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ORIGINAL PAPER

Chromium uptake by giant reed under rhizobacterial inhibition

S. Shaheen[1] · Q. Mahmood[1] · A. Pervez[1] · N. Mirza[1] · Z. A. Bhatti[1] · T. Hayat[1] · S. S. Meryem[1] · F. Ullah[1]

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Abstract The role of rhizospheric microbes of giant reed (*Arundo donax* L.) in Cr uptake from hydroponic culture was investigated. The control group was exposed to Cr in range of 25–100 mg L^{-1} containing a control itself (with no metal addition). The experimental group received same Cr treatments, but in addition was exposed to antibiotic treatment in order to inhibit rhizospheric bacteria. The range of Cr accumulated in the roots was 3–7.65 mg L^{-1}; in stem it ranged 2.15–42.4 mg kg^{-1}; while in leaves, the range of Cr content was 13.7–15 mg kg^{-1}. Overall, Cr uptake in *A. donax* (without rhizobacterial inhibition) was root < leaf < stem. However, the amount of Cr uptake in plants with rhizobacterial inhibition was significantly less (~4.6-folds in 100 mg L^{-1} Cr treatment) than those without such inhibition clearly highlighting that rhizobacterial inhibition decreased the Cr uptake. The experimental results clearly demonstrated that the inhibition of the rhizobacterial populations had great influence on the Cr uptake. However, Cr uptake could not be completely inhibited as some metal uptake was observed after the rhizobacterial inhibition although it was significantly less than the Cr uptake of plants without such inhibition.

Keywords Bioremediation · Chromium contamination · Growth · Heavy metals · Metal toxicity · Phytoremediation · Rhizobacteria

✉ Q. Mahmood
mahmoodzju@gmail.com

[1] Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan

Introduction

In modern era, industrialization is considered as the backbone of economy, but is also an important source of pollution. Toxic heavy metals are continually being added by anthropogenic activities like mining, smelting, industrialization, fuel combustion, increased roadway traffic and agricultural processes like land application of inorganic fertilizers, biosolids, agrochemicals, spread of animal manure and waste dumping (Herawati et al. 2000; Caussy et al. 2003; Caggiano et al. 2005; Blaylock et al. 1997; Mukesh et al. 2008). Metals are naturally inherited from parent material, and their concentrations are continuously increasing due to anthropogenic activities. The threat of heavy metals to human and animal health is aggravated by their long-term persistence in the environment (Gisbert et al. 2003). Chromium, which is generated by various industries, occurs in different oxidation states, but Cr(III) and Cr(VI) are the most significant. Trivalent Cr occurs naturally in the environment and is an essential nutrient for animals (Bahijri and Mufti 2002).

Phytoremediation, an emerging low-cost and environmental friendly technology for decontamination of soils, is defined as the process of utilizing plants to absorb, accumulate and detoxify contaminants in soil through physical, chemical and biological processes (Wenzel et al. 1999). Removal of heavy metal contamination on soils is difficult. Existing physical or chemical methods of soil cleanup are expensive and often result in destruction of soil structure and fertility. Using bioaccumulation in specialized plant species may provide an effective and in situ way of removing heavy metals from contaminated soils (McGrath and Zhao 2003). Many attempts have been made to study the role of rhizospheric bacteria of hyperaccumulating plants to enhance the plant tolerance against heavy metal

toxicity and heavy metals uptake by the plants (Whiting et al. 2001; Chen et al. 2005). Many studies have confirmed that the application of microorganisms in the rhizosphere is beneficial for plants growing in contaminated soils (Wang et al. 2009).

Plant growth-promoting rhizobacteria (PGPR) have been reported to be the key elements for plant establishment under stress conditions. Their use in agriculture can favor a reduction in agrochemical use and support eco-friendly crop production (Ahemad and Kibret 2014). PGPR can help the improvement of plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors. They can also inhibit soilborne plant pathogens by producing growth-promoting chemical substances and inducing plant resistance (Tak et al. 2013; Titah et al. 2013). Different plant growth-promoting rhizosphere bacteria, including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas*, *Enterobacter* group have been used for their beneficial effects on plant growth. Several studies clearly showed the effect of PGPR on growth of different crops at different climates, soils and temperatures (Sessitsch et al. 2013; Jamil et al. 2014; Titah et al. 2014). Microbial populations are known to affect trace metal mobility and availability to the plant, through release of chelators, acidification, and redox changes. The presence of rhizosphere bacteria has been reported to increase the concentrations of Zn, Cu, Pb or Cr in plants. Improvement of the interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals, and is considered to be an important component of phytoremediation technologies (Brígido and Glick 2015; Liu et al. 2015; Glick 2015; Shinwari et al. 2015). Plant growth-promoting bacteria may facilitate plant growth either indirectly or directly. There are several ways in which plant growth-promoting bacteria can directly facilitate plant growth. They may fix atmospheric nitrogen and supply it to plants which is a minor component of the benefit that the bacterium provides to the plant; synthesize siderophores which can sequester iron and other metals from the soil and provide it to plant cells which can take up the bacterial siderophore–metal complex (Glick 2015; Shinwari et al. 2015; Tak et al. 2013); synthesize phytohormones such as auxins, cytokinins and gibberelins, which can act to enhance various stages of plant growth; solubilize minerals, such as phosphorus, making them more readily available for plant growth; and synthesize the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can lower plant ethylene levels.

Plants growing in metal-enriched environments usually take up metals to varying degrees in response to external and internal factors (Reid et al. 1986). The presence of antibiotics in the growth medium may have adverse effects on the metal uptake by the hyperaccumulator plants (Xiong et al. 2008). *Arundo donax* L. commonly called as giant reed has been recently recognized as a useful bioresource to remediate metals from contaminated soils and water (Mirza et al. 2011; Kausar et al. 2012; Sabeen et al. 2013). Previously no study attempted to investigate the effects of antibiotics on the inhibition of rhizobacteria and plant metal uptake. The objective of the present research was to investigate the role of microorganisms in the rhizosphere of giant reed that could enhance decontamination of Cr-contaminated environment. The experiment was conducted during 2012–2013 at the bioremediation laboratory of department of Environmental Sciences at COMSATS Institute of Information Technology, Abbottabad, Pakistan.

Materials and methods

The hydroponics experiment

The work has been done in randomized block design (RBD) along with three replications, having equal amount (250 ± 5 g) of plant of species collected from a wetland near Nawan Shehr in Abbottabad City. Plants of control group were given Cr(VI) treatments in range of 25–100 mg L^{-1} in the hydroponics cultures. The experimental group also received same Cr(VI) treatments along with antibiotic treatment in order to inhibit the growth of rhizospheric bacteria. Thus, total ten treatments were administered, i.e., five without antibiotic and five with antibiotic. For the antibiotic treatment, ampicillin was selected because of following reasons: (a) It has the ability to inhibit both Gram-positive and Gram-negative bacteria, (b) its property to interfere with synthesis of bacterial cell wall and c) expected minimal effect on plant physiology (de Souza et al. 1999). The amount of antibiotic ampicillin [0.1 mg mL^{-1} ampicillin (de Souza et al. 1999)] was administered according to Xiong et al. (2008).

Data were collected regarding plant height, number of plants per pot, number of leaves, and number of nodes and inter nodes, and tillers before the starting of experiment (Table 1). All the replicates were prepared, and the plant was

Table 1 Average values of various morphological parameters before experiment

Parameter	Values
Number of plants	5 ± 1
Plant height	32 ± 14 cm
Number of leaves	28 ± 3
Number of nodes	37 ± 5
Number of tillers	14 ± 3

given the Hoagland's solution and the respected treatment. All the pots were kept under controlled environment. The plants were grown under greenhouse conditions with natural light, day/night temperature of 25/20 °C and humidity of 50–80 %.

Nutrient media

The growth medium of the giant reed plants was Hoagland's solution which was prepared according to Hoagland and Arnon (1938). After taking desired amounts of various nutrients, the volume was made 1 L using distilled water.

Chlorophyll content

In plant cells, chlorophyll (chl) is an essential green pigment for CO_2 fixation during the process of photosynthesis. In various plant species, Cr toxicity causes chlorosis and necrosis (Cervantes et al. 2001). The concentrations of chla, chlb and total chl (total chl = chla + chlb) were determined spectrophotometrically in 80 % acetone. For pigment estimation, 100 mg leaves were crushed with 1 ml of 80 % acetone and kept in dark for 15–30 min, and centrifuged at 4 °C for 15 min (3000 rpm). After that, green colored supernatant was separated from the pellet (almost color less). Volume of supernatant was raised up to 10 ml with 80 % acetone. Absorbance of separated material was detected at 663 nm for chlorophyll a, at 645 nm for chlorophyll b, and at 470 nm for carotenoid concentration estimation (using 80 % acetone as a blank control). Then, further calculations of various pigments were done by using formulae developed by Ianculov et al. (2005) and Brezeanu and de fotosinteza (2005).

Plant growth evaluation

Plants fresh weight was noted both at the beginning and end of the experiment. Plant growth was calculated based on the difference in fresh weight at the start and end of the experiment. Growth parameters like root elongation, number of nodes and internodes, and number of tillers along with toxicity symptoms were determined in the experiment. To evaluate root growth, the maximum root length of each plant was noted at the start and end of experiment. The root elongation was calculated for the difference between the final and initial lengths. For shoot growth analysis, the number of nodes and internodes as well as number of tillers were determined both at the start and end of experiment, and their differences were carefully calculated. In addition to these root and shoot growth parameters, toxicity symptoms of all samples were determined carefully both during and after experiment.

Harvesting of the plants

After 27 days, the plants were taken out from the pots and were placed on a filter paper in shade for a week to be completely shade dried.

Separation of plant parts and grinding

After a week for the calculation of the dry weight plant parts like roots, leaves and stems were separated. Each part was grinded in order to get the uniform size.

Digestion of the plants

After termination experiment, the plant samples (0.5 g each) were collected and analyzed for the concentrations of Cr in different plant parts. The plant samples were shade dried and ground in a grinder machine (WestPoint: WF-9291, France) to less than 1 mm size before analysis, and that was followed by digestion (Zhao et al. 1994). The digested solutions were diluted with deionized water, and the flask was filled up to 50 ml.

Analysis of chromium

After digestion of various plant parts, 50 mL of water samples were filtered through filter paper. Then, water samples (5 mL) were collected from all the pots, and amount of Cr absorbed in different parts of the plant, i.e., root, stem and leaves was determined in leftover solution through Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Translocation factor

It is related with the movement of any substance or metal from root to stem and to leaves through transportation process is called translocation. Translocation factor is calculated by applying the following formula.

$$\text{Translocation Factor TF} = \text{Cr concentration in shoots} / \text{Cr concentration in roots}$$

Bioaccumulation factor

Bioaccumulation refers to the total concentration of metal that has been stored or trapped within different parts of the plants, and it is measured by following formula.

$$\text{Bioaccumulation Factor BF} = \text{Cr concentration in shoots} / \text{Cr concentration in solution}$$

Statistical analysis

All determinations were performed in triplicate, and mean values are presented in the results. Statistical comparisons of the mean values were performed by analysis of variance (ANOVA), followed by Duncan's multiple range test ($p < 0.05$), using SAS 8.3 software (SAS Ins. Inc., Cary, USA). Using computer program Sigma Plot^{TM} version 10, the graphical work was carried out.

Results and discussion

Plant chromium content

The Cr concentrations in the various parts of the plant (*A. donax*) without any antibiotic treatment have been presented in Fig. 1. The range of Cr accumulated in the roots was 3–7.65 mg kg^{-1} for all the treatments. A similar trend of increasing Cr accumulation was also noted for stem and leaves. The maximum Cr accumulation was observed for stem which ranged 2.15–42.4 mg kg^{-1}; the highest accumulation took place at 100 mg L^{-1}. In leaves, the range of Cr content was observed to be 13.7–15 mg kg^{-1}. In leaves, the Cr accumulation did not show greater variations as indicated in Fig. 1. The left over Cr concentration in the solution ranged 5.4–34.9 mg L^{-1}. It was observed from the overall results that Cr uptake in *A. donax* plant was root < leaf < stem (Fig. 1).

The Cr concentrations in plants (*A. donax*) treated with antibiotic to cause inhibition of rhizobacterial growth are presented in Fig. 1. In root, the range of Cr accumulated was 1.6–3.7 mg kg^{-1}. Likewise, the Cr concentrations in stem and leaves were far below than that of those plants which were not treated with antibiotic (Fig. 1). The Cr concentration ranged 1.5–3.9 and 5.6–6.0 mg kg^{-1} in stem and leaves, respectively. However, significantly lower Cr accumulation in plants treated with antibiotic is clear indication of damage to the absorption process of the plants. The pattern of Cr accumulation in the plants with antibiotic treatment was root < stem < leaf, respectively.

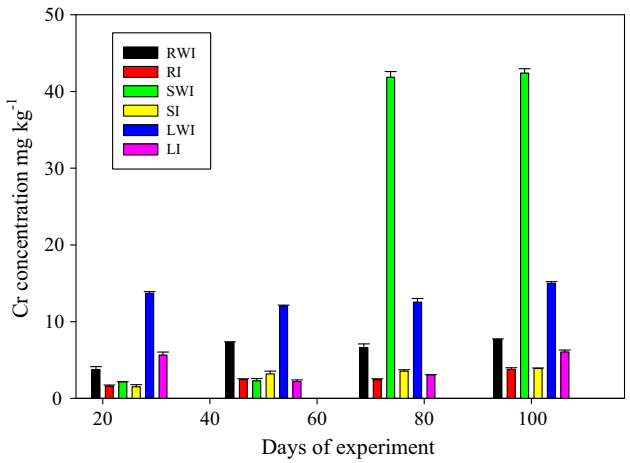

Fig. 1 Concentration of Cr in various plant organs with or without antibiotic inhibition. *RWI* root without inhibition, *RI* root under inhibition, *SWI* stem without inhibition, *SI* stem under inhibition, *LWI* leaf without inhibition, *LI* leaf under inhibition

The present study was carried out on the effects of rhizobacteria on the Cr uptake and growth characteristics of *A. donax* L plant. *A. donax* L. has been recently discovered as useful bioresource to treat metal-contaminated environments (Mirza et al. 2010a, b; Mirza et al. 2011; Kausar et al. 2012; Sabeen et al. 2013). The plants are employed to treat wastewaters or contaminated soils which may often contain various antibiotics which can inhibit the rhizobacterial populations. The rationale to conduct this experiment was to judge the ability of this spp. to survive under the presence of antibiotics which could inhibit the rhizobacterial populations. Different research works have demonstrated that the plant tolerance against heavy toxicity and heavy metals uptake could be enhanced by defining the role of rhizospheric bacteria of these hyperaccumulating plants (Whiting et al. 2001; Chen et al. 2005; Sharma et al. 2007a, b). Likewise, many studies have confirmed advantages and applications of beneficial microorganisms which reside in the rhizosphere of plants of heavy metals containing soils (Wang et al. 2009).

The results of the present investigation clearly demonstrated that the inhibition of the rhizobacterial populations had great influence on the metal uptake. Although, it could not be completely inhibited as we observed some of the metal uptake after the rhizobacterial inhibition, it was significantly less than the Cr uptake values in plants without such inhibition. Previous studies have shown that in mobilization or restriction of various heavy metals, many rhizobacteria are tolerant and therefore play important roles in phytoremediation (Gadd 1990). However, the number of rhizobacterial populations can be affected due to very high metal concentrations in the growth medium. It was previously reported that the high levels of heavy metals in contaminated soils have important impacts on population size of microorganism, community structure and overall activity of the soil microbes because population of rhizobacteria was several orders of magnitude greater than that in the bulk soil. Experiments showed that in the rhizosphere of *D. fusca*, the number of bacteria reached 1.0×10^7 CFU/g. This comparatively low bacterial count shows the presence of heavy metals in high concentrations (Abou-Shanab et al. 2003). Chaudri et al. (1992) also found that in their cadmium treatments, rhizobium populations were reduced at concentrations >7 mg/kg soil. Such a reduced number of rhizobacterial populations can decrease

the metal uptake by *A. donax* as well. However, it was interesting to note that in spite of rhizobacterial inhibition, there was some metal uptake which may be attributed to the high concentration gradient of these metals between outside and interior of the root cells. Once absorbed, these metal ions could be translocated to the aerial parts of the plants.

Xiong et al. (2008) investigated the possible role of the rhizospheric bacteria in heavy metal removal by *Sedum alfredii* Hance, from zinc-, cadmium-, copper- and lead-contaminated environment using antibiotic. The results indicated that in the uptake of nitrogen and phosphorus by *S. alfredii*, rhizospheric bacteria might have played an important role and resulted into an improved photosynthesis which ultimately increases the chlorophyll content in the leaves and plant biomass. At the same time, root elongation significantly retarded under the treatment with antibiotic, which suggested that rhizospheric bacteria protected the roots against heavy metal toxicity. The metal concentrations in the roots, stems and leaves of *S. alfredii* were much higher than those exposed to antibiotic. Thus, the rhizospheric bacteria were considered as beneficial in plant tolerance to heavy metal toxicity and also enhanced the metal removal from contaminated waste water (Xiong et al. 2008).

The current study results suggest that although rhizobacteria were inhibited, still *A. donax* could be able to absorb Cr from its hydroponic culture. There might exist some genetic control of Cr absorption in *A. donax* L. Transporter genes involved in cellular uptake of metals from their growth medium have been identified by researchers (Plaza et al. 2007; Verbruggen et al. 2009). Transport properties of plant metal transporters mediate metal entry into the cytoplasm (Kramer et al. 2007).

Bioconcentration factors

Bioconcentration factors for plants without antibiotic treatment

Figure 2 presents the bioaccumulation factors (BF) and translocation factors (TF) in the presence of microbial interaction. BF values ranged 0–3.0 at various applied Cr concentrations; the maximum value of bioaccumulation factor was 3.0 at 75 mg L^{-1}, while the highest TF value was 8.00 at 75 mg L^{-1}. The above result shows that BF and TF factors were over the reference value (1.0) for hyperaccumulation.

Bioconcentration factors with antibiotic treatment

The BF and TF values for the plants whose rhizobacterial populations were inhibited by the antibiotic application are presented in Fig. 2. BF values were below 1 for all Cr

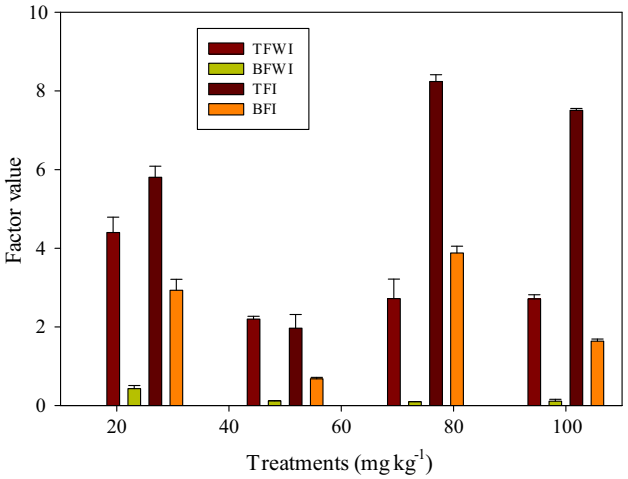

Fig. 2 Bioconcentration values for *A. donax* at various Cr treatments with or without rhizobacterial inhibition. *TFWI* translocation factor without inhibition, *BFWI* bioaccumulation factor without inhibition, *TFI* translocation factor under inhibition, *BFI* bioaccumulation factor under inhibition

treatments, and the maximum value of BF was 0.2 at 25 mg L^{-1}. The highest translocation factor value was about 4.5 at 25 mg L^{-1}. It is interesting to note that in absence of bacterial interaction, bioaccumulation decreased below 1.0 as compared to the values for the plants with active rhizobacterial populations.

Growth performance

Fresh weights of plants without antibiotic treatment

The effect of various Cr concentrations on the fresh weight of *A. donax* plants in the presence of microbial interactions are displayed in Table 2. Initially, the plants' weights were 250 ± 5 g, but fresh weights of plants after the experiment consistently decreased along increasing Cr concentrations in the growth medium. The plants of control group had the maximum values of fresh weight that was about 320 g (Table 2).

Fresh weight with antibiotic treatment

Table 2 displays the effect of Cr concentration on fresh weight of *A. donax* plants. The initial fresh weights of plants were also taken approximately 250 g for the same Cr concentrations along antibiotic treatment. The growth was significantly lower than the plants whose rhizobacterial populations were not inhibited. The plants of control group showed the maximum value of fresh weight that was about 263 g, and with an increase in concentration, decreased the value of fresh weight, respectively, and therefore, the minimum value of fresh weight that remained constant was about 255 g at 100 mg L^{-1}.

Table 2 Statistical significance of the growth performance of giant reed under various Cr treatments

	Treatment	Leaves	Tillers	Nodes	Plant height	Fresh weight	Dry weight	Root length
Without antibiotic	1	3.33a	8cd	32e	14.33bc	244.5b	62.6j	9.83c
	2	7b	4a	19.67bc	16d	242.17a	57.87h	11.95d
	3	2.66a	6b	16a	12.92ab	249.42e	56.4g	19.33h
	4	6.66b	4a	21c	15.1cd	246.58cd	52.7f	7.08b
	5	13c	10ef	15a	13.88bc	245.75bc	50.80e	8.91c
With antibiotic	6	2.66a	7bc	33.66e	14bc	247.42d	60.38i	14.33f
	7	19.66d	10ef	19bc	15.17cd	252.25f	43.36d	13e
	8	3.66a	11.33f	24.33d	12.17ab	244.92b	37.28c	34.95i
	9	6b	9.33de	21.33c	13.17ab	252.17f	34.37b	16.91g
	10	20.33d	3.33a	18.33b	15.17cd	251.92f	27.90a	2.25a

Different letters indicate significant differences between treatments ($p < 0.05$)

Dry weight

The dry weight is an important parameter which indicates the actual biomass produced during actual growth period or under stress, so it may indicate the ability of the plants to withstand stress conditions. Table 2 describes the amount of dry weight produced in *A. donax* both in the presence and absence of microbial interactions. The dry weights of plants were in the range of 50–62 g in case of plants without inhibition of rhizosphere, while it ranged 27–45 g in case of plants whose rhizobacteria were inhibited with antibiotic (Table 2).

Plant height

Plant height is another growth indicator, so plant height of both types of plants were measured and presented in Table 2. It was clear that Cr initially improved the growth of plants up to the concentration of 25 mg L^{-1} in hydroponics culture; however, then at relatively higher concentrations that was 50 and 75 mg L^{-1}, the value of the plant height slowly reduced. The plant height ranged 24 cm to 32 cm for those exposed to various Cr concentrations. As seen in the Table 2, the minimum plant height was observed at 100 mg L^{-1} Cr exposure. In case of plants with inhibited rhizosphere, the values of plants heights ranged 16 cm to 26.5 cm. The maximum value of plants height was about 26.5 cm at 25 mg L^{-1} Cr concentration.

Chlorophyll concentration

The effects of Cr on chlorophyll a (chla), chlorophyll b (chlb) and ratio of chlorophyll a to b are presented in the Fig. 3. The chla at its maximum value in control group of plants was 22 mg g^{-1} without antibiotic, but its value decreased continually with an increase in Cr concentration in medium. Thus, the minimum value of chla content was observed to be 15 mg g^{-1} in leaves at 100 mg L^{-1} Cr concentration. The maximum concentration of chlb was found in plants exposed to 25 mg L^{-1} Cr; after that, the amount of chlb gradually decreased to 4.2 at 100 mg L^{-1} Cr exposure. Likewise, the ratio of chla/chlb remained almost constant at variable Cr concentrations of 0–100 mg L^{-1}.

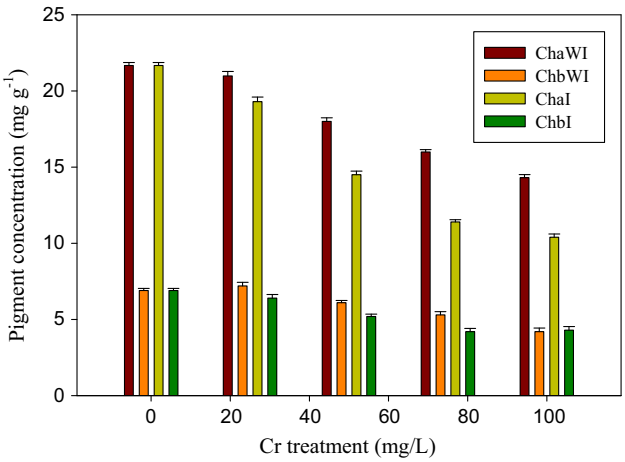

Fig. 3 Effect of Cr on pigment concentration with or without rhizobacterial inhibition. *ChaWI* chlorophyll a without inhibition, *ChbWI* chlorophyll b without inhibition, *ChaI* chlorophyll a after inhibition, *ChbI* chlorophyll b after inhibition

For the plants with inhibited rhizospheric bacteria, the maximum value of chlorophyll a in control group of plants was 22 mg g^{-1}, while the minimum value of chlorophyll a

content was observed to be 10 mg g^{-1} in leaves at 100 mg L^{-1} Cr concentration. The amount of chlb was the maximum in plants of control group and was about 7 mg g^{-1}; likewise, the minimum value of chlb content was observed to be 5 mg g^{-1} in leaves at 75 and 100 mg L^{-1} Cr concentration. The results of chl a/chl b remained almost constant at variable Cr concentrations (Fig. 3).

Toxicity symptoms

A number of toxicity symptoms appeared in the plants after getting Cr treatments without antibiotic and with antibiotic applications (Table 3). These toxicity symptoms included the appearance of stunted plants growth, yellowing of leaves, burning tips and development of malformed leaves. Plants showed normal growth in control group, but it varies with respect to the treatments at different Cr concentrations. At Cr concentration of 25 mg L^{-1}, it showed normal growth without antibiotic, but showed stunted growth with antibiotic. At 50 mg L^{-1}, plants without antibiotic displayed toxicity symptoms of stunted growth, but with antibiotic, chlorosis and burned tips appeared. At Cr concentration of 75 mg L^{-1}, plants also displayed stunted growth, and at the same concentration with antibiotic, plants displayed chlorosis and burned tips, while at 100 mg L^{-1} Cr concentration, chlorosis and burned tips have been observed both with and without antibiotic treatments.

Experimental results show that dry weights of the plants linearly decreased with increasing Cr treatment (Table 2). The fresh weights of plants after the experiment consistently decreased along increasing Cr concentrations in the growth medium. Overall results showed that the dry weights of plants were significantly less in the absence of rhizospheric microbial activity. The perfect plant for phytoextraction should be fast growing, generate a huge quantity of biomass, show tolerance and accumulate high concentrations of metals in shoots. Most frequently known heavy metal accumulators belong to the family *Brassicaceae* (Kumar et al. 2009). Since 1904, when the 'rhizosphere' term was first utilized by Hiltner (1904), many research works have been carried out on rhizosphere processes of plants; however, little attention has been paid to the microbial community of rhizospheres of plants growing on metal-contaminated sites. Soil microorganisms including free-living as well as symbiotic rhizobacteria play essential role in the rhizosphere of all higher plants. The overall results revealed that a rhizospheric microbe's interaction is possible because of higher microbial density and their metabolic activity in the rhizosphere, even in metal-contaminated soils (van der Lelie 1998). Thus, microbiological activity in plants rhizosphere stimulates the plant growth by using plant root exudates as nutrition.

It has been argued that metal solubility can also directly influence by rhizobacterial changing of heavy metal speciation in the rhizosphere. With the exception of organic-bound copper in AM (arbuscular mycorrhiza), other speciations were constant in the rhizosphere of AM and non-AM treatments. Some heavy metals like copper were activated by inducing rhizobacteria (Huang et al. 2005). Rhizosphere comprises a diverse group of free-living rhizobacteria that have the ability of mitigating toxic effects of heavy metals on the plants as well as promoting host plant growth and development in heavy metal-contaminated soils (Belimov et al. 2005). Since iron deficiency inhibits both chloroplast development and chlorophyll biosynthesis, the plants that are grown in the presence of high levels of heavy metals become chlorotic due to the low iron content of plants (Imsande 1998). However, microbial iron-siderophore serves as an iron source for plants if taken up by plants (Bar-Ness et al. 1991; Reid et al. 1986; Wang et al. 1993). It was therefore reasoned that the provision of siderophore-producing bacterium might be the best way to prevent plants from becoming chlorotic in the presence of high levels of heavy metals. It was investigated that germination and growth of *Triticum*

Table 3 Toxicity symptoms under various Cr treatments

Chromium treatment	Without antibiotic	With antibiotic
Control	Normal growth	Normal growth
25 mg L^{-1}	Normal growth	Stunted growth
50 mg L^{-1}	Stunted growth	Chlorosis and burned tips
75 mg L^{-1}	Stunted growth	Chlorosis and burned tips
100 mg L^{-1}	Chlorosis and burned tips	Chlorosis and burned tips

aestivus in the presence of potassium bichromate could be stimulated in presence Cr-resistant pseudomonads, isolated from paint industry effluents (Hasnain and Sabri 1996). In this case, the bacterial improvement of seedling growth was related with reduced Cr uptake.

The Cr concentrations accumulated in various parts of the plant were quite promising. It was observed from the overall results that Cr metal uptake in *A. donax* plant (without rhizobacterial inhibition) was root < leaf < stem. However, the amount of Cr uptake in plants with rhizobacterial inhibition was significantly less than those without such inhibition. The plants showed their ability to tolerate high concentrations of Cr when their rhizobacterial communities were intact. As shown by BF and TF of plants (without rhizobacterial inhibition), the values of these bioconcentration factors were always higher than baseline recommended value of 1, which suggested that plant is quite capable of bioremediation of Cr contamination.

Conclusion

The present study investigated the effects of antibiotic on the Cr uptake by *A. donax* plants. The plant showed decreased Cr absorption significantly less than plants without antibiotic application. In both types of plants, maximum Cr content was observed in stems. It was suggested that passive absorption of the metal in the inhibition of rhizobacteria resulting in severe growth restriction indicated by reduced fresh and dry weights, reduced amount of chlorophylls and appearance of toxicity symptoms.

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