



Short communication

Potential of *Arundo donax* to treat chromium contaminationShabana Kausar^a, Qaisar Mahmood^{a,*}, Iftikhar Ahmad Raja^a, Afsar Khan^b, Sikandar Sultan^c, Mazhar Amjad Gilani^d, Shahida Shujaat^e^a Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan^b Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan^c Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan^d Department of Chemical Engineering, COMSATS Institute of Information Technology, Defence Road, Off Raiwind Road, Lahore, Pakistan^e Department of Chemistry, Lahore College for Women University, Jail Road, Lahore, Pakistan

ARTICLE INFO

Article history:

Received 10 November 2011

Received in revised form 4 February 2012

Accepted 11 February 2012

Available online 12 March 2012

Keywords:

Bioaccumulation

Bioremediation

Chromium pollution

Cr-contaminated waters

Giant reed

ABSTRACT

The ability of *Arundo donax* against chromium pollution was tested. *A. donax* plants were grown under greenhouse conditions in nutrient solution having Cr (0, 50, 100, 200, 400, 600, and 900 $\mu\text{g L}^{-1}$) for three weeks. Shoots and roots dry matter production, growth parameters, plant physiological and morphological characters were measured. The maximum Cr content in roots was 206.33 $\mu\text{g g}^{-1}$. Likewise, Cr concentration in stem was 262.6 $\mu\text{g g}^{-1}$ at 900 $\mu\text{g L}^{-1}$ supplied Cr. In case of leaves, the maximum Cr concentration was 206.33 $\mu\text{g g}^{-1}$ at 900 $\mu\text{g L}^{-1}$ supplied Cr. The IC_{50} values of antioxidants using ABTS and DPHH standards showed that both time and increasing Cr concentrations affected the production of antioxidants indicated by lower IC_{50} value on 21st day. *A. donax* may be used in remediation of Cr contaminated wastewaters.

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1. Introduction

Heavy metals (HMs) are among the major environmental contaminants which pose a severe threat to human and animal health by their long-term persistence in the environment (Halim et al., 2003). People can be exposed to chromium through breathing, eating or drinking and through skin contact with chromium or chromium compounds. Chromium (VI) is dangerous to human health, mainly for people who work in the steel, leather and textile industry. Chromium (VI) is known to cause various health effects such as skin rashes, upset stomachs and ulcers, respiratory problems, weakened immune system, kidney and liver damage, mutations, lung cancer and even death.

Phytoremediation, is a growing field of research in environmental studies because of the advantages of its environmental friendliness, cost effectiveness, and the possibility of harvesting the plants for the extraction of absorbed contaminants that cannot be easily biodegraded for recycling among others (Malik, 2007). The use of low cost, fast growing indigenous plants with efficient biomass producing plant species such as *Arundo donax* is highly desirable for phytoremediation of metal contaminated sites and

waters. It is cultivable throughout Asia, Southern Europe, North Africa, and the Middle East for thousands of years with local names of “Nurr”, “Nurru” or “Nurro” (Susarla et al., 2002; Mirza et al., 2010, 2011; Dhir and Srivastava, 2011). It is considered as one of the most biologically productive of all communities. This research work was carried out in order to assess phytoremediation potential of *A. donax* for chromium contamination and to investigate the morphological and physiological responses of the plant to various chromium treatments.

2. Materials and methods

2.1. Plant collection

The plant material used for the present study was *A. donax* L. which was collected from near PMA road Abbottabad, Khyber Pakhtunkhwa, Pakistan. The soil-grown plants used for hydroponics tests were taken from growth of young meristematic buds grown in sterile aqueous medium (Mirza et al., 2010).

2.2. Experimental design

The experiments were laid out in randomized complete block design (RCBD) with three replications for plants grown in hydroponics. The average fresh weight of 250 g of the plant (*A. donax*)

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was taken and planted in the pots. On the average, each pot had 4 ± 1 plants with 115 ± 39 leaves, 30 ± 8 nodes and 12 ± 3 tillers. Treatments in triplicates included 0, 50, 100, 200, 400, 600 and $900 \mu\text{g L}^{-1}$ Cr given in the Hoagland's solution under controlled environment. All the chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.3. Anti-oxidant activity

To determine antioxidant activity another parallel set of experiments was conducted. In that experiment four replicates each of 250 g against each treatment were planted in a pot. Each pot was given Hoagland's solution and the respective treatment of chromium mentioned above. After each week, one set was taken out and new leaves were counted. These plants were placed outside for shade drying.

All the plants were ground to get the uniform size and dipped in methanol for 4–5 days. The plant extract was filtered and methanol was recovered by passing it through rotary evaporator. The thick left over extract was taken out in Petri dishes and placed in fume hood. The thick jelly-like extract was further analyzed. DPPH radical scavenging activity of crude extract and various fractions was estimated by standard DPPH assay protocol (Blais, 1958). Total antioxidant activity was evaluated applying an improved ABTS radical cation decolorization assay by Re et al. (1999).

2.4. Bioaccumulation factors and analytical procedures

The phytoextraction ability of *A. donax* plants was assessed using both the translocation factor (TF) and the bioaccumulation factor (BF) according to Mirza et al. (2010).

The harvested plants were separated into stems, leaves, and branches; and the fresh weight was recorded. For dry weight determination, plant material was oven-dried at 70°C for 24 h. Then weighed and ground with pestle and mortar, and passed through 0.1 mm nylon sieve. Plant samples were digested through wet digestion for metals determination using the $\text{HNO}_3/\text{HClO}_4$. The digested material was analyzed for Cr content through Perkin-Elmer Atomic Absorption Spectrometer-700. 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™ v.10 carried out graphical work.

3. Results and discussion

In roots the chromium uptake linearly increased with increasing supplied chromium concentration. It was shown that root metal contents at various supplied chromium concentrations significantly ($p < 0.05$) differed within the treatments. For stem, the maximum chromium uptake occurred at $900 \mu\text{g L}^{-1}$ which was $262.8 \mu\text{g g}^{-1}$. Accumulation of chromium in different plant parts is depicted in Fig. 1.

The stem chromium concentration at $900 \mu\text{g L}^{-1}$ was significantly ($p < 0.05$) different from rest of the treatments except for $600 \mu\text{g L}^{-1}$ where it was $147.0 \mu\text{g g}^{-1}$ (Fig. 1). For leaf chromium content, the maximum uptake occurred at $900 \mu\text{g L}^{-1}$ which was $206.33 \mu\text{g g}^{-1}$ and was significantly ($p < 0.05$) different from all other treatments. Overall, results showed that the accumulation pattern of chromium in various plant organs was as under: root > stem > leaf (Fig. 1).

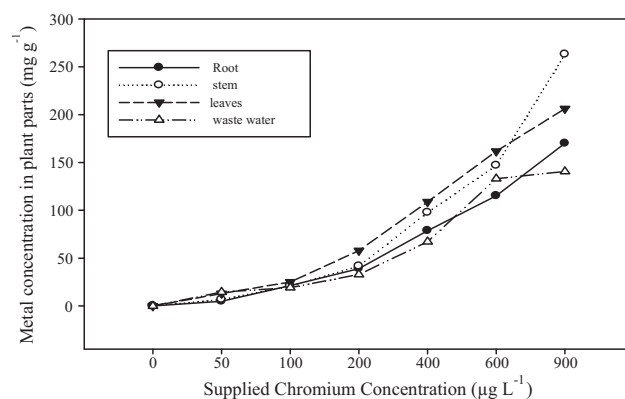


Fig. 1. The accumulation of chromium in various plant parts in hydroponics experiment.

3.1. Effects of chromium on photosynthetic pigments

The chlorophyll A content was the maximum at chromium concentrations of $200 \mu\text{g L}^{-1}$ which was significantly ($p < 0.05$) different from the maximum supplied chromium content. The maximum chlorophyll A content was observed to be 1.893 mg g^{-1} leaves at $200 \mu\text{g L}^{-1}$ supplied chromium. The amount of chlorophyll A was significantly ($p < 0.05$) different from other treatments. The chlorophyll B was depicting a different trend as compared to the amount of chlorophyll A. The chlorophyll B content was the maximum (1.187 mg g^{-1}) at $200 \mu\text{g L}^{-1}$ supplied chromium. As far as carotene content was concerned, amount was the maximum (1.56 mg g^{-1}) at $100 \mu\text{g L}^{-1}$. Further increase in the supplied chromium caused a significant ($p < 0.05$) decrease in carotene content.

3.2. Effect of chromium on bioaccumulation and translocation factors in hydroponics

Both bioaccumulation and translocation factors are presented in Fig. 2. The translocation and bioaccumulation factors increased as a function of Cr concentration in the nutrient solution. Overall, the values of BF and TF were in the range of 2.0–4.0, respectively, for various Cr treatments. The highest estimated values were TF = 4.05 and BF = 2.99 for the Cr treatment of $100 \mu\text{g L}^{-1}$. Both factors were above the reference value (1.0) for hyper-accumulation.

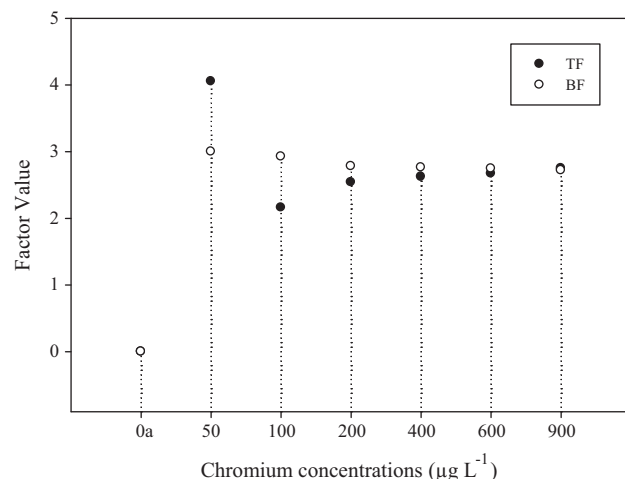


Fig. 2. Relative translocation and bioaccumulation factors at various supplied chromium concentrations.

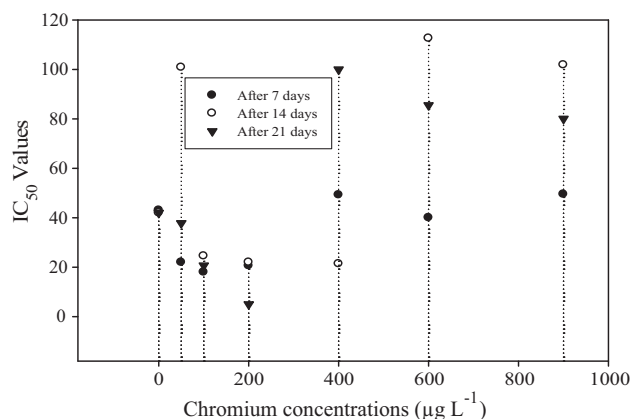


Fig. 3. The effect of chromium concentrations on the IC₅₀ values against antioxidant activity of ABTS radicals.

3.3. Antioxidant tests

The present study involved the determination of most commonly used organic radicals for the evaluation of antioxidant efficiency of complex mixtures which are the radical cation derived from 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH). These radical cations could be generated by enzymatic, chemical, and electrochemical means. IC₅₀ values were calculated based on various determinations of these antioxidants by supposing that reduced IC₅₀ values would indicate a greater oxidative stress in the plant caused by absorbed metal content (Figs. 3 and 4).

The IC₅₀ values of ABTS showed that both the time and increasing Cr concentrations strongly inhibited the production of ABTS (Fig. 3) which was indicated by lower IC₅₀ value on 21st day. A similar trend was obvious for DPPH up to 200 µg L⁻¹ supplied Cr (Fig. 4). However, a relative increase in IC₅₀ was observed at higher supplied Cr contents (>200 µg L⁻¹) showing that the concentration of these antioxidants might be greater with increasingly higher Cr exposure.

The potential of *A. donax* to serve as a phytoremediation plant is dependent on the condition that it meets the general characteristics of phytoremediation plants. Overall, the values of BF and TF were in the range of 2.0–4.0, respectively, for various Cr treatments. Both factors were above the reference value (1.0) for hyper-accumulation.

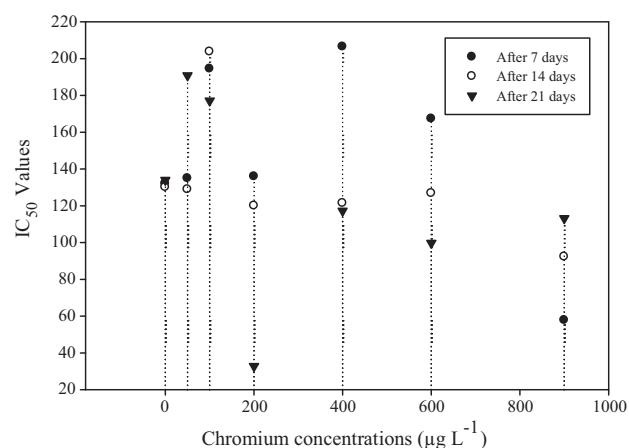


Fig. 4. The effect of chromium concentrations on the IC₅₀ values against antioxidant activity of DPPH radicals.

A plant is a hyper accumulator, if it meets the following criteria:

1. Concentrations of heavy metals in plant shoots should reach hyper accumulating level, which is different for different heavy metals e.g., it is more than 1000 mg kg⁻¹ for Pb and Cu (Baker and Walker, 1989; Brown et al., 1994), for As (Ma et al., 2001), for Ni and Co (Brooks, 1998) and for Cr (Lombi et al., 2001), and more than 10,000 mg kg⁻¹ for Zn (Brown et al., 1994).
2. Concentrations of heavy metals in shoots should be 10–500 times greater than those in normal plant (Shen and Liu, 1998).
3. HM concentration in shoots should be invariably greater than in roots (Brown et al., 1994).
4. An enrichment coefficient (the ratio of metal concentration in shoot to that in soil) should be greater than one (Brown et al., 1994).

ATPases are involved in the overall metal-ion homeostasis and tolerance in plants (Williams et al., 2000). Once the metal ions have entered the roots, they can either be stored in the root or forwarded to the shoot, primarily through the xylem. The rate of metal translocation to the shoot may depend on metal concentration in the root (Prezemeck and Haase, 1991) suggested a phytochelatin-mediated metal binding in the xylem sap as a possible mechanism for metal translocation. Low molecular weight chelators such as citrate (Lee et al., 1977) and free histidine as in *Alyssum lesbiacum* (Kramer et al., 1996) were associated with this process. Other chelating compounds like malate, citrate, and histidine may also have a role in the metal-ion-mobility in plants (Von Wiren et al., 1999).

Plant tolerance to a particular metal is governed by an inter-related network of physiological and molecular mechanisms, to be understood essentially for developing plants suitable for phytoremediation of the contaminated sites (Singh et al., 2003).

Modification or over expression of enzymes involved in the synthesis of GSH and PCs might be a good approach to enhance the HM tolerance and consequently the phytoremediation potential in plants. Glutathione is also a precursor of phytochelatins, low molecular mass peptides produced by plants to immobilize heavy metals. The protection against oxidative stress by scavenging ROS is mediated by components of enzymatic and non-enzymatic antioxidant system of ascorbate–glutathione pathway (Mahmood et al., 2010).

4. Conclusions

Root metal contents at various supplied chromium concentrations significantly differed within the treatments. For stem, the maximum chromium accumulation occurred at 900 µg L⁻¹ which was 262.8 µg g⁻¹ while the maximum accumulation in leaf was 206.33 µg L⁻¹ at same supplied Cr. In general, the amounts of these pigments increased with the increasing Cr content in the growth medium. The use of *A. donax* for the treatment of moderately Cr contaminated environments (up to 500 µg L⁻¹) may be a cost efficient biotechnology.

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