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Toxic effects of chromium on the aquatic cyanobacterium *Oscillatoria* sp and removal of chromium by biosorption

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Abstract

"*Chromium*" is a non-essential metal for microorganisms and plants and a serious pollutant in diverse environment conditions. The hexavalent form of the metal Cr(IV) is considered to be more toxic than relatively harmless Cr(III) form. Certain microorganisms in the environment are able to tolerate high levels of Cr due to their resistance mechanism. In the present work we report the toxic effect of chromium on *Oscillatoria sp* and the removal of hexavalent using the cyanobacterium *Oscillatoria sp.,* by biosorption method. *Oscillatoria sp.,* was grown in BG-11 medium containing different concentration of Cr. Effect of Cr on cellular metabolism was studied by estimating the amount of chlorophyll *a*, carotenoids, c. phycocyanin, allophycocyanin, sugars, free amino acids and proteins at various metal concentration. An increase in metal concentration caused a decrease in the growth of the *Oscillatoria sp*., and also decreased the cellular contents like chlorophyll *a,* carotenoids, c.phycocyanin, allophycocyanin, sugars, free amino acids and proteins. Biosorption of chromium by *Oscillatoria sp.,* was carried out using living cells, heat killed cells and pre-treated cells. Results showed that high amount of metal were adsorbed by heat killed and living cells of *Oscillatoria sp*. The experimental conditions used in the present work are simple and have low operational cost. The proposed biosorption method is economically feasible and eco-friendly in nature.

Keywords: Cyanobacteria, *Oscillatoria*, Biosorption, Chromium, Metal toxicity

INTRODUCTION

Environmental pollution and contamination have become a key focus of concern during the recent times. Environmentally friendly processes need to be developed to clean up the environment without creating harmful waste products. Cyanobacteria are photosynthetic microorganisms and one of the largest and most important groups of prokaryotes on Earth (Saiqa Razi and Shahida Hasnain, 2006). Water contamination by heavy metals is often irreversible and in general it is tacit that the exposure to heavy metals leads to the establishment of a tolerant microbial population. Moreover, hexavalent chromium is considered to be the most hazardous form of chromium due to its solubility, mobility, and toxicity, as well as its carcinogenic and mutagenic properties. Bioavailability of Cr(VI) is largely a function of its ability to cross biological membranes, its powerful oxidizing capabilities and its interference with electron transport in respiration and photosynthesis. Heavy metals are widespread in the environment as they are non-degradable and thus persistent (Jasviri *et al.,* 2002). Microbial biomass has been used for removal of heavy metals. The use of microbial biomass is cost effective for industrial waste water treatment (Akthar *et al.,* 1995). The advantage of microbial biomass is that it can passively bind large amount of metals. This process is called as biosorption, which is possible by both living and non-living biomass. In biosorption process, the biomass is more effectively used and exploited rather than in bioaccumulation (Rani Gupta *et al.,* 2000). Biosorption which is regarded as an emergent technology is now considered an ideal alternative method for decontamination of metal containing effluents. The advantage of biosorption technique is its low operating cost and that it will minimize the volume of chemical and biological sludge to be disposed.

Recent research indicates that many microorganisms can accumulate large concentration of metals (Ramteke *et al*., 2000). Hence, the use of microbial cells for remediation of heavy metals offers a potential alternative to existing methods of decontamination of heavy metals. Algae, fungi and bacteria used for accumulation of heavy metals have been studied and its application in the biological processes in removal of heavy metals from wastewater are more effective (Kuyucak and Volesky 1989). The biomass is capable of absorbing and adsorbing metal ions from aqueous solution even when the cells have been killed (Garnham *et al*., 1992).

Cyanobacteria are also useful in removing harmful metals from the environment. *Spirulina platensis*, a cyanobacterium has been shown to contain detectable levels of mercury and lead when grown under contaminated conditions (Slotton *et al.,* 1989), implying that this cyanobacterium can take up the toxic metals from the target environment. Studies confirmed that this cyanobacterium both adsorbs and takes up metal ions (Bender *et al.,* 1994). *Oscillatoria sp.,* (filamentous) and *Synechocystis sp.,* (unicellular), were also able to tolerate up to 200µg K_2CrO_4 mL^{-1} on BG11 medium under laboratory conditions (Hasnain *et al.,* 2005). A study also indicated that carboxyl groups on cyanobacterial biomass are responsible for binding with various ions (Gardea-Torresdey *et al.,* 1990). The biosorption of Hg^{2+} under batch stirred reaction system by two cyanobacterial strains *Spirulina platensis* and *Aphanothece*

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flocculosa, showed that 98% of mercury was removed from the solution (Amber *et al*., 2008). Cyanobacterial strains play a key role in the removal of chromium toxicity from leather industries and *Spirulina fusiformis* was capable of removing 93-98 % of the metal toxicity (Pandi *et al.,* 2009). The biosorption characteristics of arsenite and monomethyl arsonate from aqueous solution by *Acidithiobacillus ferrooxidans* BY-3 were studied and the results showed that both metals involved pseudo second order kinetics (Lei yan *et al*., 2010). Recently chemically modified biomass (treating 0.1m/L HCL) of cyanobacteria *Microcystis* was used for the removal of anionic $Sb(OH)_6^-$ by biosorption method (Fuhong sun *et al*., 2011). The biological role of cyanobacterial strains in the removal of toxicity by heavy metal is rather scanty to completely understand the mechanism involved.

In the present study the biomass of the cyanobacterial strain *Oscillatoria sp*., was used to remove the heavy metal chromium toxicity at various concentrations. The biochemical results showed that increase in metal concentration cause the decrease in the growth rate of *Oscillatoria sp*., and also decreased the cellular contents.

MATERIALS AND METHODS

Organism and growth conditions

Oscillatoria sp., a filamentous cyanobacterium, was obtained from the Culture Collection Centre, Centre for Advanced study in Botany, University of Madras, Guindy campus, Chennai. Cultures were grown at 24 ± 1ºC in a thermostatically controlled room illuminated with cool white fluorescent tubes (Philips 40 W) providing an irradiance of 50 µE/m2/s in a 12h: 12h light/dark regime.

Heavy metal stock solutions

3.7g of K_2CrO_4 was dissolved in 100 mL of glass distilled water. This stock solution was used as a heavy metal stock solution. From this stock different concentration of chromium (1, 2, 3, 4, 5, 10µg/mL) were taken for the further analysis.

Effect of chromium on growth of *Oscillatoria* sp.,

The cultures were maintained and subcultured using BG_{11} medium (Rippka *et al*., 1979) with different concentration of chromium. *Oscillatoria sp*., was cultured and shaken with glass beads to get a uniform cell suspension and growth was measured by in terms of fresh weight.

Estimation of pigments

Oscillatoria sp., was grown in different concentrations of heavy metal solution (chromium). Medium without chromium served as control. At the end of growth period estimation of pigments like chlorophyll *a*, carotenoid was estimated using the method of Mackinney, 1941. Absorbance was measured using 10 mm width cuvette at 663 nm and 480 nm. Similarly c.phycocyanin (PC) and allophycocyanin (APC) were also estimated by Bennet and Bogoras, 1973.

Estimation of sugars and free amino acids

For the estimation of sugars and free amino acids 100mg of the sample was taken. The estimation of sugars and amino acids both are down by the modified method of Sadasivam and Manickam, 1996. The solution was cooled rapidly and the absorbance was read at 630 nm and 570 nm.

Estimation of protein

Proteins were estimated following the method of Lowry *et al.,* 1951. *Oscillatoria sp.,* cells were centrifuged at 10000 rpm/min^{-10}. The pellets were washed twice with Tris-HCL buffer (pH 7.5) and re-suspended in 5 mL of the buffer. Cells were disrupted in a motor and pestle for 10 minutes, then centrifuged and protein in the filtrate was precipitated with 10% Trichloroacetic acid. The precipitate was removed by centrifugation at 10000 rpm/min^{-10} and dissolved in 1 mL of 0.1 N NaOH. After 30 minutes, 5mL of solution alkaline copper reagent was added to 1mL of the sample followed by 0.5 mL of Folin phenol reagent and optical density (O.D) was read at 650 nm in a spectrophotometer after 10 minutes. Amount of protein in the sample was calculated with a standard prepared using Bovine Serum Albumin (BSA).

Biosorption of Chromium by *Oscillatoria sp.,*

Biosorption of chromium by *Oscillatoria sp* was done by three methods first one is living cells that means fresh biomass, uptake of heavy metal (chromium) in the living cells of *Oscillatoria sp.,* was monitored by amending the basal media with different concentrations (5 and 10µg/mL) of the metals and the algal culture was incubated at an irradiance of 50µE/m^2/s and 24 ± 1ºC in a 12h: 12h light/dark regime. Heat killed cells were prepared by taking fresh biomass and incubated in hot water and maintained at 100°C for 5 minutes. For pre-treated cells, the fresh biomass was suspended in 0.1 N NaOH and incubated for 20 minutes. The biomass was later suspended in solutions containing different concentrations of chromium. The biomass was left for 1hr in the respective solutions. After an hour cells were harvested and centrifuged at 10000 rpm/min^{-10}. The clear solution were collected and analyzed for metal using Atomic Absorption Spectrophotometer by the method described by Singh *et al.,* 1989.

RESULTS AND DISCUSSION

Effect of chromium on growth of *Oscillatoria* sp.,

It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alterations (Rocchetta *et al*., 2006). The result of this investigation show that the growth of *Oscillatoria* sp*.,* decreased as the concentration of metal increased (1, 2, 3, 4, 5, 10µg/mL). The metal concentration was low (1µg/mL and 2µg/mL) the growth of *Oscillatoria* sp., is not affected. As the metal concentration increased (10µg/mL) the growth was found to be affected (Table 1). Davies *et al*., (1978) stated that the metal content of organisms depends on the concentration of the ambient water as well as on bioavailability. Banerjee and Mishra, 2002 have also shown the mitigating effect of immobilization on cyanobacteria subjected to heavy metal stress. The growth of species, decreased as the concentration of metal increased. According to Daniel *et al*., (1979) cyanobacteria of the genus *Oscillatoria* were the most tolerant to different metal concentrations.

Table 1. Effect of chromium on growth of *Oscillatoria sp*.,

Concentration of chromium (µg/mL)	Growth Measurement
C	+++
1	+++
2	+++
3	++
4	++
5	+
10	+

Effect of Cr on photosynthetic pigments

The result showed that chlorophyll *a,* carotenoids, phycocyanin and allophycocyanin concentration decreased in *Oscillatoria* sp., as the metal concentration increased (1, 2, 3, 4, 5, 10 µg/mL). At increasing concentration, chlorophyll *a* gradually decreased from 1.765 to 0.517 mg/L. For cartenoids it decreased from 26.4 to 14.5 mg/L (Fig 1 and 2). Similarly for c.phycocyanin and allophycocyanin the percentage decrease over control was found to be high in 5µg/mL. At increasing concentration of metal both c.phycocyanin and allophycocyanin concentration gradually decreased (Table 1 and 2). The toxic effects of lethal concentration of Cr^{6+} on photosynthetic pigments were more pronounced in *A. nidulans* as compared to resistant strain Cr^{r}l8. Pigment content has been identified as a valuable parameter for defining toxicity of heavy metals [Bolanos *et al*., 1992, Rai *et al*., 1981, Rai *et al.,* 1991, Raizada *et al.,* 1990, Sorentinom *et al.,* 1979]. It has been demonstrated that in the presence of heavy metal ions like Zn^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+}, photosynthetic electron transport chain of cyanobacteria is inhibited [Mohanty *et al*., 1985, Murthy *et al*., 1999]. Decrease in photosynthetic pigments of *A. nidulans* in presence of Cr^{6+}, thus, this indicates that biosynthesis of these pigments is affected by metal ions. On the other hand, biosynthesis of photosynthetic pigments of the resistant strain Cr^{r}l8 is not much affected in sublethal concentration of Cr^{6+}, leading to normal levels of photosynthetic pigments and growth in presence of Cr^{6+} ions.

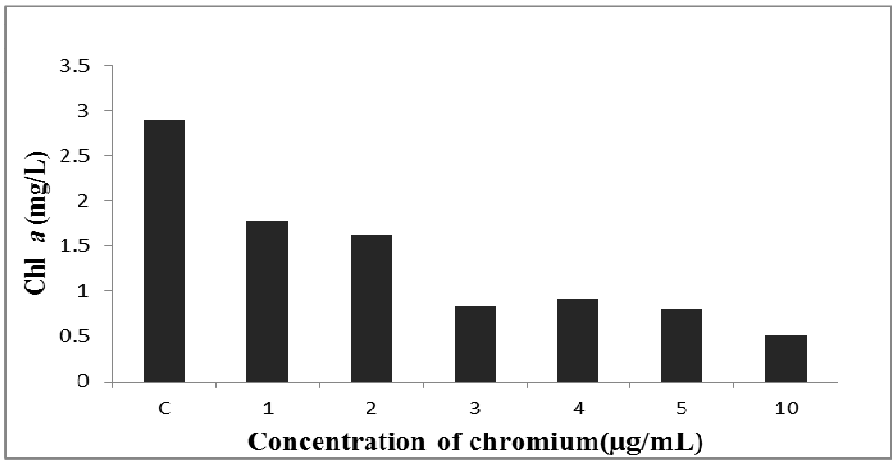

Fig1.Effect of Chromium on Chlorophyll *a* in *Oscillatoria sp*.,

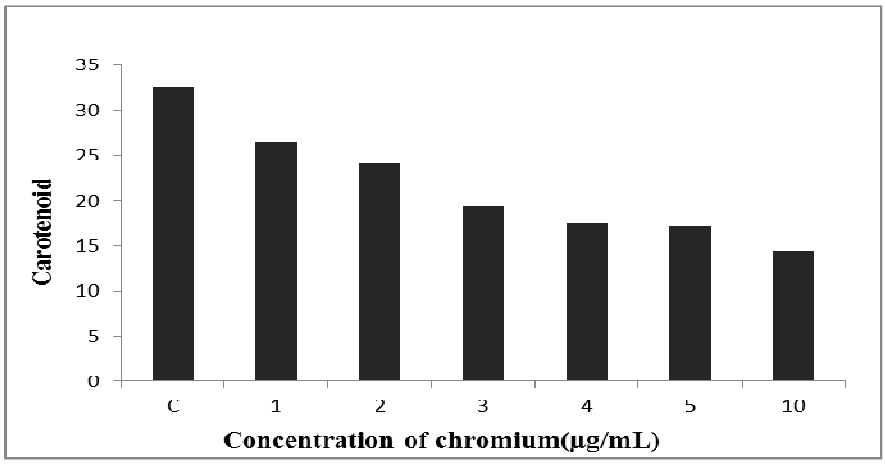

Fig 2.Effect of chromium on Carotenoids in *Oscillatoria sp*.,

Table 2. Effect of chromium on C. phycocyanin in *Oscillatoria sp.,*

Concentration of chromium (µg/ml)	C. phycocyanin(mg/mL)	% decrease over control
C	0.040	-
1	0.033	17.5
2	0.032	20
3	0.030	25
4	0.029	27.5
5	0.022	45
10	0.023	42.5

Table 3. Effect of chromium on allophycocyanin in *Oscillatoria sp.,*

Concentration of chromium (µg/mL)	Allophycocyanin (mg/mL)	% decrease over control
C	0.401	-
1	0.037	90.77
2	0.029	92.76
3	0.021	94.76
4	0.020	95.01
5	0.018	95.51
10	0.019	95.26

Effect of Cr on sugars and free amino acids

Many algae accumulate Cd, Cu, Pb, Zn and other metals which affect their general physiological and biochemical characteristics (Gupta and Singhal, 1995). The result showed that as the concentration of chromium (1-10µg/mL) increases the maximum amount of sugar and amino acids in 100 mg of cells decreased. The percentage over control was high when concentration of chromium was 10µg/mL. The percentage over control was low in 1µg/mL (Fig.3). The increased total carbohydrate accumulation following metals was accompanied by decrease Fathi *et al*. (2005) reported that the higher doses severely attenuate chlorophyll synthesis coupled with severe drop in protein resulting in increased carbohydrates. Torres *et al*. (1998) demonstrated that algae *Cylindrothica fusiformis* produce carbohydrate as a defense mechanism against copper toxicity in stationary phase when cells are exposed to 0.5 mg Cu L^{-1}.

Estimation of protein

The result showed that as the concentration of chromium increases the amount of protein in the cells decreased (5.0 – 2.0). The percentage decrease over control was high in (62.96%) when concentration of chromium was 10µg/mL. the percentage decrease over control was low in (3.70%) when the concentration of chromium was 1µg/mL (Fig 3). The accumulation of protein at low heavy metal concentrations may be one of the ways through which the algae can abolish their toxic effects, or to increase respiration leading to the utilization of carbohydrate in favor of protein accumulation (Osman *et al*., 2004). Whereas, the suppression of protein accumulation may be attributed to shortage of carbon skeleton results from low photosynthetic rate. Such results are in accordance with those of Fathi *et al*. (2000). However, some authors (Osman *et al*., 2004; Tripathi and Gaur, 2006; Barbara and Michael, 1994) reported that the toxic action of heavy metals on the enzymatic reactions responsible for protein biosynthesis.

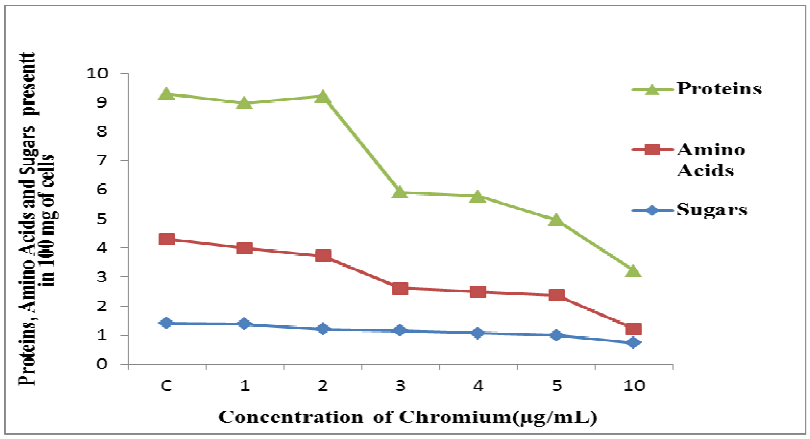

Fig 3.Effect of Chromium on Proteins, Amino acids and Sugars in *Oscillatoria* sp.,

Biosorption of Chromium by *Oscillatoria sp.,*

The results were tabulated based on living cells, Heat killed cells, Pre-treated cells. The high amount of metal was adsorbed (1.90) in heat killed cells of *Oscillatoria sp*., in 10µg/mL concentration and 5µg/mL the high amount of metal was adsorbed (0.86) in living cells (Table 4). Fisher *et al*., 1984 stated that heat killed cells accumulated as much metal as the live cells, further indicating that bioaccumulation of metal in the algae proceeded by non-metabolic adsorption also. Similarly Rabsch and Elarachker 1980 heatkilled cells of *Coscinodiscus granii* accumulated 3 times more Cd and 4 times more Zn than did in live cells. Ahuja *et al*., 1999 also reported that, in a fresh water cyanobacterium, *Oscillatoria anguistissima*, showed a very high capacity for the biosorption of zinc at 641 mg g^{-1}. The uptake of the metals was higher in immobilized condition in comparison to free cells, due to the presence of abundant hydroxyl groups in the alginate, which bind the metal ions. Similar effects were observed by Brun *et al.,* 1998; Gloaguen *et al.,* 1996; Ye *et al*., 1997.

Table 4. Biosorption of chromium by *Oscillatoria sp.,*

Treatment	Initial concentration (µg/mL)	Final concentration (µg/mL)	Metal uptake by biomass	Metal removal (%)
Living cells	5	4.14	**0.86**	17.2
	10	9.06	0.94	9.4
Heat killed cells	5	4.19	0.81	16.2
	10	8.91	**1.09**	10.9
Pre-treated cells	5	4.72	0.28	5.6
	10	9.27	0.73	7.3

CONCLUSIONS

The results of this study indicated that the biomass of *Oscillatoria* sp., was suitable for the development of efficient biosorbent for the removal of heavy metal chromium from waste water. Sequentially, cyanobacteria grown in metal polluted environments had proven the ability to tolerate the high concentration of metals ions Cu, Cd and Zn. Our results has showed that high amount of metal was adsorbed in heat killed cells of *Oscillatoria* sp., in 10µg/mL and 5µg/mL the high amount of metal was adsorbed in living cells. In fact, the pre-treated cells have shown less effect in the removal of chromium at various concentrations. An increase in metal concentration caused a decrease in the growth of the *Oscillatoria* sp., and decreased the cellular contents like chlorophyll *a,* carotenoids, C.phycocyanin, allophycocyanin, sugars, free amino acids and proteins. The ability provided a preliminary data screening the potential of microbial biomass to be used as a biosorption for the removal of metal ions from contaminated waters. This will be useful to understand the absence/presence of chromium toxicity in waste water from industrial effluents, the bioabsorption tolerance of cyanobacteria could be a useful tool for characterizing chromium and other heavy metals contamination in the environment to monitor the bioremediation progressively.

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