Removal and bioaccumulation of heavy metals from aqueous solutions using freshwater algae

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ABSTRACT

Four freshwater algae, including Cladophora glomerata, Oedogonium westii, Vaucheria debaryana and Zygmena insigne, were tested for their bioaccumulation capacity for cadmium (Cd), chromium (Cr) and lead (Pb) in a controlled environment with an average temperature of 18 °C, and light/dark duration of 12:12 h. Experiments were performed in aqueous solutions containing selected heavy metals (HM) (ranging from 0.05 to 1.5 mg L⁻¹) with 0.5 g of living algae at 18 °C and pH 6.8. The results indicated that C. glomerata was observed to be the most competent species for the removal of Cr, Cd and Pb from aqueous solutions. HM removal trends were in the order of Cd > Cr > Pb while the removal efficiency of selected algae species was in the order of C. glomerata, O. westii, V. debaryana and Z. insigne. The bioaccumulation capacity of C. glomerata, V. debaryana and Z. insigne was observed for different HM. Removal of HM was higher with low levels of HM in aqueous solutions. The results indicated that C. glomerata, O. westii, V. debaryana and Z. insigne had significant (P ≤ 0.01) diverse bioaccumulation capacity for Cr, Cd and Pb.

Key words | bioaccumulation, Cladophora glomerata, heavy metals, Oedogonium westii, Vaucheria debaryana, Zygmena insigne

INTRODUCTION

Industrialization and urbanization have generated huge amounts of wastewater (WW) that is frequently discharged into surrounding environments, such as the aquatic ecosystem, without prior treatment. WW contains high concentrations of toxic heavy metals (HM), which are highly persistent in nature. HM can be accumulated in the tissues of living organisms through transport and transformation and can result in long-term adverse effects on biological systems (Mehta & Gaur 2005; Singh et al. 2007; Deng et al. 2008; Lim et al. 2010; Abdel-Aty et al. 2013).

HM-like Cr is an essential element and plays an important role in biological systems, but it causes toxic effects when its bioaccumulated concentration exceeds the permissible limits. Cd and Pb are non-essential elements and are highly toxic even when present in trace amounts in environmental matrices (Krishna et al. 2009). So it is very important to overcome this problem by investigating a suitable and economically feasible technique to remove these contaminants from biological systems.

Algae are frequently available and can be easily cultivated in aquatic ecosystems. Previous research has illustrated that algae absorb nitrate, phosphorus and HM, which leads to improved water quality (Li et al. 2010).

It is well recognized that various fresh and marine water algae are capable of sequestering HM selectively from aqueous solutions and storing metals such as calcium (Ca), cobalt (Co), magnesium (Mg), selenium (Se), copper (Cu), Zinc (Zn), Cr and Pb within their cells by active biological transport (Afkar et al. 2010; Kumar & Gaur 2011; Chen et al. 2012).

Blue green algae (cyanobacteria) were listed in the top 10 natural methods of removing HM from aqueous solutions (Chojnacka et al. 2005; Patrick 2004). Freshwater filamentous green algae (Stigeoclonium sp.) have a relatively higher sorption efficiency for Zn and mostly live in mining WW (Pawluk-Skowronska 2001). Metal sorption efficiency remained high for algae biosorbents even with very low concentrations of metal in aqueous solutions (Mehta & Gaur 2005; Tastan et al. 2012; Piotrowska-Niczyporuk et al. 2012).
Algae’s biosorbent advantages can compensate for the disadvantages related to the commercial treatment of WW in which the sorption efficiency reduces at very low metal concentrations (Eccles 1999). Most of the published research is related to removal efficiency using non-living algae biomass, which showed that dead cells may sorb more metals than living cells (Mehta & Gaur 2005; Singh et al. 2012).

Previously, different algae species (living and non-living) were studied for their ability to remove HM from aqueous solutions (Ajjabi & Chouban 2009; Tuzen & Sari 2010; Abdel-Aty et al. 2013; Laib & Leghouche 2012).

In this study, benthic filamentous macro algae, such as C. glomerata, O. westii, V. debaryana and Z. insigne, were selected as testing materials due to their high adaptability, wide distribution and ease of cultivation all over the world to investigate their removal efficiency for Cd, Cr and Pb at different HM concentrations and to compare the HM bioaccumulation capacity of these algae species to determine a feasible environmentally friendly method to treat contaminated water, such as industrial WW. Furthermore, using algae, this study will help to achieve the HM target limits as set by different organizations for the discharge of WW into the aquatic ecosystem.

**MATERIALS AND METHODS**

**Sampling, identification and cultivation of algae**

*O. westii* was collected from the pond ecosystem in Islamia College University, Peshawar, Pakistan, while *C. glomerata*, *V. debaryana* and *Z. insigne* strains were isolated from local freshwater ponds, present in Peshawar City, Pakistan. The collected strains were washed thoroughly with tap water to remove all visible soil particles and then finally washed with distilled water. These algae were examined under a microscope, taxonomically identified using the published literature (Prescott 1962), which was confirmed by a taxonomist in the Department of Botany, University of Peshawar, Pakistan. These algae were then cultivated in distilled water for 3 weeks at room temperature under natural light and used for subsequent experimental work.

**Preparation of solutions**

Stock solutions of HM were prepared using Cd(NO₃)₂, Cr(NO₃)₃·12H₂O and Pb(NO₃)₂ (analytical grade) in double deionized water (DDW). The initial concentrations of Cd, Cr and Pb ranged from 0.05 to 1.5 mg L⁻¹. The culture medium was modified Gorham’s medium dilute (1:5) consisting of nutrient solutions such as NaNO₃ (496 mg L⁻¹), K₂HPO₄ (59 mg L⁻¹), MgSO₄ 7H₂O (75 mg L⁻¹), CaCl₂ 2H₂O (36 mg L⁻¹), Fe citrate (6 mg L⁻¹), Na₂SiO₃9H₂O (59 mg L⁻¹), Na₂CO₃ (20 mg L⁻¹), citric acid (6 mg L⁻¹) and EDTA (1 mg L⁻¹) in DDW. Before use, the medium was sterilized by autoclaving at 121°C for 10 minutes and the pH was adjusted to 6.8 with 1 M NaOH (Fitzgerald, 1968).

**Experimental design**

The experiment was carried out in Erlenmeyer flasks (250 mL) containing 180 mL of Gorham’s medium to which 25 mL each of Cd, Cr and Pb solutions of the desired concentrations of 0.05, 0.1, 0.5, 1.0 and 1.5 mg L⁻¹ were added (pH 6.8). To avoid HM contamination, all glassware was soaked in 10% HNO₃ for 24 h, washed with DDW and then oven dried at 80°C prior to use. Living algae such as *C. glomerata*, *O. westii*, *V. debaryana* and *Z. insigne* (0.5 g of each) were added to each flask. One set without HM was included as a control treatment. Each experiment was conducted in quadruplicate. During the incubation period of 7 days, the temperature was kept at 18°C, while light/dark duration was 12:12 h in a controlled and clean environment with continuous aeration and where 3,000 flux light intensity was continuously provided using fluorescent tube lamps.

**HM analysis**

After 7 days of incubation, algae samples were removed from each culture, washed three times with 5 mM edetic acid (EDTA) then with DDW to remove superficially bounded metals. The samples were then shade dried followed by drying in oven at 80°C for 1 h. HM were extracted from dried algae biomass using a modified method of Rybak et al. (2012). Briefly, 0.5 g of powdered algae was taken in a beaker containing 10 mL solution of HNO₃ (65%) and H₂O₂ (30%) (3:1). Then, beakers were placed on a hot plate at 110°C until the acid solution became colorless; it was then filtered through Millipore filter paper (0.40 µm) into a volumetric flask (50 mL) and the final volume was made up using DDW. HM concentrations in algae biomass were measured using an atomic absorption spectrometer (Analyst 700, Perk Elmer, Waltham, Massachusetts, USA) in the Centralized Resource Laboratory, University of Peshawar, Pakistan.

**Quality control**

For accuracy and precision, reagent blanks and standard reference materials were used in each batch. Plant reference
material (GBW10015 (GSB-6)) was obtained from the National Research Center for Standards in China. The recovery rates of these metals were satisfactory and ranged from 92.4 ± 8.2 to 103 ± 10.7%. The highest recovery was obtained for Cd, while the lowest recovery was achieved for Cr followed by Pb.

DATA ANALYSIS

Bioaccumulation measurement

Bioaccumulation capacity (q) (mg metal ions g⁻¹ active biomass or living cells) was determined using the following equation (Flouty & Estephane 2012):

\[ q = \frac{C_{\text{int}}}{m} \times V \]  \tag{1}

where \( C_{\text{int}} \) is the intercellular metal concentrations (mg L⁻¹) after bioaccumulation; \( m \) is the amount of the active biomass (g) and \( V \) is the volume of the aqueous solutions (L).

Table 1 summarizes the uptake of Cd, Cr and Pb by *O. westii*, *C. glomerata*, *V. debaryana* and *Z. insigne* grown at different concentrations (0.05–1.5 mg L⁻¹). In *O. westii*, the uptake rates of Cd, Cr and Pb ranged from 0.038 to 0.230, 0.015 to 0.261 and 0.027 to 0.261 mg L⁻¹, respectively during 1 week. The removal trend was in the order of Cr>Pb>Cd from aqueous solutions when the concentrations of selected metals were low, but overall, its efficiency decreased with increasing concentrations of metals when compared to other algae species (see Figure 1).

### Statistical analysis

The data were statistically analyzed using the statistical package SPSS 16.0, while graphs were prepared with the Sigma Plot (10.0 version), presenting the mean values using independent cultures of freshwater algae.

RESULTS AND DISCUSSION

Removal efficiency

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Similarly, *C. glomerata* has higher efficacy for taking up each HM from aqueous solutions. The metal removal trend was in the order of Cd > Cr > Pb from aqueous solutions at low concentration, demonstrating that *C. glomerata* has potential for removing Cd and Cr. However, its performance was not very good at the lowest concentration (0.05 mg L\(^{-1}\)) for removing Pb. *C. glomerata* worked better and showed more efficiency at higher concentrations for removing Pb from aqueous solutions (Figure 1).

For *V. debaryana*, the trend for metal removal was in the order of Cr > Pb > Cd from aqueous solutions when selected metal concentrations were high (see Figure 1). *Z. insigne* uptake rates were in the order of Cd > Pb > Cr from aqueous solutions at high concentration, indicating that this algae has significant \((P \leq 0.01)\) potential for the removal of Cd. However, it performed better for Pb at high concentrations; at low concentrations, its efficiency was decreased (Figure 1). The results of our study showed that bioaccumulation in the selected algae was highly dependent on the initial metal concentrations.

At low metal concentrations, these algae showed effectiveness for Cd, Cr and Pb bioaccumulation, but at high metal concentrations their bioaccumulation decreased. *Z. insigne*, *C. glomerata* and *V. debaryana* were observed as the best Cd sorbents (Figure 1). The results of this study are consistent with those reported by Tien (2002). However, Zhou et al. (1998) found that the sorption of Cd by *Sargassum kjellmanianum* and *Laminaria japonica* were not altered with the initial metal concentrations. Gupta & Rastogi (2008) studied green algae *Spirogyra* species, and mentioned that a high dose can result in high bioaccumulation capacity due to the availability of more sorption sites. These results are in agreement with the results obtained in this study. Similarly, Bajguz (2011) observed that *C. vulgaris* achieves the highest reduction in HMs during bioaccumulation at high concentration. All metal ions present in the solutions would interact with the binding sites at lower concentrations and thus facilitate maximum adsorption. More ions are left unabsorbed in the solution due to saturation of binding sites at higher concentrations (Nirmal & Oommen 2012).
Bioaccumulation capacity (q)

Table 2 summarizes the bioaccumulation capacity of C. glomerata for Cd, Cr and Pb, which ranged from 15.5 to 78.5, 13 to 173.2 and 4.3 to 86.4 mg/g, respectively. The results indicated that C. glomerata was more efficient in the bioaccumulation of Cd compared to other HMs such as Pb and Cd. C. glomerata bioaccumulation rates were 56.2, 61.2 and 42.4% for Cd, Cr and Pb, respectively, at metal concentrations of 0.1 mg L\(^{-1}\) in aqueous solutions. The result showed that C. glomerata has significant (P < 0.01) bioaccumulation capacity for HM at 0.1 mg L\(^{-1}\) concentration in aqueous solutions.

The bioaccumulation capacity of O. westii for Cd, Cr and Pb ranged from 13.9 to 82.8, 5.4 to 94 and 9.72 to 94 mg/g, respectively (Table 2). The results indicated that O. westii was more efficient in bioaccumulation of Pb when compared to other HM, such as Cd and Cr. The result showed that O. westii has significant (P < 0.01) bioaccumulation capacity for HM at 0.1 and 0.05 mg L\(^{-1}\) concentrations in aqueous solutions as compared to other levels of metals.

The bioaccumulation capacity of V. debaryana for Cd, Cr and Pb ranged from 10.1 to 69.8, 9 to 197.6 and 5.04 to 82.4 mg/g, respectively (Table 2). The results indicated that V. debaryana has the highest bioaccumulation capacity for Cr and Cd exposed to different concentrations of aqueous solutions as compared to other algae species.

The bioaccumulation capacity of Z. insigne for Cd, Cr and Pb ranged from 10.1 to 69.8, 9 to 197.6 and 5.04 to 82.4 mg/g, respectively (Table 2). These results indicated that Z. insigne was more efficient in bioaccumulation of Cr as compared to Pb and Cd at 1.5 mg L\(^{-1}\) concentration. Z. insigne showed maximum bioaccumulation capacity for Cd (30.2%) at 0.1 mg L\(^{-1}\) followed by Cr (71.5%) and Pb (42.4%) at 0.5 mg L\(^{-1}\) concentration, as compared to other algae species.

The HM used in this study are frequently present in industrial and domestic WW which lead to contamination of the aquatic ecosystems and raise several ecological and human health problems (Pribyl et al. 2008; Shen et al. 2009). Lower concentrations of HM were tested in these experiments because Cd usually occurs at very low concentrations in water (Shen et al. 2009). Pakistan EPA had established maximum allowable concentrations of 0.1, 1.0 and 0.5 mg L\(^{-1}\) for Cd, Cr and Pb, respectively, for industrial WW. These metals (Cd, Cr and Pb) or their compounds have been used in a number of industries such as plastic, cable batteries, paints, steel and glass industries. The effluent from these industries can cause contamination of aquatic environments.

The results of this study confirmed that C. glomerata, O. westii, V. debaryana and Z. insigne were highly competent in metal uptake during the incubation period (7 days). These algae species survived in the stress environment caused by the presence of toxic HM; therefore, this could be considered as a positive indication for algae to be used for phycoremediation of contaminated water.

According to the results obtained in this study, O. westii has a significant removal effect for Cd from aqueous solution, and this removal efficiency reached 47.9%. This particular species of algae showed great effectiveness at low metal concentrations in aqueous solutions; therefore, this algae can be used as an economically feasible option for metal removal from contaminated water.

In addition, high adaptability, wide distribution, ease of cultivation and harvesting are major characteristics of O. westii, which position it as a potential agent for the treatment of contaminated water.

All the freshwater algae grew well under the experimental conditions described above, with no apparent
yellow and dead parts after 7 days of culturing in the solutions containing HM. In conclusion, the absorption of HM from a water body using freshwater algae was relatively stable. It can be used to develop high capacity biosorbent materials for the removal and recovery of HM ions from WW streams.

The cell surface is the focal site of metal binding in algae (Andrade et al. 2005), and sorption of HM involves the exchange of metal ions with surface-bound cations or protons (Mehta & Gaur 2005). Our research showed that Z. insigne has the maximum bioaccumulation capability for Cr (see Figure 1) followed by C. glomerata, even when the concentration increased to a very high level in aqueous solution. Raiz et al. (2004) reported that Cd binding by Sargassum sp. involves complexation, whereas Adhiya et al. (2002) stated that Cd biosorption to Chlamydomonas sp. involves chelation with carboxylic groups. However, most of the research mainly focused on the removal efficiency using dead algae biomass. Deng et al. (2006, 2008) used non-living Cladophora sp. to adsorb Pb and Cd and it has been stated that dead cells could adsorb more metals than living cells (Mehta & Gaur 2005; Singh et al. 2007).

Our results are in agreement with those reported by Ji et al. (2012). They observed that living algae have a higher removal capability for HM than non-living biomass because dead algae may gradually fall to the bottom of the water body and result in an increase of sediment. In comparison, living algae are cheap, widely available and work better than dead algae biomass (Ji et al. 2012).

**CONCLUSIONS**

This experiment investigated the bioaccumulation capacities of C. glomerata, O. westii, V. debaryana and Z. insigne for Cd, Cr and Pb when tested as living material. The bioaccumulation capacities were influenced by the initial metal concentration in the aqueous solutions. Maximum bioaccumulation was recorded at low concentrations of selected metals in aqueous solutions. C. glomerata, V. debaryana and Z. insigne take up more Cr when the initial metal concentrations increased in the aqueous solutions. It is extremely important to note that these algae could not only survive up to a period of 7 days but also maintain their unique abilities to remove HM from aqueous solutions. The high removal efficiency of these living algae, low labor input, low transportation cost and high yields of biomass under cultivation make this treatment technology suitable for the removal of toxic HM from aqueous solutions.

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