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# Heavy Metal Resistance *Chlorella* spp., Isolated from Tannery Effluents, and Their Role in Remediation of Hexavalent Chromium in Industrial Waste Water

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Metal resistant Cyanobacteria and algae have been reported in a number of studies (Kumar 1994; Verma and Singh 1995; Sandau et al. 1996; Haq and Shakoori 1998). Multiple metal resistance of algae has also been reported (Verma and Singh 1995). The mechanism of metal resistance in algae is different from that of bacteria. The usual metal resistance and metal processing in algae involves biosorption, adsorption and bioaccumulation (Verma and Singh 1995; Sandau et al. 1996). Reduction of metals like that of hexavalent chromium (CrVI) by algae is not well documented. Reduction of CrVI is the usual mechanism of CrVI detoxification in bacteria (Ohtake et al. 1990; Yamamoto et al. 1993; Turick et al. 1996; Shakoori et al. 1999). Microorganisms reduce CrVI to CrIII. CrIII is less toxic as compared with CrVI. A large number of studies have emphasized the use of bacteria and some other microorganisms for detoxification of metal ions present in the industrial effluents and sewage wastes. The use of bacteria for environmental cleanup or bioremediation of polluted water is economical, safe and sustainable (Eccles 1995). Studies have suggested the use of a selection strategy for indigenous CrVI-reducing bacteria in a bioprocess particularly for in situ bioremediation of CrVI contaminated soils, sediments and water (Turick et al. 1996; Ohtake and Silver 1994).

The objective of this study was to isolate and characterize a metal tolerant algal strain from tannery effluents and to determine its capability to reduce CrVI. The CrVI reduction by the algal strain suggested the importance of algae in bioremediation and environmental cleanup operations.

## MATERIALS AND METHODS

Five CrVI polluted water samples were obtained from the ponds receiving tannery effluents near the city of Lahore (Pakistan). The samples were transferred to the laboratory in half filled screw capped bottles. They were acclimatized to the laboratory conditions by keeping them in the laboratory for 24 hours. The samples

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were inoculated in Bold basal liquid medium in 100 mL conical flasks (Haq and Shakoori 1998). Antibiotics, ampicillin (25μg/mL), chloramphenicol (35μg/mL) and gentamicin (25μg/mL) were used to prevent the growth of bacteria. The culture flasks were kept in daylight for 12 hours on the average at room temperature. The pH of the medium was adjusted at 7.0. The growth of alga was observed as greening of the culture. Small drops of the algal culture were observed under a compound microscope. The algal culture contained only one type of cells which were identified using the method described in "Standard Methods for the Examination of Water and Wastewater" (Anonymous 1980). The absence of bacteria was confirmed after spreading the algal culture on Luria-Bertani agar plates. No bacterial colony appeared after overnight incubation at 37°C.

Resistance of the alga to four metal ions i.e. CrVI, $Cu^{2+}$, $Pb^{2+}$ and $Cd^{2+}$ was checked by addition of the respective metal salts ($K_2Cr_2O_7$, $CuSo_4.5H_2O$, $PbNO_3$ and $CdCl_2$) in the medium. The concentrations of these metal ions were 12μg/mL, 10μg/mL, 14μg/mL and 9μg/mL, respectively. Metal ions were sterilized separately and added to the medium when the temperature of the salt medium was slightly less than 60°C. The growth of the culture was checked by counting the number of algal cells in the medium as described earlier (Haq *et al.* 1998). The growth was compared with that of the control culture which contained no metal ions added. The growth curves of the algal strain were determined by counting the cells of alga in the culture every day for 10-14 days. The growth curves were determined with and without addition of metal ions.

The CrVI reduction capability of the alga was checked by growing the algal culture in medium containing $K_2Cr_2O_7$ (10μg/mL) and by estimation of the amount of CrVI in the medium at zero day, after 7, 14, 21 and 28 days. The control experiment contained chromium, but no alga. The estimation of CrVI was done by diphenyl carbazide method (Chakrabarty and Mishra 1992; Kunicka et al. 1992).

## RESULTS AND DISCUSSION

The algal culture obtained was pure and no other microorganisms were observed. The algal cells were round, non flagellate, non motile and 3-6μm in diameter. The size of the chloroplast was 2-2.5μm in diameter. The shape of the chloroplast was crescent to horse-shoe shape. On the basis of morphological characteristics the algal isolate was identified as belonging to the genus *Chlorella.*

The growth curve pattern showed a gradual increase in the number of cells in the culture when the culture was not treated with any metal. When the culture was treated with a metal the increase in number of cells slowed down. The control culture for treatment with $Cu^{2+}$ contained $5.0x10^5$ cells per mL which increased to $11.804x10^5$ after 10 days. However, when $Cu^{2+}$ (10μg/mL) was added the number increased from $4.92x10^5$ to $7.04x10^5$ cells/mL in 10 days. The control culture for treatment with $Pb^{2+}$ contained $5.0x10^5$ cells /mL of the culture which increased to $15.76x10^5$ and when Pb2+ (14μg/mL) was added the number of cells increased from $4.28x10^5$ to $6.76x10^5$. The control culture for treatment with CrVI contained $8.04x10^5$ cells /mL of the culture which increased to $14.04x10^5$ in 12 days and when CrVI (12 μg/mL) was added the number of cells increased from $8.0x10^5$ to $11.84x10^5$ in 12 days. The control culture for treatment with $Cd^{2+}$ contained $5.72x10^5$ cells /mL of the culture which increased to $10.48x10^5$ and when $Cd^{2+}$ (9 μg/mL) was added the number of cells increased from $5.68x10^5$ to $9.08x10^5$ in 9 days. The growth curves are shown in Figure 1. The metal ions had inhibitory effect on the growth of alga, which was expressed in the form of decrease in growth rate of the culture.

The algal culture was grown in medium containing Cr at a concentration of 10μg/ mL. The reduction in the amount of Cr in the medium was 90% after 7 days, 93% after 14 days, 95% after 21 days and 97% after 28 days as shown in Figure 2.

During sewage waste treatment operations it is the usual practice to use bacteria, yeast or protozoa for sludge digestion. The operation degrades and removes the organic wastes. However the BOD of the sewage water increases during this operation as bacteria or other heterotrophic microorganisms deplete the oxygen dissolved in water. The last step of sewage waste treatment is to lower the BOD of treated water. This is achieved either by bubbling of air (oxygen) or by growth of algae in water in lagoons. The growth of algae provides oxygen, lowers BOD and renders water appropriate for reuse, aquaculturing and release in the natural water bodies. Parallel to this procedure is the industrial waste treatment as suggested by our lab (Haq and Shakoori 1998). The model proposed involves the use of algae for industrial waste treatment as the advance or final stages. It would not only process the reduced amount of toxic metals but also lower the BOD of treated water which is the final requirement of wastewater treatment.

Metal resistant algae have been reported in waste waters and metal polluted environment Wong et al 1997; Mukherjee 1998). Wong and Trevors (1988) reported toxic effect of CrVI to algae varying between 20 and 10,000 $\mu gL^{-1}$. The algal strain being reported in this paper can tolerate dichromate about 12,000 $\mu gL^{-1}$. These kinds of algae process and detoxify heavy metal ions usually through the process of bio-sorption, adsorption and bio-accumulation (Sandau et al. 1996;

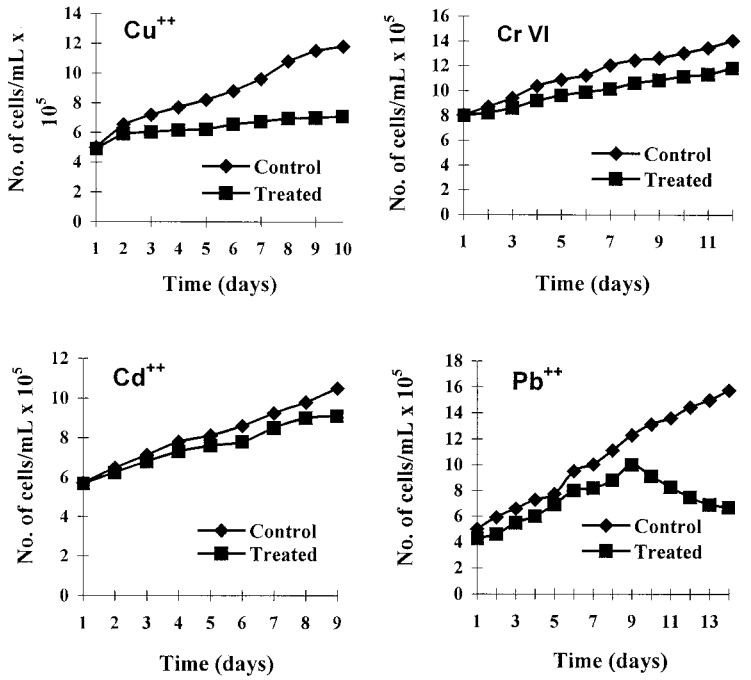


**Figure 1.** Growth curves of *Chlorella* in media containing different heavy metal ions.

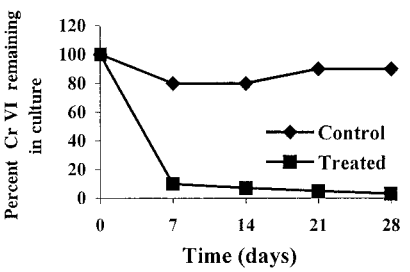


**Figure 2.** Percentage reduction of Cr VI by an algal isolate, *Chlorella,* growing in $K_2Cr_2O_7$ containing (treated) medium. The control contained Cr VI, but no algae.

Verma and Singh 1995; Kumar 1994). The reduction of chromium although well documented for bacteria has not been frequently reported for algae. In this study we have reported the isolation of *Chlorella* alga which is resistant to highly toxic dichromate ions and can reduce hexavalent chromium (CrVI). This capability of the alga can be exploited for metal detoxification operations at the advance stages when there is a need for lowering of BOD of treated water. The CrVI detoxification ability of the alga is slower as compared with that of bacteria but at lower concentrations this ability was very efficient in reducing almost 90% of CrVI in first seven days and even higher in the last 21 days.

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