www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

Archives of Environmental Protection
Vol. 44 no. 2 pp. 73–82

PL ISSN 2083-4772
DOI 10.24425/119705

DE GRUYTER OPEN

Phytoextraction of Cr by maize (*Zea mays* L.). The role of plant growth promoting endophyte and citric acid under polluted soil

Javed Ali[1], Amna[1], Tariq Mahmood[2], Kashif Hayat[1], Muhammad S. Afridi[1], Fawad Ali[1], Hassan J. Chaudhary[1]*

[1]Quaid-i-Azam University, Pakistan
Department of Plant Sciences
[2]Hazara University Mansehra, Pakistan
Department of Genetics

*Corresponding author's e-mail: hassaan@qau.edu.pk

Keywords: maize, phytoextraction, chromium, citric acid, *Burkholderia vietnamiensis*.

Abstract: High chromium (Cr) toxicity has turned into a serious environmental concern. Cr contaminated agronomic soils negatively affect the growth and yield of crops. Current research was conducted to enhance the phytoextraction potential of maize by using *Burkholderia vietnamiensis* and citric acid (CA). Plants were subjected to three concentrations of Cr (0.86, 350, and 500 ppm). A pot experiment was conducted under greenhouse conditions with completely randomized design (CRD). After 72 days of experiment, plants were harvested to analyze the morphological and biochemical attributes of soil, bacteria and plant. Results revealed that plant fresh, dry biomass, root, shoot length and chlorophyll contents significantly increased by 56%, 50%, 58%, 78% and 60%, respectively, at 500 ppm Cr concentration in combine treatment of *B. vietnamiensis* and CA. Maize plants treated with both *B. vietnamiensis* and CA significantly increased the bioaccumulation (BA) of Cr up to 50% and translocation factor (TF) by 31%. Furthermore, superoxide dismutase (SOD), proline and peroxidase dismutase (POD) activities in leaves were markedly increased by 30%, 42% and 15%, respectively, when treated with CA. Current study reveals that exogenous co-application of *B. vietnamiensis* + CA enhance plant growth by alleviating heavy metal stress and accelerate the phytoextraction of Cr. Taking into account the heavy metal tolerance and accumulation capacity, *Zea mays* is suitable for phytoremediation of contaminated soils in combination with *B. vietnamiensis* and CA.

Introduction

Economy of third world countries like Pakistan, India, Brazil, Bangladesh, and Sri Lanka is dependent on agriculture setup (Ramos et al. 2002). Irrigation of agricultural fields with industrial wastewater is a common practice nowadays, as it overcomes the need of water, nutrients and minerals that directly increase the plant growth (Ensink et al. 2004, Yasmeen et al. 2014). This practice results in deposition of toxic wastes in open agriculture fields without any appropriate remediation or recovery methods (Scholz and Lucas 2003). Certain metals and metalloids, such as chromium, arsenic, lead, copper, zinc, cadmium, mercury and nickel are constantly being added to agricultural soils either by irrigation system or waste disposal (Baran and Wieczorek, 2015:Khan 2005) that pose threat to food safety and also potential health risks (Ali et al. 2015a). Cr (VI) is on the 17th position among other hazardous substances (ATSDR 2015). Under normal conditions, Cr concentrations in soil remain lower than the toxic levels (1ppm), while in some cases this concentration exceeds 5 ppm (Zayed and Terry 2003). Cr exists in two stable forms, i.e. Cr (VI) and Cr (III). The former is considered as the most hazardous to all life forms, while the latter is an essential component of human balanced diet (Yu et al. 2016). The requirement of Cr as a nutrient for plants is about 0.5 to 5.0 ppm g^{-1} however, 5 to 100 ppm g^{-1} Cr in soil is considered toxic (Ali et al. 2015a). Over-accumulation of Cr causes certain phytotoxic effects in plants that lead to DNA damage, metabolism inhibition, chlorosis, delayed seed germination, premature leaf falling, low crop yield and loss of enzymatic activities (Gill et al. 2015, Yu et al. 2016). Cr is also considered toxic to animals and humans, leading to a variety of health problems due to carcinogenic and mutagenic effects (Yu et al. 2016).

Increased Cr phytotoxicity in soil is receiving great attention in public as well as scientific community (Yáñez and Rodrigo 2002). Its remediation is necessary to overcome the persistent effects caused by the leaching of this metal. Bioremediation technology and various in-situ physicochemical methods have been recommended in the last three decades. However, most of the conventional methods (physicochemical methods) are expensive, time-consuming, limited to relatively small areas, alter the physical, chemical and biological soil

properties and last but not the least produce secondary pollutants (Wuana and Okieimen 2011). These limitations have prompted the scientific community to develop cheaper and alternative techniques to decontaminate the soils. A possible strategy to cope with Cr contamination, known as phytoremediation, in which plants are used to remediate contaminated soils and to ease the improvement of soil texture, was formulated a decade ago. Phytoremediation is use of plants and associated soil microbes to reduce the concentration or toxic effects of contaminants in the environment (Markowicz et al. 2016). It offers a long term, natural, viable, and economic alternative to all other conventional strategies. Of all the phytoremediation approaches, phytoextraction is a technique in which toxic metals are taken up from the soil via roots and translocated to above-ground parts of the plants (Rafati et al. 2011). Phytoextraction is a relatively slow and steady process as it takes ample time to reduce metal contents to an acceptable level within a limited time frame, most probably due to the slow growing and small sized metal hyperaccumulator plants. To overcome these limitations, some improvements have been made in the last few years that increase the bioavailability of heavy metals. Use of fast growing natural or genetically modified hyper accumulating plants with extensive root system, proper chelating agent, or augmentation of microbes plays an important role in addition with above mention method (Ullah et al. 2015). Soil amendments with metal chelating agents like Ethylene Diamine Triacetic Acid (EDTA), Hydroxyethylene diaminetriacetic acid (HEDTA), Diethylenetriamine Pentaacetic Acid (DTPA), Ethylene Glycol Tetraacetic acid (EGTA), Hydroxylamine, Citrate and Nitrilotriacetic (NTA) enhance the bioavailability and absorbance of heavy metals by plant roots. These measures are worth considering with the probability of success lying underneath (Khan et al. 2000).

Rhizobacteria with heavy metal tolerance and plant growth promotion are potential candidates to inhabit the rhizosphere, penetrate the roots and activate certain plant tolerance mechanisms against various environmental stresses (Akram et al. 2016). *Staphylococcus arlettae* strain Cr11 promoted plant growth by reduction of Cr (VI) to less toxic form (Sagar et al. 2012). *Staphylococcus sciuri* strain SAT-17 exhibited plant growth promoting properties such as indole-3-acetic acid production, phosphate solubilization, and deaminase activity of 1- aminocyclopropane-1-carboxylic acid. (Akram et al. 2016). Further, it reduced the production of reactive oxygen species, enhanced antioxidant enzyme activity and increased biomass of *Zea mays* plants under heavy metal stress. Indole acetic acid production by *Pseudomonas* and *Acinetobacter* strains, leads to improved growth and eventually enhanced metals uptake by crop plants (Jankowski et al. 1995). *Burkholderia cepacia,* an antagonistic PGPR, is also used for bioremediation of heavy metals. Additionally, many other *Burkholderia* species such as *B. vietnamiensis* make strong association with plants to harness plant roots, stem and leaves by fixing atmospheric nitrogen and protect the plants from biotic and abiotic stresses (Khan 2005).

Current pot experiment was conducted to investigate the alone and combine effects of *B. vietnamiensis* strain FY92 and citric acid to enhance *Z. mays* growth and its bioaccumulation potential under Cr stress. Indeed, this is the first report explaining the physiological responses of *Z. mays* in association with *B. vietnamiensis* along with CA. This study will evoke attention towards the establishment of long-term plans which will involve synergistic microbial and chelate assisted phytoremediation technique for the effective remediation of Cr-affected soils.

Materials and methods

Experimental work

A completely randomized design (CRD) pot experiment was conducted to investigate the synergistic effects of *B. vietnamiensis* and CA on the growth and Cr uptake of the maize plant. The experiment was consisted of four treatments, untreated soil (Control), inoculated with bacteria (T1), inoculated with bacteria and chelator (T2), and treated with chelator (CA, 5 mmol kg^{-1}) (T3). The experiment was performed in triplicate for each treatment. Eight surface--sterilized seeds were placed in each pot measuring 13×11 cm^2 filled with 1.5 kg autoclaved soils at a depth of 2 cm (Chen et al. 2013, Khan and Bano 2016). Thinning of plants was done to 5 plants per pot after two weeks of germination and the plants were carefully watered (50 ml) on the daily basis (Chen et al. 2013). The Cr solution of concentration (500 ppm) was applied after two weeks of seed germination then continued at one--week interval. However, 5 mmol kg^{-1} of CA was applied after one week of Cr treatment.

Chemical characteristics of soil

Soil samples were collected from Ittehad Steel Re-Rolling Mills, Islamabad (33.7167°N, 73.0667°E) and Quaid-i-Azam University, Islamabad (33.7472°N, 73.1389°E). These soil samples were analyzed for their physiochemical characteristics such as soil pH (McLean, 1982), soil organic matter (Nelson and Sommers, 1982), and electrical conductivity (EC) (McLean, 1982). The analysis for macro-nutrients, i.e., Nitrate-Nitrogen (Estefan et al. 2013), Potassium (K) (Estefan et al. 2013) and Phosphorus (P) (Nguyen et al. 1992, Qureshi et al. 2012) was also done. Quantification of Cr in samples was done by atomic absorption spectrophotometer (AAS). All data for physiochemical characterization and nutrient analysis is presented in Table 1.

Table 1. Physiochemical properties and nutritional state of the experimental soil S2. (Industrial site).

Properties	**soil**
Soil texture	Loam
Soil pH	7.28
Soil EC (dS m^{-1})	0.35
Organic matter (%)	0.711
Total K^{++}	27.29 (ppm)
Total mg^{++}	71.11 (ppm)
Total Ca^{++}	673.15 (ppm)
Total Fe	133.67 (ppm)
N	13.24 (ppm)
P Reference soil Cr	10 (ppm) 0.869 (ppm)
Industrially contaminated soil (Cr)	130 ppm

Seed sterilization and microbial inoculation

Seeds of *Z. mays* (advance germplasm line: Islamabad Gold) were obtained from National Agriculture Research Center (NARC), Islamabad, Pakistan. Surface sterilization of seeds was done with 75% ethanol for 5 min followed by $HgCl_2$ (0.1%) for 1 min. Subsequently, the seeds were washed three to five times with autoclaved distilled water (Akram et al. 2016).

The bacteria, *B. vietnamiensis* FY92 was taken from Plant-Microbe Interactions Lab, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. The 16S rRNA gene sequencing was submitted to NCBI with the accession number of *B. vietnamiensis* (KR025475). *B. vietnamiensis* inoculum was prepared in separate 250 ml flasks containing 100 ml of sterilized LB broth and incubated in a shaker incubator at 120 rpm at 37°C for 48 hrs. The culture was then centrifuged at 10000 rpm for 15 min and washed twice with sterile distilled water. For bacterial inoculation, seeds were soaked in bacterial suspension adjusted to OD_{600}=1.000 [10^8–10^9 colony-forming units (CFU) mL^{-1}] or in sterilized water for control and subsequently planted in plastic pots (Chen et al. 2013).

Soil samples were dried, ground, mixed well and sieved by passing through sterilized 2-mm mesh. The pots were filled with sterilized soil for sowing maize seeds with different treatments.

Reclamation of inoculated bacteria

The presence of *B. vietnamiensis* was verified from roots and shoots using the method of Qin et al. 2014 and Luo et al. 2011 in inoculated plants as well as its absence in non-inoculated plants. The inoculated maize Islamabad variety Gold plants after 72 days were sterilized and cut into 0.2×0.5 cm small sections, respectively. Then, the samples were plated onto nutrient agar media and incubated for 2–5 days at 28°C. The colonies were identified for their morphological characteristics and compared with the original inoculated strains. (Qin et al. 2014, Luo et al. 2011).

Plant analysis

Morphological parameters

Z. mays plants were harvested after 72 days of sowing. The plants were carefully removed from the pots, and roots were washed several times with distilled water. Morphological parameters such as plant height, shoot root length, fresh and dry weight were measured.

Photosynthetic pigments test Chlorophyll fluorometer

Photosynthetic efficiency was analyzed using a portable plant efficiency analyzer Pocket Pea (HansaTech Instruments, PPEA-130016). The applied fluorometer automatically calculated and displayed a value for the fluorescence parameter Fv/Fm (Martina).

Photosynthetic pigments

The photosynthetic pigments were extracted as described by (Sumanta et al. 2014). The equations for chlorophyll a, chlorophyll b and carotenoids are as follows:

Chlorophyll a= 1.07 (OD 663) – 0.09 (OD 645)
Chlorophyll b= 1.77 (OD 645) – 0.28 (OD 663)
Carotenoids= OD 470 * 4

Antioxidant activities

Superoxide dismutase (SOD) and Peroxidase activities (POD) were noted according to the methods of Bano and Bhatt (2010) Reddy et al. (1995), respectively.

Proline test

The proline contents of maize leaves were determined by the methods of Bates et al. (1973) and Khan and Bano (2016).

Metal analysis of plants by wet acid digestion method

Plant nutrient analysis and metal uptake were determined by wet acid digestion (Wan et al. 2012). 1 g dried plant material was transferred to 100 ml flask after fine grinding with pestle and mortar. Then, 10 ml Nitric-Perchloric acid (HNO_3-$HClO_4$, at 3:1 ratio) was supplemented to plant material in the flask and left overnight. After the initial digestion, the flasks with the material were then transferred to the fume hood, and the temperature was raised to 70°C for 60 minutes. The temperature was steadily raised until the brown fumes turned into white fumes. The mixture was then permitted to cool for a few minutes and diluted carefully with distilled water. The extract was then filtered through filter paper (Whatmann No. 42) and the volume of filtrate was raised up to 50 ml with distilled water. Filtered samples were then used to determine the concentrations of desired metal, i.e. Cr and other macro--elements by flame atomic absorption spectrometry (Varian FAAS-240, Triad Scientific and New Jersy, USA) (Khan and Bano 2016).

Calculation of bioaccumulation coefficients (BAC), bioconcentration factor (BCF) and translocation factor (TF)

The bioaccumulation factor and bioconcentration factor provide an index of the ability of the shoots and roots to accumulate the metal with respect to the metal concentration in the soil (Anwer et al. 2012, Malik et al. 2010, Putwattana et al. 2015). The translocation factor was calculated accordingly (Ahmad et al. 2015).

Statistical analysis

The experimental data obtained in this study were analyzed statistically using statistics software (STATISTICA version. 8.1). Two-way analysis of variance (ANOVA) was used to determine the significant differences among the means based on Fisher's least significant difference (LSD) procedure. Correlation coefficients and dendrograms were also obtained to study the interrelationship of the measured parameters.

Results

Chemical characteristics and nutrient analysis of soil

Basic chemical characteristics and nutrient analysis of soil are given in Table 1. The organic matter of reference soil was 0.711%. Reference soil was slightly acidic with pH of 7.28, and EC values were 0.35 (dS m^{-1}). Nitrate-N and phosphorous were 13.24 and 10 ppm, respectively. The concentrations of K^{++}, Ca^{++}, and Fe for reference soil were 27.29, 71.11, 673.15 and 133.67 ppm, respectively. Heavy metal analysis of the two

soils showed higher concentration of Cr in the industrially contaminated soil (S2 soil). However, Cr concentrations for reference soil and S2 soils were 0.869 ppm and 130.25 ppm, respectively as shown in Table 1.

Morphological and biochemical analysis of plant

Plant biomass was significantly influenced by the application of *B. vietnamiensis* and citric acid. The morphological attributes such as root and shoot length, and fresh and dry weight were enhanced in plants with combined treatment of *B. vietnamiensis* + citric acid as compared to non-inoculated (Table 2). In the normal (reference) soil, the synergistic effect of *B. vietnamiensis* + citric acid significantly increased root and shoot length, and fresh and dry weight followed by increase in these morphologic attributes with the application of *B. vietnamiensis* (T1) and citric acid (T3) as compared to its control (Table 2). In Cr spiked (S1) soil, *B. vietnamiensis* (T1) considerably amplified shoot length, fresh and dry weight after T2 except for root length that was enhanced by T3. In S2 soil, shoot length, and fresh and dry weight were enhanced by T2 as compared to its control, whileT3 enhanced root length. Interestingly, the combined application of *B. vietnamiensis* and citric acid (T2) significantly increased shoot length, and fresh and dry weight compared to the control in different soil samples, thus IT showed maximum growth as compared to the solitary treatment of bacteria or CA. The solitary treatment of CA, however, increased root length in all soil samples. In general, inoculated plants showed better growth than non-inoculated or CA-treated plants under control and Cr contaminated soils. The pattern observed for enhanced plant growth and biomass was T2> T1 > T3 > C.

Photosynthetic pigments

An Fv/Fm value of less than 0.8 shows that the photosynthetic robustness of the plant is compromised due to Cr uptake (Table 2). The photosynthetic pigments were reduced in industrial contaminated as well as Cr treated soil. A significant decline in chlorophyll content was recorded in plants grown on contaminated soils compared to the plants in reference soil. However, (T1) and (T2) effectively increased chlorophyll contents of plants relative to control. Moreover, treated plants showed the highest chlorophyll content in all soil samples, though the chlorophyll contents were reduced in CA-treated plants. Like plant biomass, T1 and T2 showed the maximum increase in chlorophyll contents under Cr contaminated soil (Table 2).

Antioxidant enzymes

Treatment with CA (T3) increased the SOD activity by 47% compared to control (untreated) soil (Fig. 1, 2). Similarly, the POD activities increased by 75% with the increase in Cr concentration relative to control soil. The inoculation with *B. vietnamiensis* increased the SOD and POD of maize leaves in Cr spiked soil (S1). The response of *B. vietnamiensis,* and CA treatment (T2) on maize was variable. Combination of both increased the SOD activity by 4.1% in heavy metal treated plants as compared to control (Figs 1 and 2).

Proline content

Proline contents of *Z. mays* enhanced with an increase in metal concentrations in various soil samples (Fig. 3). Plants treated with CA had higher proline content than *B. vietnamiensis* inoculated and non-inoculated plants. CA treated plants enhanced proline content up to 28.87%, 23.75%, and 16.48% for

Table 2. Effect of bacterial isolate *Burkholderia vietnamiensis* on different growth parameter of *Zea mays* plant under citric acid (CA) and bacterial inoculated treatments

Reference Soil Agricultural soil of QAU*							
Treatments	**Shoot Length**	**Root Length**	**Fresh Weight**	**Dry Weight**	**Chloro a**	**Chloro b**	**Carotenoids**
Control	41.07±0.41	27.04±0.03	5.10±0.02	5.422±0.02	17.03±0.01	9.05±0.01	5.04±0.01
T1	52.17±0.03	28.13±0.13	12.03±0.00	11.49±0.05	18.44±0.20	12.11±0.03	5.06±0.01
T2	57.43±0.04	29.01±0.02	16.01±0.00	12.33±0.01	7.92±0.02	6.65±0.01	3.02±0.00
T3	51.05±0.03	30.35±0.33	11.26±0.01	16.12±1.03	10.18±0.04	5.02±0.00	2.03±0.00
S1 Agricultural soil of QAU spiked with 500 mg kg-1							
Treatments	**Shoot Length**	**Root Length**	**Fresh Weight**	**Dry Weight**	**Chloro a**	**Chloro b**	**Carotenoids**
Control	9.13±0.03	10.27 ±0.01	2.13±0.01	5.80±0.04	14.13±0.04	4.13±0.02	2.58±0.01
T1	38.05±0.03	22.09 ±0.32	7.04±0.00	2.76±0.01	19.81±0.03	6.62±0.02	4.19±0.02
T2	42.04±0.03	23.07± 0.03	8.86±0.01	7.24±0.0	17.08±0.03	6.12±0.03	3.31±0.00
T3	36.15±0.03	24.03 ±0.03	5.80±0.04	1.36±0.02	11.65±0.04	1.17±0.01	2.34±0.04
S2 Industrial soil							
Treatments	**Shoot Length**	**Root Length**	**Fresh Weight**	**Dry Weight**	**Chloro a**	**Chloro b**	**Carotenoids**
Control	7.05±0.01	3.46±0.03	0.30±0.04	0.30±1.34	11.80±0.05	4.19±0.05	2.17±0.03
T1	11.21±0.01	4.04±0.32	0.10±0.02	0.08±0.11	16.07±0.01	10.76±0.01	4.35±0.05
T2	15.00±0.00	5.18±0.02	1.21±0.03	0.42±0.07	12.02±0.00	7.57±0.03	2.31±0.05
T3	10.11±0.03	8.03±0.03	0.42±0.06	0.10±0.21	8.049±0.00	3.91±0.04	2.16±0.02

Growth was measured at 72 days after seed germination under three different soil conditions. SL – shoot length, RL – root length, FW – fresh weight, DW – dry weight, Chloro – Chlorophyll. The values are mean ±SD (n = 3) at $p < 0.05$ (LSD test in two-way ANOVA)

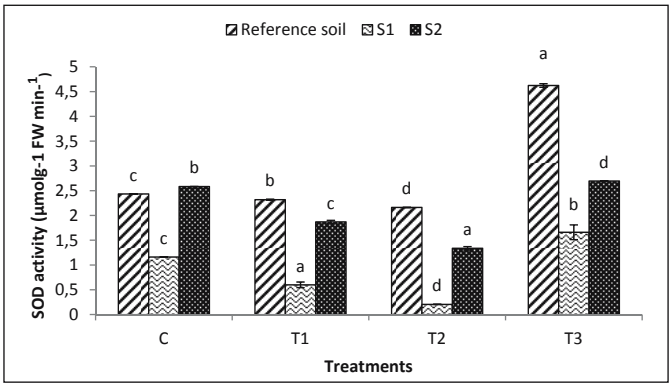

Fig. 1. Total plant length of *Zea mays* L. in different soil. Results are shown as mean ± SEM (n = 3). Error bars show standard error. Means with different letters are statistically different at $p < 0.05$ (LSD test in two-way ANOVA)

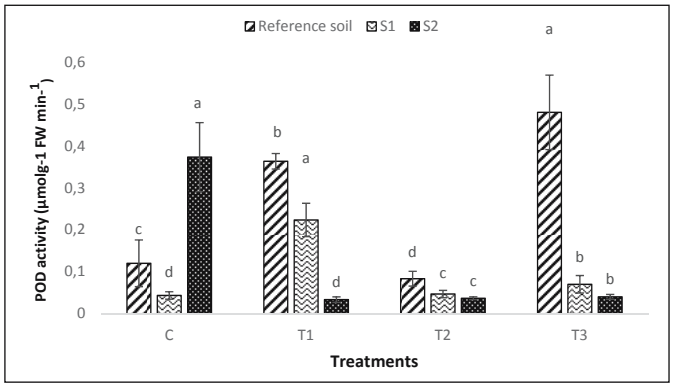

Fig. 2. Shoot length and root length of *Zea mays* L. in different soil. Results are shown as mean ± SEM (n = 3). Error bars show standard error. Means with different letters are statistically different at $p < 0.05$ (LSD test in two-way ANOVA)

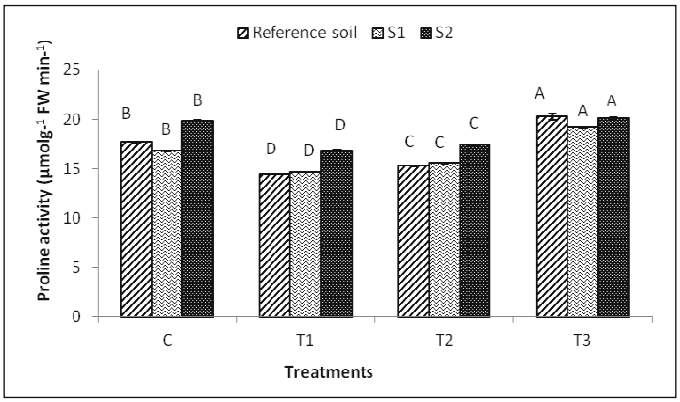

Fig. 3. Fresh and dry weight of *Zea mays* L. in different soil. Results are shown as mean ± SEM (n = 3). Error bars show standard error. Means with different letters are statistically different at $p < 0.05$ (LSD test in two-way ANOVA)

reference, S1, and S2 soils, respectively. Moreover, the highest proline was expressed with T3 in each soil. The maximum proline contents for T3 were observed as 20.26, 19.16 and 20.13 µg/g for reference, S1, and S2 soils, respectively. The increasing order of proline content was T3 > T1 > T2 > C.

Bioaccumulation coefficient (BAC), bioconcentration factor (BCF) and translocation factor (TF)

Increased accumulation of heavy metals was observed in tissues of *Z. mays* with the elevated levels of metals in the soil (Table 3). Higher concentration of metals was observed in roots of *Z. mays* than in shoots except in normal soil. In the shoot, 94.23% of increased Cr accumulation was observed in comparison to uninoculated plants. Although the much higher concentration of heavy metals was found in roots, a significant amount of metals was still observed to be accumulated in shoots. Unlike biomass, the effect of CA on metal uptake was greater than the application of *B. vietnamiensis* in normal soil. In comparison with inoculated plants, CA increased the accumulation of Cr by 74.06%. Furthermore, higher accumulation was detected in CA-treated plants followed by highest accumulation of Cr in plants inoculated with *B. vietnamiensis* + CA. Likewise, higher accumulation of Cr was observed in inoculated and citric acid treated plants compared to the un-inoculated and untreated plants in soil S1 and S2 soil. Results of the current study revealed the co-application of *B. vietnamiensis,* and CA as the most effective approach for the accumulation of Cr in plant organs. Highest BAC values of Cr noted for reference, S1 and S2 were 3.32, 2.24 and 4.09, respectively (Table 3). The pattern for BAC values was S2 > reference soil > S1. Similar to BAC, the maximum values for BCF were also obtained. BCF is an effective indicator of the efficiency of a plant for the accumulation of heavy metals (Table 3). The highest root BCF values for Cr were noted as 1.99, 1.15 and 1.18 in different soil (Table 3). The descending order of root BCF values was reference soil > S2 > S1. TF describes the ability of a plant in translocating the elements from root to shoot of a plant. The TF values for Cr varied in all the contaminated soils used for the experiment. The maximum TF values found for Cr were 5.2, 1.9 and 1.3 (Table 3). The order for TF values was observed as reference soil > S1 > S2.

Correlations and dendograms for the interrelationship of measured parameters

Table 4 in Reference Soil (Agricultural soil of QAU) shows the Pearson's correlation coefficients for the measured parameters at $p \geq 0.05$ level. The Inoculation of PGPE showed significantly

Table 3. Bioaccumulation coefficient, bioconcentration and translocation factor of *Zea mays* L. under three different soil conditions

Reference Soil Agricultural soil of QAU*			
Treatments	**BAC**	**BCF**	**TF**
Control	0.743± 0.00042	0.503± 0.0047	0.372± 0.0008
T1	1.214± 0.00047	1.119± 0.0151	1.0855± 0.0144
T2	2.618± 0.01809	1.953± 0.0074	1.703± 0.0027
T3	3.327± 0.01815	1.996± 0.0033	1.203± 0.0767
S1 Agricultural soil of QAU spiked with 500 mg Cr kg^{-1}			
Treatments	**BAC**	**BCF**	**TF**
Control	1.014± 0.002	1.021± 0.004	0.993± 0.002
T1	1.755± 0.006	1.150± 0.003	1.317± 0.001
T2	2.249± 0.004	1.333± 0.005	1.956± 0.004
T3	1.351± 0.001	1.054± 0.005	1.282± 0.007
S2 Industrial soil			
Treatments	**BAC**	**BCF**	**TF**
Control	1.283± 0.0027	0.765± 0.0005	0.848± 0.0004
T1	3.286± 0.0092	1.179± 0.0006	1.125± 0.0028
T2	4.090± 0.0134	1.180± 0.0007	1.306± 0.0010
T3	2.718± 0.0188	0.889± 0.0022	0.848± 0.0005

Table 4. Pearson's correlation coefficients showing the significant correlations among the measured parameters at p=0.05

Variables	Shoot Length	Root Length	Fresh Weight	Dry Weight	Soil Cr	Root Cr	Shoot Cr
Ref Shoot Length							
S1 Shoot Length							
S2 Shoot Length							
Ref Root Length	0.618						
S1 Root Length	0.973*						
S2 Root Length	0.201						
Ref Fresh Weight	0.998**	0.592					
S1 Fresh Weight	0.957*	0.869					
S2 Fresh Weight	0.756	0.193					
Ref Dry Weight	0.722	0.964*	0.689				
S1 Dry Weight	-0.216	-0.395	0.069				
S2 Dry Weight	0.35	-0.321	0.802				
Ref Soil Cr	0.631	0.972*	0.618	0.893			
S1 Soil Cr	0.826	0.681	0.954*	0.353			
S2 Soil Cr	0.974*	0.253	0.596	0.134			
Ref Root Cr	0.826	0.93	0.817	0.908	0.958*		
S1 Root Cr	0.691	0.526	0.87	0.551	0.974*		
S2 Root Cr	0.865	-0.091	0.366	0.052	0.919		
Ref Shoot Cr	0.984*	0.681	0.987*	0.74	0.725	0.89	
S1 Shoot Cr	0.758	0.638	0.895	0.455	0.949	0.967*	
S2 Shoot Cr	0.906	-0.229	0.637	0.446	0.869	0.917	

*, ** and *** for p ≤ 0.05, ≤ 0.01, ≤ 0.001, Respectively
Ref: Soil Agricultural soil of QAU, S1: Agricultural soil of QAU spiked with 500 ppm, S2: Industrial soil.

strong correlations with most of the studied parameters. Root length showed significantly positive correlation with most of the parameters especially Dry Weight (r=0.998), and soil Cr (r=0.972). Shoot length positively correlated with Shoot Cr (0.984) and Plant Fresh Weight (r=0.998). Plant Fresh Weight also has a strong positive correlation with Shoot Cr (r=0.987). Similarly, Soil Cr has a positive correlation with root Cr (r=0.958) under reference soil. Under the conditions of soil 1, shoot length has a strong positive correlation with root length (r=0.973) and Plant fresh weight (r=0.957). Root Cr has a positive correlation with soil Cr (r=0.974) and Shoot Cr (r=0.967). Further, soil Cr has a positive correlation with fresh plant weight (r=0.954). Under the conditions of soil 2, a significant positive correlation of shoot length was noted with amount of Cr in soil (r=0.974).

Discussion

Chromium (Cr) toxicity is one of the most lethal environmental issues that hinder plant growth and contaminates the food chain (Ali et al. 2015b). Phytoremediation is an affordable environmental strategy to lessen the Cr concentration in contaminated soils and offers a sustainable alternative to get rid of heavy metals from soil (Ali et al. 2013b). For efficient phytoextraction of heavy metals, native flora has been screened (Khan et al. 2014). The tolerance capacity of each plant depends upon its species, or genotype and the surrounding heavy metal concentration (Leitenmaier and Küpper 2013). The first parameter to select for bacterial isolate as a potential candidate is to evaluate its tolerance towards certain concentration of heavy metals, which quantitatively is considered as up to 500 ppm generally, an isolate exhibited more than one plant growth-promotion characteristic. Plant growth-promoting bacteria directly or in-directly have the capability to enhance heavy metals uptake by plants through various mechanisms. (Glick 2010).

In addition to a normal outlook of plants in response to abiotic stress, certain morphological and agronomic criteria like shoot and root length as well as fresh and dry weight of the plants are needed to be analyzed to convincingly conclude if a plant is tolerant to the stress or not. Rogers et al. (2012) reported that the root length markedly increased by CA application under Cr stress. Our findings revealed that properties of plant growth and biomass were remarkably declined with enhanced supply of Cr in the soil. It is possible that this decline in plant growth results from some ultra--structural modifications in different plant organs (Gill et al. 2015). The application of CA resulted in more root length than the application of bacteria only in a Cr-stressed soil (Table 2). The decrease in biomass was suggested to be possible because of such factors as chelate-induced metal mobility, chelate toxicity or toxicity of chelate-metal complex. Previously under Cr stress, reduction in morphological parameters of wheat and other plant species was observed (Gill et al. 2015). Moreover, it has been already reported that shoot growth has stunted by the increased Cr concentration (Faisal et al. 2005). Enhancement or decline in growth and biomass with CA application can be traced by analyzing the genetic diversity of plant species, environmental conditions, the application time of CA, and exposure time to stress. In comparison with control treatments alone, the application of *B. vietnamiensis,* and CA significantly increased plant biomass and shoot length (Table 2). This enhancement in biomass may be either due to decreased Cr uptake or increased nutrient uptake. More work, however, still needs to be done to unravel this mechanism. It is also known that addition of heavy metal like Pb affects root and shoot growth of *Zea mays* L (Hussain et al. 2013). Nevertheless, the plant grew better when diazotroph was used in combination with heavy metals. Our results are in agreement with previous studies (Paungfoo-Lonhienne et al. 2014), which reported that inoculation of plants with *B. vietnamiensis* strains promoted plant growth and subsequently increased shoot biomass. Plant growth promotion by *Burkholderia* species is attributed because of biological nitrogen fixation (BNF), combined heavy metal tolerance capability or other plant growth promoting mechanism. Plant-microbe interaction, therefore, has high potential to be exploited for phytoremediation efficiency of polluted soil (Naveed et al. 2014).

Photosynthetic pigments of *Zea mays* become reduced with increased Cr concentrations (Table 2). Previous reports support our results that increased Cr concentrations reduced the photosynthetic pigment contents in various plant species (Gill et al. 2015). The decrease in chlorophyll content is assigned to both direct and indirect factors, like inhibition of chloroplast ultrastructure modifications and decline/decomposition of photosynthetic pigments via acceleration of the chlorophyllase activity (Hegedüs et al. 2001) (Gill et al. 2015). Variation in gas exchange parameters (Ali et al. 2011), decomposition of chlorophyll and decrease in carotenoid contents were higher when treated with heavy metal especially in un-inoculated, and CA-treated plants (Table 2).

The best self-defense mechanism in heavy metal-stressed plants is the activation of antioxidant enzymes. The production of SOD and POD in metal-treated plants relates to the findings of (Ali et al. 2015), where increased antioxidant enzyme activity was observed as compared to control when Cr contaminated soils were amended with CA. (Figures 1, 2, 3). In plants this overexpression of antioxidant enzymes depends upon exposure to Cr stress, and might be an effective way for plants survival with the capability of increased metal accumulation. (Haouari et al. 2012). It is logical that the inherent capability of plants to quench the excessive oxidative burst under intense Cr stress is not enough until it is supplemented with the exogenous application of CA to cope with metallic stress (Mishra and Koehler 2006, Meng et al. 2009). Our results showed that *Z. mays* treated with CA scavenged the oxidative stress via enhancing the antioxidant enzymatic activity.

In the present study, plants treated with CA contained significantly enhanced Cr uptake. CA application has enhanced the uptake of Cr in different parts of the plant (Table 3) (Gill et al. 2015). It is noticed that the accumulation of Cr in *Z. mays* directly depends upon the available amount of Cr in the soil. Further, the application of *B. vietnamiensis* + CA enhanced the plants Cr uptake relative to its control and all other abiotic treatments alone (Table 3). In *Z. mays* the direct relation was observed in the total uptake of Cr and biomass obtained in the presence of *B. vietnamiensis* + CA. Our results of metal tolerance ability of *Z. mays* are consistent with the findings of (Li et al. 2014) indicating that different kinds of grasses have strong tolerance ability to higher concentrations of Cr when treated with both biotic and abiotic metal scavengers.

Normally plant roots accumulate more heavy metals as compared to shoot as observed in *Vicia faba* (Shahid et al. 2012) and *Hordeum vulgare* (Ali et al. 2015). In roots the enhanced Cr sequestration might be due to the reason that roots are directly exposed to heavy metal stress which might be due to Cr precipitation in roots as an insoluble salt or because of its fixation with other molecules like celluloses, pectins, hemicelluloses, and sugar. (Shahid and Abbasi 2011). The enhanced nutrient uptake due to CA + *B. vietnamiensis* may contend with Cr uptake. The reason behind the enhanced Cr transportation may be the plant growth promoting activities of the inoculated strain that increase plant biomass to accumulate more heavy metal (Husen et al. 2013). Based on the present results, it is proposed that the use of plant growth-promoting bacteria resistant to Cr defend plant against the Cr inhibitory effects present in soil and assist plant in Cr transportation from soil to plants parts. *Burkholderia sp.* inoculated maize plants significantly increased the uptake of Pb and Cd as compared to uninoculated plants (Ullah et al. 2015). These results suggest for the incorporation of *B. vietnamiensis* + CA component in the phytoremediation of Cr by *Z. mays*.

Our findings suggest for a strong correlation among plant fresh and dry biomass as well as root and shoot length in both inoculated and uninoculated plants (Audet and Charest 2007). Our model of S1, S2, PGPE inoculated and chelator treated soil, showed a strong correlation with the 'enhanced uptake' of heavy metals by the root and shoot as was noticed in the study done by (Brown et al 1994, Audet and Charest 2007) where Cd and Zn were significantly correlated with the root and shoot uptake concentration of the heavy metal because a positive and linear curve was observed which tended to reach a plateau in the soil containing high concentration of heavy metals. We have designated zones of Enhanced Uptake and Metal-Binding which show greater Cr uptake in PGPE-inoculated and chelator-treated plants than non-PGPE plants in the soils both low and high in the Cr levels, respectively.

Conclusion

Various plant parameters including growth, photosynthetic pigments, and biomass increased by the enhanced enzymatic activity of antioxidants when *B. vietnamiensis* and CA were applied as reclamation measures and increased the Cr concentration in various plant parts of *Z. mays*. Thus, the use of *B. vietnamiensis* together with CA is suggested for efficient reclamation of unwanted metal from the soil. The conclusion to our findings is that various plant parameters like growth, photosynthetic pigments, and biomass increased by enhancing antioxidant enzyme activity with the application of *B. vietnamiensis* and CA, whereas in various plant parts Cr concentration was increased which ultimately elevated metal uptake in *Z. mays*. Our findings revealed that in Cr contaminated soils, Cr accumulation and phytoremediation has greatly enhanced by *B. vietnamiensis* and CA.

Acknowledgements

We are grateful to National Agriculture Research Center (NARC) Islamabad, Pakistan for the support by providing the maize seed carrying out the research work. We also sincerely pay thanks to Quaid-i-Azam University Islamabad for providing funds for current research work (URF-2016).

References

Ahmad, I., Akhtar, M.J., Asghar, H.N., Ghafoor, U. & Shahid, M. (2016). Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake, *Journal of Plant Growth Regulation*, 35, pp. 303–315.

Akra, M.S., Shahid, M., Tariq, M., Azeem, M., Javed, M.T., Saleem, S. & Riaz, S. (2016). Deciphering Staphylococcus sciuri SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (Zea mays L.), *Frontiers in Microbiology* , 7, 867.

Ali, S., Bharwana, S. A., Rizwan, M., Farid, M., Kanwal, S., Ali, Q., Ibrahim, M., Gill, R.A. & Khan, M. D. (2015). Fulvic acid mediates chromium (Cr) tolerance in wheat (Triticum aestivum L.) through lowering of Cr uptake and improved antioxidant defense system, *Environmental Science and Pollution Research*, 22, pp. 10601–10609.

Angus, A.A., Agapakis, C.M., Fong, S., Yerrapragada, S., Estrada-De Los Santos, P., Yang, P., Song, N., Kano, S., Caballero-Mellado, J. & De Faria, S.M. (2014). Plant-associated symbiotic Burkholderia species lack hallmark strategies required in mammalian pathogenesis, *PloS one*, 9, e83779.

Audet, P. & Charest, C. (2007). Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives, *Environmental Pollution,* 147, 3, pp. 609–614.

Bano, M. & Bhatt, D.K. (2010). Ameliorative effect of a combination of vitamin E, vitamin C, α-lipoic acid and stilbene resveratrol on lindane induced toxicity in mice olfactory lobe and cerebrum, *Indian Journal of Experimental Biology*, 48, pp. 150–158.

Baran, A. & Wieczorek, J. (2015). Application of geochemical and ecotoxicity indices for assessment of heavy metals content in soils, *Archives of Environmental Protection*, 41, pp. 54–63.

Bates, L., Waldren, R. & Teare, I. (1973). Rapid determination of free proline for water-stress studies, *Plant and soil*, 39, pp. 205–207.

Brown, S.L., Chaney, R.L., Angle, J.S. & Baker, A.J.M. (1994). Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc-and cadmium-contaminated soil, *Journal of Environmental Quality*, 23, 6, pp. 1151–1157.

Chen, Z.-J., Sheng, X.-F., He, L.-Y., Huang, Z. & Zhang, W.-H. (2013). Effects of root inoculation with bacteria on the growth, Cd uptake and bacterial communities associated with rape grown in Cd-contaminated soil, *Journal of Hazardous Materials*, 244, pp. 709–717.

Ensink, J.H., Mahmood, T., Van der Hoek, W., Raschid-Sally, L. & Amerasinghe, F.P. (2004). A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one?, *Water Policy*, 6, pp. 197–206.

Estefan, G., Sommer, R., and Ryan, J. (2013). Methods of soil, plant, and water analysis. *A manual for the West Asia and North Africa region*, pp. 170–176.

Faisal, M., Hameed, A. & Hasnain, S. (2005). Chromium-resistant bacteria and cyanobacteria: impact on Cr (VI) reduction potential and plant growth, *Journal of Industrial Microbiology and Biotechnology*, 32, pp. 615–621.

Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P., Yang, S., Ali, S. & Zhou, W. (2015). Chromium-induced physio-chemical and ultrastructural changes in four cultivars of Brassica napus L., *Chemosphere*, 120, pp. 154–164.

Glick, B.R. (2010). Using soil bacteria to facilitate phytoremediation, *Biotechnology Advances*, 28, pp. 367–374.

Haouari, C.C., Nasraoui, A.H., Bouthour, D., Houda, M.D., Daieb, C.B., Mnai, J. & Gouia, H. (2012). Response of tomato (Solanum lycopersicon) to cadmium toxicity: growth, element uptake, chlorophyll content and photosynthesis rate, *African Journal of Plant Science*, 6, pp. 1–7.

Hegedüs, A., Erdei, S. & Horváth, G. (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress, *Plant Science*, 160, pp. 1085–1093.

Husen, P., Tarasov, K., Katafiasz, M., Sokol, E., Vogt, J., Bauppmart, J., Nitsch, R., Ekroos, K. & Ejsing, C.S. (2013). Analysis of lipid experiments (ALEX): a software framework for analysis of high--resolution shotgun lipidomics data, *PloS one*, 8, e79736.

Hussain, A., Abbas, N., Arshad, F., Akram, M., Khan, Z.I., Ahmad, K., Mansha, M. & Mirzaei, F. (2013). Effects of diverse doses of Lead (Pb) on different growth attributes of Zea-Mays L, *Agricultural Sciences*, 4, pp. 262–265.

Jankowski, C.R., Vo, H.-D. & Lippmann, R.P. (1995). A comparison of signal processing front ends for automatic word recognition, *IEEE Transactions on Speech and Audio processing*, 3, pp. 286–293.

Khan, A., Kuek, C., Chaudhry, T., Khoo, C. & Hayes, W. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation, *Chemosphere*, 41, pp. 197–207.

Khan, A.G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation, *Journal of Trace Elements in Medicine and Biology*, 18, pp. 355–364.

Khan, M.U., Sessitsch, A., Harris, M., Fatima, K., Imran, A., Arslan, M., Shabir, G., Khan, Q.M. & Afzal, M. (2014). Cr-resistant rhizo-and endophytic bacteria associated with Prosopis juliflora and their potential as phytoremediation enhancing agents in metal-degraded soils, *Frontiers in Plant Science*, 5, 755.

Khan, N. & Bano, A. (2016). Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation, *International Journal of Phytoremediation*, 18, pp. 211–221.

Leitenmaier, B. & Küpper, H. (2013). Compartmentation and complexation of metals in hyperaccumulator plants, *Frontiers in Plant Science*, 4, 374.

Li, C., Xiao, B., Wang, Q., Yao, S. & Wu, J. (2014). Phytoremediation of Zn-and Cr-contaminated soil using two promising energy grasses, *Water, Air & Soil Pollution*, 225, 2027.

Luo, S.-L., Chen, L., Chen, J.-L., Xiao, X., Xu, T.-Y., Wan, Y., Rao, C., Liu, C.-B., Liu, Y.-T. and Lai, C. (2011). Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator Solanum nigrum L. and their potential use for phytoremediation, *Chemosphere*, 85, pp. 1130–1138.

Malik, R.N., Husain, S. & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan, *Pakistan Journal of Botany*, 42, pp. 291–301.

Markowicz, A., Płaza, G. & Piotrowska-Seget, Z. (2016). Activity and functional diversity of microbial communities in long-term hydrocarbon and heavy metal contaminated soils, *Archives of Environmental Protection*, 42, pp. 3–11.

Martina, L. Acclimation of photosynthesis to different growth temperatures in Betula pendula, degree project for Bachelor of Science with a major in Biology, Department of Biological and Environmental Sciences University of Gothenburg.

McLean, E. (1982). Soil pH and lime requirement, *Methods of soil analysis. Part 2. Chemical and microbiological properties*, pp. 199–224.

Meng, H., Hua, S., Shamsi, I.H., Jilani, G., Li, Y. & Jiang, L. (2009). Cadmium-induced stress on the seed germination and seedling growth of Brassica napus L., and its alleviation through exogenous plant growth regulators, *Plant Growth Regulation*, 58, pp. 47–59.

Miché, L. & Balandreau, J. (2001). Effects of rice seed surface sterilization with hypochlorite on inoculated Burkholderia vietnamiensis, *Applied and Environmental Microbiology*, 67, pp. 3046–3052.

Mishra, P. & Koehler, M.J. (2006). Technological pedagogical content knowledge: A framework for teacher knowledge, *Teachers college record*, 108, 1017.

Najeeb, U., Jilani, G., Ali, S., Sarwar, M., Xu, L. & Zhou, W. (2011). Insights into cadmium induced physiological and ultra-structural disorders in Juncus effusus L. and its remediation through exogenous citric acid, *Journal of Hazardous Materials*, 186, pp. 565–574.

Naveed, M., Mitter, B., Yousaf, S., Pastar, M., Afzal, M. & Sessitsch, A. (2014). The endophyte Enterobacter sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics, *Biology and Fertility of Soils*, 50, pp. 249–262.

Nelson, D. & Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter, *Methods of soil analysis. Part 2. Chemical and microbiological properties*, pp. 539–579.

Nguyen, C., Yan, W., Le Tacon, F. & Lapeyrie, F. (1992). Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus Laccaria bicolor (Maire) PD Orton, *Plant and Soil*, 143, pp. 193–199.

Paungfoo-Lonhienne, C., Lonhienne, T.G., Yeoh, Y.K., Webb, R.I., Lakshmanan, P., Chan, C.X., Lim, P. E., Ragan, M.A., Schmidt, S. & Hugenholtz, P. (2014). A new species of Burkholderia isolated from sugarcane roots promotes plant growth, *Microbial Biotechnology* , 7, pp. 142–154.

Putwattana, N., Kruatrachue, M., Kumsopa, A. & Pokethitiyook, P. (2015). Evaluation of organic and inorganic amendments on maize growth and uptake of Cd and Zn from contaminated paddy soils, *International Journal of Phytoremediation*, 17, pp. 165–174.

Qin, S., Zhang, Y.J., Yuan, B., Xu, P.Y., Xing, K., Wang, J. & Jiang, J.H. (2014). Isolation of ACC deaminase-producing habitat--adapted symbiotic bacteria associated with halophyte Limonium sinense (Girard) Kuntze and evaluating their plant growth--promoting activity under salt stress, *Plant Soil*, 374(1–2), pp. 753–766.

Qureshi, M., Ahmad, Z., Akhtar, N., Iqbal, A., Mujeeb, F. & Shakir, M. (2012). Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth, *The Journal of Animal & Plant Sciences*, 22, pp. 204–210.

Rafati, M., Khorasani, N., Moattar, F., Shirvany, A., Moraghebi, F. & Hosseinzadeh, S. (2011). Phytoremediation potential of Populus alba and Morus alba for cadmium, chromium and nickel absorption from polluted soil, *International Journal of Environmental Research*, 5, pp. 961–970.

Ramos, R.L., Jacome, L.B., Barron, J.M., Rubio, L.F. & Coronado, R.G. (2002). Adsorption of zinc (II) from an aqueous solution onto activated carbon, *Journal of Hazardous Materials*, 90, pp. 27–38.

Reddy, L., Nigam, S. & Reddy, A. (1995). Stability of pod yield in foliar disease-resistant groundnut varieties, *International Arachis Newsletter*, 15, pp. 9–11.

Rodriguez, E., Santos, C., Azevedo, R., Moutinho-Pereira, J., Correia, C. & Dias, M.C. (2012). Chromium (VI) induces toxicity at different photosynthetic levels in pea, *Plant Physiology and Biochemistry*, 53, pp. 94–100.

Rogers, A., McDonald, K., Muehlbauer, M.F., Hoffman, A., Koenig, K., Newman, L., Taghavi, S. & Lelie, D. (2012). Inoculation of hybrid poplar with the endophytic bacterium Enterobacter sp. 638 increases biomass but does not impact leaf level physiology, *GCB Bioenergy*, 4, pp. 364–370.

Sagar, S., Dwivedi, A., Yadav, S., Tripathi, M. & Kaistha, S.D. (2012). Hexavalent chromium reduction and plant growth promotion by Staphylococcusarlettae Strain Cr11, *Chemosphere*, 86, pp. 847–852.

Scholz, W. & Lucas, M. (2003). Techno-economic evaluation of membrane filtration for the recovery and re-use of tanning chemicals, *Water Research*, 37, pp. 1859–1867.

Shahid, M., Dumat, C., Silvestre, J. & Pinelli, E. (2012). Effect of fulvic acids on lead-induced oxidative stress to metal sensitive Vicia faba L. plant, *Biology and Fertility of Soils*, 48, pp. 689–697.

Shahid, M.N. & Abbasi, N.A. (2011). Effect of bee wax coatings on physiological changes in fruits of sweet orange CV. "blood red", *Sarhad Journal of* Agriculture, 27, pp. 385–394.

Sumanta, N., Haque, C.I., Nishika, J. & Suprakash, R. (2014). Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents, *Research Journal of Chemical Sciences*, 4(9), pp. 63–69.

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S. & Yang, X. (2015). Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review, *Environmental and Experimental Botany*, 117, pp. 28–40.

Wan, Y., Luo, S., Chen, J., Xiao, X., Chen, L., Zeng, G., Liu, C. & He, Y. (2012). Effect of endophyte-infection on growth parameters and Cd-induced phytotoxicity of Cd-hyperaccumulator Solanum nigrum L, *Chemosphere*, 89, pp. 743–750.

Wuana, R.A. & Okieimen, F.E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation, *Isrn Ecology*, Article ID 402647, 20 pages.

Yáñez, A. & Rodrigo, G.J. (2002). Intranasal corticosteroids versus topical H1 receptor antagonists for the treatment of allergic rhinitis: a systematic review with meta-analysis, *Annals of Allergy, Asthma & Immunology*, 89, pp. 479–484.

Yasmeen, T., Ali, Q., Islam, F., Noman, A., Akram, M.S. & Javed, M.T. (2014). Biologically treated wastewater fertigation induced growth and yield enhancement effects in Vigna radiata L, *Agricultural Water Management*, 146, pp. 124–130.

Yu, X.-Z., Feng, Y.-X. & Liang, Y.-P. (2016). Kinetics of phyto-accumulation of hexavalent and trivalent chromium in rice seedlings, *International Biodeterioration & Biodegradation*, 128, pp. 72–77.

Zayed, A.M. & Terry, N. (2003). Chromium in the environment: factors affecting biological remediation, *Plant and Soil*, 249, pp. 139–156.