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Tolerance and Uptake of Cadmium and Nickle by *Chlorella* sp., Isolated from Tannery Effluents

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Abstract: The alga, *Chlorella*, showed tolerance against Cd^{2+} (10 µg/ml), and Ni^{+2} (12 µg/ml). Cadmium and nickel processing capability of alga was worked out. The reduction in the amount of Cd^{2+}after 7, 14, 21 and 28 days of culturing was 76%, 80%, 88% and 96%, respectively. *Chlorella* could also remove 78% Ni^{+2} after 7 days, 82% after 14days, 88% after 21 days and 94% after 28 days from the medium. The resistance of alga against heavy metals present in industrial effluents indicates that the alga has acquired efficient means of resisting, tolerating or processing metal ions The heavy metal uptake ability of *Chlorella* can be exploited for metal detoxification and environmental clean-up operations.

Key words: Heavy metal resistance, bioremediation**,** *Chlorella.*

INTRODUCTION

Industrialization has led to increased emission of pollutants into ecosystems (Diagomanolin *et al*., 2004). Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for production of food crops. Farm productivity has decreased in toxic metal polluted areas (Gosavi *et al*., 2004). Accumulation of toxic metals e.g. Hg, Cu, Cd, Cr and Zn in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses (Thiele, 1995). Elevated levels of such metal ions are generally toxic and cause major damage to cell (Inouhe *et al*, 1996).

Cd^{2+} contamination in surface water comes mainly from phosphatic fertilizers used in agricultural operations, which is reflected in municipal water supplies drawing water from river sources. The major route of exposure of Cd^{2+} to humans is via the consumption of vegetables homegrown on Cd contaminated soil. It is well known that soil pH is one of the main soil properties controlling bioavailability of Cd in plants (Millis *et al*., 2004). Cd^{2+} is carcinogenic, embryotoxic, teratogenic and mutagenic and may cause hyperglycemia, reduced immunopotency and anaemia, due to its interference with iron metabolism (Sanders, 1986). The toxicity of Cd has also been well documented in other eukaryotes (Rainbow, 1995; Unger and Roesijadi, 1996).

Nickel is a problematic heavy metal (Joho *et al*., 1995). Higher concentrations of nickel are toxic. Nickel contamination may come from desorption of the metal to natural waters from the earth's crust after the global climatic change or from growing electroplating/steel industries. It can cause contact dermatitis, particularly in young women using nickel-containing ear rings. Acute inhalation exposure to nickel can cause metal fume fever and acute exposure to nickel carbonyl can cause pneumonitis. Nickel compounds are found to be nephrotoxic, hepatotoxic, immunotoxic and teratogenic (Ross, 1995).

Among microorganisms, bacteria, yeast, algae and protozoa are generally the first category to be exposed to heavy metals present in the environment (Gelmi *et al*., 1994). Microorganisms have acquired a variety and array of mechanisms to remove or detoxify toxic metal ions. The acquisition of heavy metal tolerance in algae from polluted areas, in line with acquisition of pesticide resistance in insects or herbicide resistance in weeds, is an example of

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evolution in action (Shaw, 1990). Metal resistant Cyanobacteria and multiple metal resistant algae have been reported in a number of studies (Verma and Singh, 1995; Nishikawa and Tominaga, 2001; Rehman and Shakoori, 2003; Feng and Aldrich, 2004)). Several algae have been used for elucidating mechanisms for metal accumulation (Wilde and Benemann, 1993).

An efficient waste water treatment strategy which has emerged as a result of a large number of studies on the ability of microorganisms to detoxify industrial effluents is bioremediation. Microalgae have been utilized for bioremediation against CO_2 and heavy metal removal (Takano *et al.*, 1992). Metal uptake ability of *Chlorella* has been assessed to exploit this alga for clean-up of industrial waste waters containing metal ions.

MATERIALS AND METHODS

Culturing of Chlorella

Axenic culture already isolated from tannery effluents of Kasur and maintained in the Cell and Molecular Biology Laboratory, University of the Punjab, was used in this work. The algal cells were cultured in Bold basal liquid medium in 100 ml conical flasks (Haq and Shakoori, 1998). Antibiotics, ampicillin (25 µg/ml), chloramphenicol (35 µg/ml) and gentamicin (25 µg/ml) were used to prevent the growth of bacteria. The culture flasks were kept in day light for 12 hours at room temperature (25°C). The pH of the medium was adjusted at 7. The growth of *Chlorella* was observed as greening of the culture. Small drops (10µl) of algal culture were observed under a compound microscope.

Resistance to heavy metals

Resistance of *Chlorella* to two metal ions *i.e.* Ni^{+2} and Cd^{+2} was checked by addition of the respective metal salts ($NiCl_2$ and $CdCl_2$) in the medium. The concentrations of these metal ions were 12 and 10 µg/ml, respectively. Metal ions were sterilized separately and added to the medium when the temperature of the medium was slightly less than 60°C. The growth of the culture was checked by counting number of algal cells in the medium as described earlier (Haq and Shakoori, 1998). The growth was compared with that of the control culture, which contained no metal ions added.

Determination of growth curves

The growth curves of *Chlorella* were prepared by counting the algal cells in the culture every day for 8 days. Each count was the mean of five readings. The growth curves were determined with and without addition of metal ions in distilled water.

Metal processing ability

The metal processing capability of *Chlorella* was checked by growing it in salt medium containing $NiCl_2$ (5 µg/ml) and $CdCl_2.H_2O$ (5 µg/ml) and by estimating the amount of the metal in the medium on day 0, 7, 14, 21and 28. The culture was centrifuged (500rpm, 15min) and the supernatant was used for the estimation of metals by AA1275 atomic absorption spectrophotometer (Varian, USA).

RESULTS AND DISCUSSION

Resistance and growth curves

The growth curve pattern of *Chlorella* showed a gradual increase in the number of cells in the culture without any metal, whereas the number of cells decreased when culture was treated with a metal at 8 µg/ml. The control culture contained $7.15x10^6$ cells/ml on day 1, which increased to $27.05x10^6$ cells/ml after 8 days. For treatment with Cd^{2+} culture contained $6.7x10^6$ cells/ml which increased to $10.45x10^6$ cells/ml after 8 days. However, the number increased from $7.1x10^6$ to $16.0x10^6$ cells/ml in the presence of Ni^{+2} after 8 days (Fig. 1). The metal ions slow down the growth of the organism after 5-6 days of exposure.

Cadmium and nickel processing

Chlorella could efficiently process Ni^{+2} and Cd^{+2} from the medium. The algal culture grown in the medium containing Cd^{+2} (5.0µg/ml) could reduce 76% of cadmium from the medium after 7 days, 80% after 14 days, 88% after 21 days and 96% after 28 days. It could also reduce nickel 78% after 7 days, 82% after 14 days, 88% after 21 days and 94% after 28 days from the medium containing Ni^{+2} at a concentration of 5.0 µg/ml (Fig. 2).

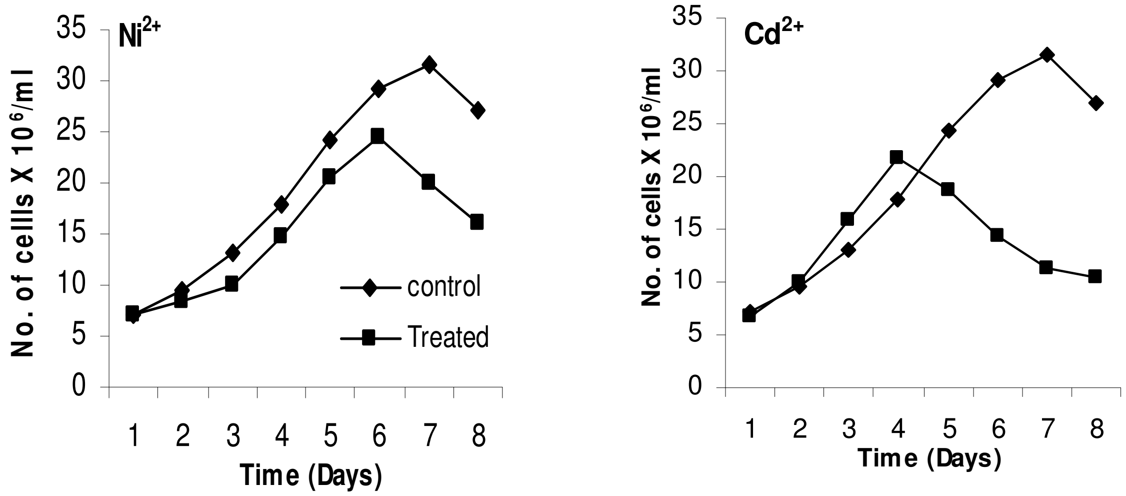

Fig.1. Growth curves of *Chlorella* in media containing Ni^{+2} and Cd^{+2} ions.

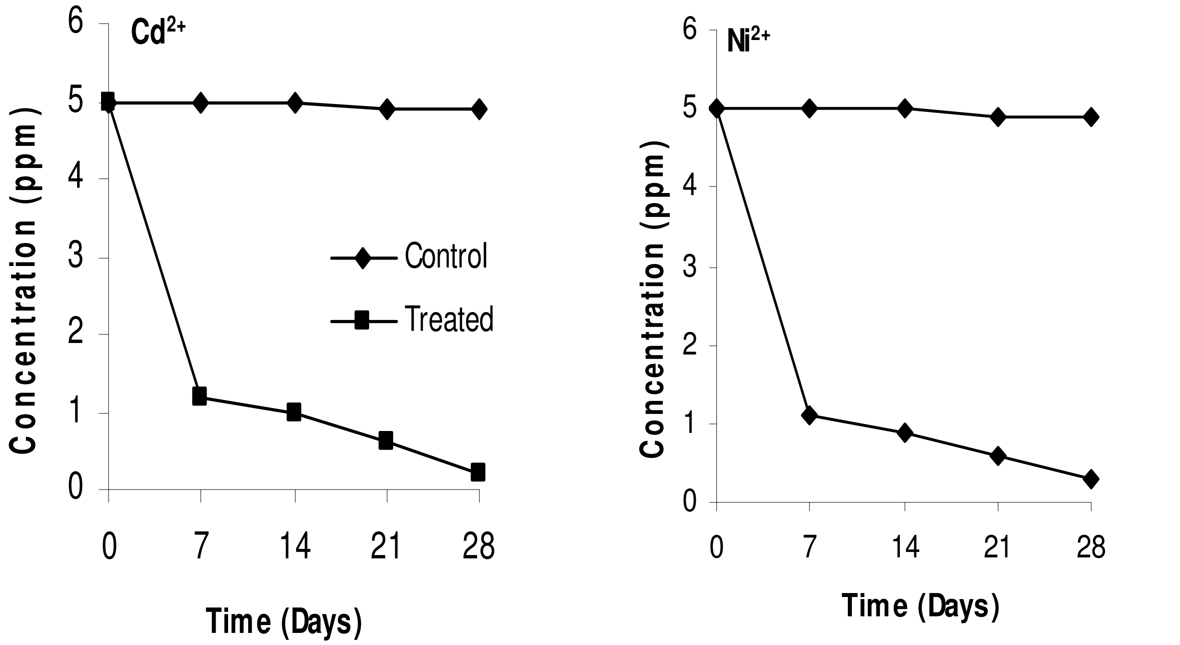

Fig. 2. Uptake of Cd^{+2} and Ni^{+2} by *Chlorella* from the medium containing heavy metals. The controls did not contain algal cells.

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. 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.

Microbiological detoxification of polluted water is economical, safe, and sustainable (Eccles, 1995). Microorganisms have been used to remove metals from polluted industrial and domestic effluents on a large scale. Algal and fungal biomasses have proven useful in biosorption studies for the removal of heavy metals from contaminated sources (Cervantes *et al*., 2001: Yan and Viraraghavan, 2003; Gosavi, *et al*., 2004). Metal resistant algae have been reported in wastewaters and metal polluted environment. These algae process and detoxify heavy metal ions usually through bio-sorption, adsorption and bio-accumulation (Gin *et al*., 2002; Boswell *et al*., 2002; Rehman and Shakoori, 2003; Davis *et al*., 2003; Chojnacka *et al*., 2004)

Reduction in heavy metal toxicity depends on the nature of the heavy metal and the organisms under stress. Metal tolerant strains of *Chlorella* secrete organic material that induces a decrease in the concentration of free metal ions in the medium (Prasad *et al*., 1998). *Chlorella,* having the potential to detoxify toxic metal ions as described in this study, can be exploited for wastewater treatment operations when there is a need for lowering of BOD of treated water.

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