Brachiaria mutica									
Uninoculated	$56^{h} \pm 3.2$	$21^{g} \pm 2.1$	$1.6^{g} \pm 0.10$	$78^{defg} \pm 5.8$	$33^{ef} \pm 2.4$	$1.9^{efg} \pm 0.11$	$181^{b} \pm 8.8$	$43^{abc} \pm 3.1$	$2.4^{bcd} \pm 0.13$
Pantoea stewartii ASI11	$62^{fgh} \pm 2.9$	$23^{g} \pm 1.3$	$1.9^{efg} \pm 0.13$	$81^{def} \pm 3.8$	$36^{de} \pm 1.8$	$2.3^{bcd} \pm 0.15$	$196^{b} \pm 6.4$	$45^{ab} \pm 2.3$	$2.7^{ab} \pm 0.21$
Microbacterium arborescens HU33	$60^{gh} \pm 3.3$	$22^{g} \pm 1.7$	$1.8^{fg} \pm 0.11$	$84^{de} \pm 4.2$	$35^{de} \pm 1.5$	$2.2^{cdef} \pm 0.11$	$189^{b} \pm 7.5$	$44^{abc} \pm 2.4$	$2.5^{bc} \pm 0.17$
Enterobacter sp. HU38	$65^{efgh} \pm 4.5$	$22^{g} \pm 1.1$	$2.0^{defg} \pm 0.14$	$89^{d} \pm 6.7$	$37^{cde} \pm 1.7$	$2.4^{bcd} \pm 0.16$	$191^{b} \pm 9.8$	$46^{ab} \pm 2.9$	$2.6^{ab} \pm 0.13$
Consortium*	$80^{def} \pm 6.7$	$29^{fg} \pm 1.6$	$2.5^{bc} \pm 0.16$	$107^{c} \pm 5.3$	$43^{abc} \pm 2.8$	$2.7^{ab} \pm 0.23$	$235^{a} \pm 8.9$	$54^{a} \pm 2.6$	$3.2^{a} \pm 0.16$

The values presented are the means ± SD of at least three measurements. Significant differences ($p < 0.05$) were determined using a two-way ANOVA followed by a Bonferroni post hoc test. Treatment groups with at least one common letter are not significantly different from each other. Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of each strain

*Mixture of *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38

Table 4 Bioconcentration and translocation factors of *Leptochloa fusca* and *Brachiaria mutica* plants grown in Cr-contaminated soil at different metal contamination levels

Treatment	50 mg Cr kg^{-1} soil			100 mg Cr kg^{-1} soil			200 mg Cr kg^{-1} soil		
	BCF			BCF			BCF		
	Roots	Shoots	TF	Roots	Shoots	TF	Roots	Shoots	TF
Leptochloa fusca									
Uninoculated	3.68	0.46	0.13	2.26	0.32	0.14	1.84	0.18	0.10
Pantoea stewartii ASI11	4.18	0.50	0.14	2.45	0.34	0.15	2.06	0.19	0.10
Microbacterium arborescens HU33	4.12	0.54	0.15	2.41	0.37	0.16	2.03	0.20	0.11
Enterobacter sp. HU38	4.10	0.50	0.14	2.43	0.35	0.15	2.05	0.21	0.11
Consortium*	5.66	0.68	0.18	3.17	0.46	0.20	2.47	0.24	0.13
Brachiaria mutica									
Uninoculated	1.12	0.42	0.38	0.78	0.33	0.42	0.77	0.22	0.28
Pantoea stewartii ASI11	1.24	0.46	0.41	0.81	0.36	0.46	0.84	0.23	0.29
Microbacterium arborescens HU33	1.20	0.44	0.39	0.84	0.35	0.45	0.86	0.22	0.29
Enterobacter sp. HU38	1.30	0.44	0.39	0.82	0.37	0.47	0.84	0.23	0.30
Consortium*	1.60	0.54	0.48	1.13	0.41	0.53	1.07	0.26	0.33

Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of each strain

BCF bioconcentration factor, *TF* translocation factor

*Mixture of *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38

Table 5 Effect of metal contamination on bacterial survival and persistence (10^4 CFU g^{-1} dry weight) in the shoot, root, and rhizosphere of *Leptochloa fusca* and *Brachiaria mutica* grown in Cr-contaminated soil at different metal contamination levels

Treatment	50 mg Cr kg^{-1} soil			100 mg Cr kg^{-1} soil			200 mg Cr kg^{-1} soil		
	RH	RI	SI	RH	RI	SI	RH	RI	SI
Leptochloa fusca									
Pantoea stewartii ASI11	$6.3^{b} \pm 0.2$	$645^{cd} \pm 26$	$0.23^{b} \pm 0.01$	$5.0^{cd} \pm 0.2$	$564^{def} \pm 39$	$0.14^{defg} \pm 0.03$	$3.3^{e} \pm 0.1$	$415^{gh} \pm 25$	$0.10^{efg} \pm 0.01$
Microbacterium arborescens HU33	$5.8^{bc} \pm 0.4$	$611^{cde} \pm 44$	$0.22^{bc} \pm 0.04$	$4.8^{d} \pm 0.3$	$512^{fg} \pm 28$	$0.19^{bcd} \pm 0.02$	$2.9^{e} \pm 0.3$	$377^{h} \pm 21$	$0.09^{fg} \pm 0.02$
Enterobacter sp. HU38	$6.6^{b} \pm 0.3$	$682^{bc} \pm 22$	$0.24^{b} \pm 0.01$	$4.5^{d} \pm 0.4$	$587^{cdef} \pm 26$	$0.16^{cde} \pm 0.02$	$3.1^{e} \pm 0.1$	$393^{h} \pm 37$	$0.08^{g} \pm 0.01$
Consortium*	$8.1^{a} \pm 0.5$	$894^{a} \pm 37$	$0.33^{a} \pm 0.02$	$6.3^{b} \pm 0.2$	$766^{b} \pm 37$	$0.24^{b} \pm 0.01$	$4.3^{d} \pm 0.2$	$514^{ef} \pm 17$	$0.15^{def} \pm 0.03$
Brachiaria mutica									
Pantoea stewartii ASI11	$4.55^{bcd} \pm 0.22$	$435^{b} \pm 21$	$0.66^{bc} \pm 0.07$	3.64ef ± 0.38	$314^{cd} \pm 18$	$0.53^{cdef} \pm 0.03$	$2.63^{g} \pm 0.16$	$264^{de} \pm 11$	$0.41^{ef} \pm 0.03$
Microbacterium arborescens HU33	$4.73^{bc} \pm 0.19$	$452^{b} \pm 27$	$0.64^{bc} \pm 0.05$	$3.53^{egf} \pm 0.16$	$332^{c} \pm 13$	$0.47^{def} \pm 0.02$	$2.89^{fg} \pm 0.30$	$252^{df} \pm 13$	$0.39^{f} \pm 0.04$
Enterobacter sp. HU38	$6.18^{a} \pm 0.42$	$594^{a} \pm 33$	$0.83^{a} \pm 0.07$	5.23b ± 0.39	$443^{b} \pm 22$	$0.73^{ab} \pm 0.06$	$4.06^{cde} \pm 0.33$	$347^{c} \pm 23$	$0.55^{cde} \pm 0.06$
Consortium*	$4.82^{bc} \pm 0.31$	$462^{b} \pm 19$	$0.62^{bcd} \pm 0.02$	$3.69^{def} \pm 0.27$	$308^{cde} \pm 16$	$0.48^{def} \pm 0.05$	$2.91^{fg} \pm 0.12$	$248^{e} \pm 16$	$0.44^{ef} \pm 0.05$

The values presented are the means ± SD of at least three measurements. Significant differences ($p < 0.05$) were determined using a two-way ANOVA followed by a Bonferroni post hoc test. Treatment groups with at least one common letter are not significantly different from each other. Soil was inoculated with 50 ml bacterial suspension (app. 10^{10} CFU ml^{-1}) of each strain

CFU colony forming unit, *RH* rhizosphere, *RI* root interior, *SI* shoot interior

*Mixture of *Pantoea stewartii* ASI11, *Microbacterium arborescens* HU33, and *Enterobacter* sp. HU38

Discussion

Heavy metal contamination in soil inhibits plant growth and biomass production (Sessitsch et al. 2013, Khan et al. 2015). In this study, Cr contamination in soil inhibited plant growth, particularly at higher levels of contamination. Similar findings have been reported earlier, whereby HMs including Cr inhibit plant growth and development (Chen et al. 2003, Prapagdee et al. 2013, Lin et al. 2014). However, in this study, inoculated endophytic bacteria were observed to reduce inhibitory effects of Cr by significantly improving growth of plants (Table 2, Figs.1, 2, 3, 4). Bacteria can enhance plant growth and protect plants from abiotic stresses, such as the presence of contamination in soil, through a wide variety of mechanisms including nitrogen fixation, phosphate solubilization, ACC deaminase activity, and production of siderophores and phytohormones (Souza et al. 2015). In this study, the inoculated endophytes possessed several plant growth promotion activities, such as ACC deaminase, siderophore, and IAA production which allowed them to contribute positively towards plant growth and development in the presence of high Cr contamination in soil. Similar findings have already been reported (Lee et al. 2004, Singh et al. 2010, Srivastava et al. 2013). Inoculated bacteria with ACC deaminase activity reduce HM stress by enzymatic hydrolysis of ACC, thereby increasing plant growth by decreasing the amount of ACC and ethylene in contaminated soils (Dell'Amico et al. 2005, Glick et al. 2007, Ma et al. 2011a). IAA produced by inoculated PGP bacteria contributes to plant growth and development by increasing root growth along with elongation of root hair and better absorption of nutrients and metals by plant roots (Taghavi et al. 2009, Prapagdee et al. 2013, Ahemad 2015). Inoculated PGP endophytic bacteria also exhibited potential of producing siderophores (metal chelating agents) that is responsible to increase chlorophyll biosynthesis by making iron available to metal-stressed plants (Ahemad 2015). Siderophores also enhance bioavailability of metals in rhizosphere by their metal binding capacity through complexation reactions (Braud et al. 2009, Ullah et al. 2015). In addition, endophytic bacteria stimulate plant defense mechanisms against pathogens (Nunes da Silva et al. 2014) and act

as biofertilizers as well (Vessey 2003, Sheng et al. 2008, Chen et al. 2010).

Bacteria reduce metal toxicity in plants grown in metal-polluted environment by increasing the uptake of trace elements (Sheng et al. 2008). In this study, the application of PGP bacterial isolates individually and in combination showed increase in plant growth and metal uptake. Plants inoculated with different PGP bacterial strains showed better growth as compared to uninoculated plants. Different inoculated bacterial strains affect plant growth and metal accumulation up to different extent, however, bacterial consortium showed maximum performance in enhancing plant growth and metal accumulation, especially in roots of both plant species grown in Cr-contaminated soils (Table 3). The significant ($p < 0.05$) increase in plant growth as well as metal accumulation observed here may be attributed to the different bacteria present in consortium which work synergistically promoting each other's beneficial effect (Dary et al. 2010, Ahemad 2015, Kumar et al. 2016).

L. fusca and *B. mutica* showed potential for phytostabilization of Cr-polluted soils. The BCF values of roots were recorded to be > 1, and of shoots to be < 1, and TF values were < 0.5. The high root BCF values along with low shoot BCF values confirm the phytostabilization potential of both plants, whereas low TF values indicate that Cr is poorly translocated within plants. Plants ability to accumulate more Cr in roots is a natural toxicity response in which Cr becomes immobilized in the vacuoles of root cells (Ramana et al. 2013, Ullah et al. 2015). Plants with high BCF values in the roots and low TF values are suitable for phytostabilization of toxic HMs in the soil (Korzeniowska and Stanislawska-Glubiak 2015). Moreover, bacteria produce phytohormones which increase plant biomass and metal uptake (Glick 2003, Sessitsch et al. 2013).

In addition to various growth-promoting characteristics, inoculant bacteria must have capacity of persistence and re-colonization in polluted environment, which is an important factor for bacterial-assisted phytoremediation. In the current study, the inoculated bacterial strains exhibited survival and persistence in the rhizosphere soil, roots, and shoots of *B. mutica* (Table 5). Several earlier studies have also reported that endophytic bacteria can colonize rhizosphere and internal tissues of host plants (Ma et al. 2011b, He et al. 2013, Yuan et al. 2014). The highest bacterial population was detected in the roots of *L. fusca* and *B. mutica* vegetated in soil with 50 mg Cr kg^{-1} as compared to the roots of plants vegetated in soil with 200 mg Cr kg^{-1}. This shows that HMs in soil adversely affect survival and persistence of bacteria (Giller et al. 1998). Furthermore, maximum competence, survival, and persistence of inoculated bacteria in endosphere may be attributed to the additional protection provided by plants against the deleterious outer environment. A similar trend of bacterial colonization pattern in the plants' endosphere was observed by different researchers (Andria et al. 2009, Afzal et al. 2012, Khan et al. 2015). In this study, the effect of endophytic augmentation in phytostabilization of HMs may be credited to the high population of potential endophytes in the rhizosphere and endosphere of plants. In a recent study, augmentation with endophytic bacteria enhanced the growth of *L. fusca* while aiding in the removal of both organic and inorganic pollutants from the tannery effluent. Moreover, bacterial augmentation decreased toxicity in the effluent as well. Higher number of Cr-resistant bacteria was isolated from the rhizosphere and endosphere of *L. fusca* inoculated with the endophytes than from uninoculated plants.

The efficacy of plants for phytostabilization of Cr is evaluated not only on the basis of metal accumulation but also the opportunity of gaining appropriate quantity of root biomass (Gołda and Korzeniowska 2016). Although, *L. fusca* roots accumulated greater amount of Cr (493 ± 23 mg kg^{-1}) than that of *B. mutica* roots (235 ± 8.9 mg kg^{-1}), the percent increase in total metal accumulation in roots per pot was found higher for *B. mutica* due to its denser root biomass than that of *L. fusca* (Figs. 3 and 4).

Conclusions

The study performed here emphasizes the applicability of endophyte-assisted phytoremediation for viable and economical restoration of Cr-contaminated soil and presents the prospects of assessing the efficacy of endophyte-assisted phytoremediation at field-scale studies. Most importantly, we have established that *L. fusca* and *B. mutica* can not only survive well in extremely contaminated soil but cleanup it as well. In this way, two entirely new additions have been made in the plant species that are well-known to be useable for remediation of Cr-contaminated lands. Bacterial consortium alleviated the negative effects of Cr contamination on plant growth and increased plant biomass, chlorophyll content, and also increased metal accumulation in *L. fusca* and *B. mutica*. Our experiment showed high values of root BCF (> 1) and low values for shoot BCF and TF (< 1) indicating the competency of both plants to tolerate and accumulate Cr in roots with less translocation to aerial parts, thus minimizing the chances of Cr uptake in the food chain.

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