

## Biosorption of Cadmium by Live and Immobilized Cells of *Spirulina Platensis*

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**ABSTRACT:** *Spirulina platensis*, a cyanobacterium of economic important was studied for the tolerance to cadmium. The bioassay studies showed the EC<sub>50</sub> value to be 1.53 mg/g. The cyanobacterium was very sensitive to low metal concentration and the productivity was also reduced. The chlorophyll pigments reduced with decreasing the algal biomass. The algal survival rate showed a marked reduction in their survival rate from 98% in the control medium to >50% at 1.6 mg/L cadmium and no growth in the culture exposed to cadmium concentration of 1.9 mg/L. The Biosorption studies showed that the algae had a great potential for adsorbing the heavy metal on to the cell. A maximum uptake of 44.56 mg/g was obtained in living cells of *Spirulina platensis* suggesting the possibility of the algae to be a good biosorbent. Culturing the algae in low metal concentrations can be utilized as potential tertiary treatment for metal containing effluent. The immobilized cell of *Spirulina platensis* was able to be more effective in absorbing the metal 47.89 mg/g to the cell. The results of the study indicate the potentiality of the algae to be a possible agent for removal of heavy metals from aqueous solutions.

**Key words:** *Spirulina platensis*, Bioassay, Sub lethal, Bioaccumulation, Biosorption, Immobilized cells

### INTRODUCTION

Effluents containing metal emerged from various electroplating, tanning, pesticide and nuclear reactor and they are present as ionic species, inorganic or organic complexes or associated with collides and suspended particulate materials. These metals cannot be degraded once released into the environment and it is an increasing problem for waste water treatment. The release of heavy metals from industries into the environment has resulted in many problems for both human health and aquatic ecosystems (Inthorn *et al.*, 1996). Algae in metal containing localities tend to concentrate metal from ambient water and pass them to higher tropic level. They form the base of the food chain and their primary productivity depends upon maintaining the level of available metal ion at a concentration between toxicity and deficiency. Accumulation of trace metals in the food chain has been considered as a major environmental hazard (Rao and

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Govindarajan, 1992). Being important in primary production the study of toxic effects of metal pollutant on algae is important. Utilization of phytoplanktonic algae with a high potential to adsorb heavy metals for the removal of residual metals from waste water resulting in high quality reusable efficient water and valuable biomass that could be used for different purpose. Algal biotreatment of industrial effluents be an improved integrated high rate algal ponding system in successive stages by the cultivation of filamentous forms of algae can be utilized successfully for the heavy metal removal (Subramanian and Uma, 1996). Cyanobacteria are known to accumulate heavy metals from industrial effluents (Brower *et al.*, 1997). Cyanobacteria are characterized by high tolerance and can exist in various extreme conditions: in hot springs, in snow, in water rich in salts, etc. Simultaneously, they are very sensitive to the pollution by heavy metals (Companalla, 2000). These oxygen-evolving organisms quickly

respond and adapt to stress conditions in general (Borbely *et al.*, 1990) and heavy metals in particular (Raveender *et al.*, 2002). They have developed natural methods of resistance towards heavy metals, viz., and a reduction in metal intake by extracellular sequestration (Silver and Misra 1988), localization / compartmentalization inside the cell or energy-dependent efflux (Verma and Singh 1991). One of the vast and various alga groups are cyanobacteria and *Spirulina platensis* is one of them. A possibility to use *Spirulina platensis* dry biomass for remediation of sewage waters from cadmium is shown by Rangsayatorn (2002). The present work was taken up to investigate the toxic effect of cadmium on the productivity of *Spirulina platensis*. The metal uptake capacity of the algae during short term exposure to varying high concentration of heavy metal and the mechanism involved in the sorption of cadmium were also investigated. The toxicity and biosorption of cadmium to live and immobilized *Spirulina platensis* cultures were investigated. The performance of the algae for cadmium accumulation was studied in a laboratory flow through system using immobilized cells packed in a column where a synthetic effluent was fed.

## MATERIALS & METHODS

The LC<sub>50</sub> were determined to a range between 1mg/L and 2 mg/L of Cadmium (1, 1.3, 1.6, 1.9 mg/L). 50mL of culture medium with and without amendments was taken in 100 mL tissue culture bottle was carried out under illumination at temperature of 31.5± 1°C in a rotary shaker. The algal biomass was estimated by 10 mL of the culture was filtered using Whatman no.1 filter paper. The fresh weight of the culture was determined and the filter paper was then dried completely in hot air oven and the dry weight of the culture was determined. The values were recorded on the 1, 4, 7 and 14<sup>th</sup> day.

The algae grown in the control and amended medium for the first seven days were harvested and washed with fresh culture medium. It was then reinoculated into toxicant free medium. The algal biomass was determined after 7 days of inoculation. The survival of algae is expressed as percent decrease in total biomass of cells in 15 days to that of control. Chlorophyll 'a' and 'b' were estimated by the method of Kundson *et al.*, (1977).

The chlorophyll content was estimated by taking 10 mL of the sample and 20mL of acetone was ground well in a mortar and pastel. It was then centrifuged and the supernatant was made up to 100 mL using 80% acetone. The absorbance was read at 645 and 663 nm using a Spectronic 20D Spectrometer. The chlorophyll content was estimated using Arnon relations. 0.01484g dry weight of *Spirulina platensis* was inoculated into 50 mL of culture media containing 1ppm of cadmium and cultured at 31.5± 1°C in a rotary shaker under illumination. The algal biomass was determined on the 1, 4, 7 and 14<sup>th</sup> day of incubation. The residual cadmium concentration was determined using AAS.

Living cells of *Spirulina platensis* 0.03468-0.0436 g dry weight were inoculated into solutions of increasing concentration of Cadmium ranging from 1, 1.3, 1.6, 1.9 mg/L. The set up was incubated at 31.5 1°C under illumination in a rotary shaker. The cells were filtered using Whatman No.42 after 24 hours of incubation. Immobilization was carried out by equal volume of *Spirulina platensis* (0.03648 g) dry weight and sodium alginate (1.5%) was mixed thoroughly and the beads were produced using 5 mL syringe. Immobilized cells were packed in a column (1.8 x 30 cm) and fed with 100 mL of cadmium solution adjusted to the required pH. The experiments were performed at 31°C and the flow rate (120 mL/hr) was regulated using multichannel peristaltic pump. The residual concentration was determined using Atomic adsorption Spectrophotometer.

## RESULTS & DISCUSSION

The bioassay studies on the cyanobacterium, *Spirulina platensis* clearly has indicated that the organism to be very sensitive to very low concentrations of cadmium. Cadmium at a concentration < 2.2 mg/L was able to inhibit the growth of the organism with in an exposure period of less than 48-72 hrs. The organism died by forming clumps productivity of the algal in the media amended with varying concentration of cadmium. The productivity of the algal in the media amended with varying concentration of cadmium (1-2 mg/L) decreased the algal productivity. The presence of cadmium at a concentration of 1 mg/g reduced the algal biomass by about 15%. Increasing concentration of the metal (1.3, 1.6 and

1.9 mg/L) reduced the productivity by 32%, 45% and 63% respectively for an exposure of 14 days (Fig.1). The chlorophyll pigment reduced with the decreasing the algal biomass. The chlorophyll 'a' content reduced from 0.0553 mg/g in control to 0.0117 mg/g in the media with 1.9 mg/g of cadmium (Fig.2). The chlorophyll 'b' content showed a decline but was not a much as the chlorophyll 'a' pigment. The pigment content declined from 0.0441 mg/g to about 0.0166 mg/g of dry biomass (Fig.3). Studies on the algal survival rate showed a marked reduction in their survival rate from 98% in the control medium to >50% at 1.6 mg/L and no growth in the culture exposed to cadmium concentration of 1.9 mg/L (Table1). The cells exposed to cadmium concentration of 1.9 mg/L showed an increased lag phase and a negligible growth after incubation of seven days. Bioaccumulation studies at sub lethal concentration (1 mg/g) showed a reduction in the algal productivity by about 15%. The cell accumulation rate reduced and the residual concentration increased (Fig.4) gradually to 0.74 mg/g on the 7<sup>th</sup> day and to a saturation level of 0.92 mg/g in the medium. In the Biosorption study at 24 hrs of contact, the metal uptake of 44.56 mg/g dry weight of biomass was obtained at 1.6 mg/L and the immobilized cells of *Spirulina platensis* showed a maximum uptake of 47.89 mg/g dry weight at 1.6 mg/L (Fig.5). Treatment of industrial effluents by an integrated high rate algal ponding system by the cultivation of filamentous algae can be utilized for heavy metal removal.

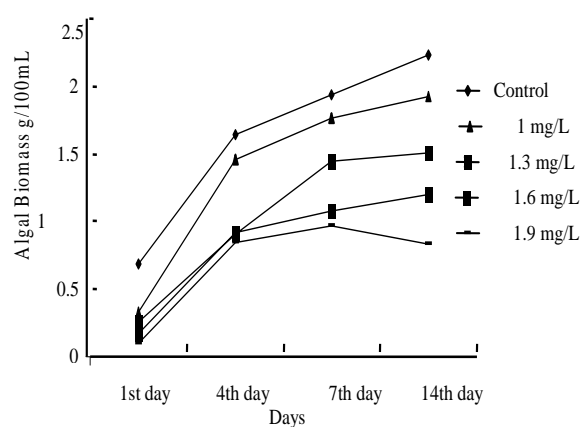


Fig. 1. Effect of Cadmium on Productivity of *Spirulina platensis*. (Values are expressed in g /100mL)

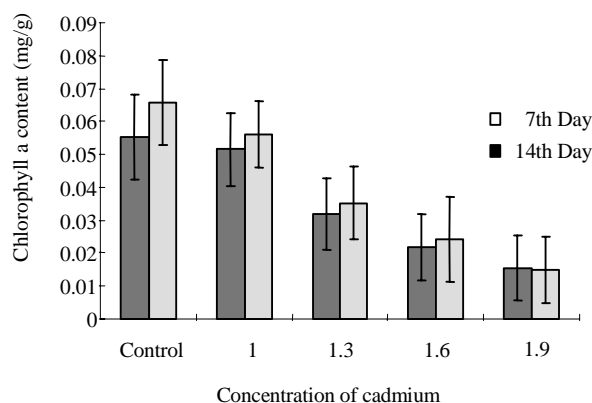


Fig.2. Effect of Cadmium on chlorophyll 'a' pigment values are expressed in mg/g of dry biomass. Error bar represents  $\pm$  S.D from the average of triplicates

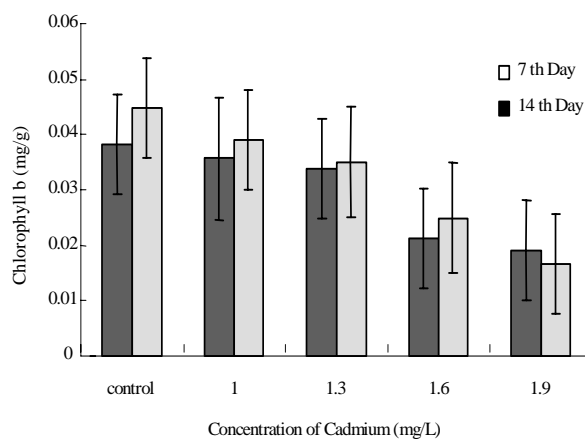


Fig.3. Effect of Cadmium on chlorophyll 'b' pigment values are expressed in mg/g of dry biomass. Error bar represents  $\pm$  S.D from the average of triplicates

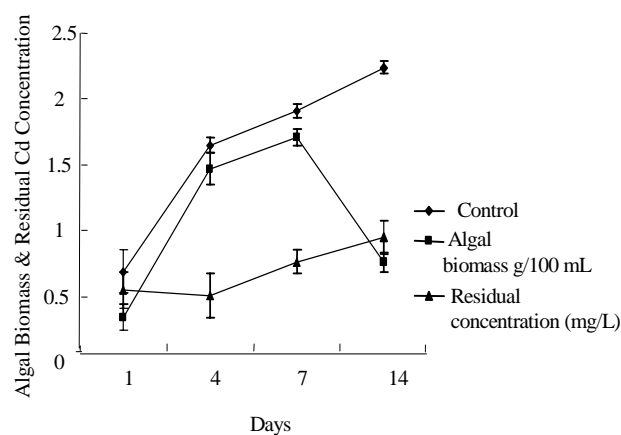
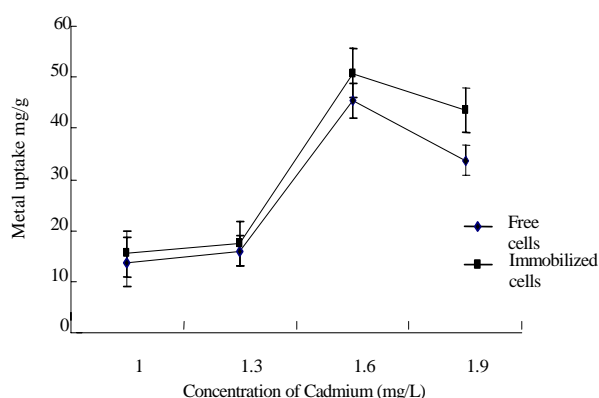


Fig. 4. Toxicity and Bioaccumulation of Cadmium at sub lethal concentration (1mg/L) in *Spirulina platensis*. Error bar represents  $\pm$  S.D from the average of triplicates



**Fig.5. Biosorption of Cadmium in *Spirulina platensis* at varying concentration by Free and Immobilized Cells. Error bar represents  $\pm$  S.D from the average of triplicates**

Utilization of cyanobacterium as a potential treatment of industrial effluents and metal recovery has been proved successful (Murugesan and Ruby, 2005; Wehrleim and Wetterm, 1994). The results obtained based on the bioassay studies the cadmium at a very low concentration of 1 mg/L has affected the algal productivity by about 15%. The effect of varying concentration of cadmium on algal productivity (1-1.9 mg/L) is represented in Fig1. The EC<sub>50</sub> concentration of cadmium required to reduce the productivity by 50% was determined to be 1.53 mg/g. Studies on metal toxicity to *Spirulina maxima* exposed to 1.2 mg/g of cadmium through out their growth phase had decreased the productivity by 30% (Augusto da Costa and de Franca, 1998). Rao and Gowrinathan (1995) reported the effects of the metal ion cadmium on the growth of marine diatoms, *Skeletonema costatum*, showed that EC<sub>50</sub> of cadmium as 1.359 mg/g. The metal ions ability to enter a chemical reaction as a positively charged ion and their capacity to bind to the enzyme prosthetic group is an important reason behind the mechanism of enzyme inhibition. Fisher and Frood (1980) while

studying the metal toxicity on algae attributed the inhibition of NADP-oxidoreductase. Inhibiting the enzyme activity increases the cellular NADPH levels resulting in a decrease in the algal growth rate. Interference with various cellular processes such as photosynthesis, metabolism of sulfate containing amino acids, uptake of NO<sub>3</sub> and NH<sub>4</sub><sup>+</sup> ions by metal ions have been reported as a reason behind the decreased algal productivity (Rao,1995). The results obtained on the effect of cadmium on chlorophyll pigment content has shown a decline with increasing concentration of the metal (Fig 2&3) indicating a possible reduction in the photosynthetic rate. Sen *et al.*, (1987) suggest that at 10 ppm Cr (VI) lowered the chlorophyll in *Pistia stratiotes* by decreasing the synthesis of chlorophyll as possible by increasing chlorophyllase activity. The lower ratio of chlorophyll 'a' and 'b' was obtained due to a greater decline in chlorophyll 'a' compared to chlorophyll 'b'. It also suggests that the metabolism was prevalent in this species (Black and Mayne, 1970). The effect of the metal ion is dependent on the varying degree of metal concentration in the cell and also the amount of ion that can transfer across the cell membrane (Sinkiss, 1979). In the present study on the survival rate of the organism after exposure to varying cadmium concentration (1-1.9 mg/L) has clearly shown a marked decline in the survival rate (Table 1). This suggests a possible inhibition of various cellular processes as well as a reduction in the multiplication rate. Gill *et al.*, (1996) studied the metal effect on the diatoms, have shown a decline in cell division rate to inhibition of nickel deposition on the cell wall. At lethal concentrations at 2.2 mg/L the *Spirulina platensis* cells died with an exposure period of 48-72 hours. The cells clumped even on continuous agitation suggesting the secretion of mucous exopolysaccharide resulting in the formation of clumps.

**Table 1. Percent decrease in the productivity and survival rate of *Spirulina platensis* on exposure to various cadmium concentrations**

Sample No.	Concentration of Cadmium (mg/L)	% decrease on the Productivity				% decrease in algal survival
		1 <sup>st</sup> Day	4 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	
1	1	52.865	10.28	8.57	12.85	21
2	1.3	64.04	40.51	25.65	31.81	38
3	1.6	75.04	44.55	45.22	45.29	57
4	1.9	86.25	45.35	50.80	62.44	--

At sub-lethal concentration the cell productivity was declined by about 15%. The organism showed a survival rate of about 78%. Studies on the accumulation of cadmium showed a drastic decline to about 0.35 mg/L during the first 48 hours of contact. There after there was an increase in the residual concentration of cadmium in the amended media (Fig.4). These results suggest a possible adoption of several mechanisms to maintain low intracellular concentration of heavy metal. The possible mechanisms is the biomethylation and transport of the metal through the cell membrane by diffusion controlled process, biosynthesis of intracellular polymer which serve as a trap for metal ion removal, precipitation of insoluble metal complexes at all cell surface (Singleton and Simmons, 1996). The cellular morphology of *Spirulina platensis* in control and the cells of the cadmium amended media did not show any marked variation.

Biosorption studies on the Cd<sup>2+</sup> uptake on a short term exposure showed its dependence on the metal concentration in the ambient medium. The metal uptake increased with an increasing up to 40 mg/L. The maximum uptake of 44.56 mg/g dry weight biomass was obtained at 40 mg/L (Fig.5). Increased concentration of cadmium did not influence the metal uptake instead showed a decline. The uptake of metal at a period of 24 hrs showed up take to be maximum during the initial period of contact at 1.6 mg/L. The reduction in the uptake may be attributed to the saturation of metal ion on the cell surface. Cadmium uptake studies in *Spirulina maxima*, have showed a similar pattern of uptake (Augusto da costa and de Franca 1998). Pandey and Singh (1993) reported the metal uptake kinetics of accumulation suggest a two fold step during uptake, rapid binding to negatively charged cell wall and metabolism dependent cation impact.

The binding of the metal ion to the cell wall compounds is the possible mechanism behind the surface adsorption of the metal ion. Capsular material or extracellular polysaccharide found in Cyanobacteria carry sulphonated (SO<sub>3</sub><sup>-</sup>) and carboxyl (COO<sup>-</sup>) charge and promote ionic and electrostatic binding of the carboxylic and uronic acid moieties with the positively charged cation (Subramanian and Uma, 1996). Studies on the

mechanism of uptake of cadmium in *Spirulina platensis* cell have clearly indicated the metal ion on the surface. Adsorption of the cadmium was about 67% on the cell surface. A cadmium uptake study in *Saccharomyces* by Volesky and May-Phillips (1995) has reported the maximum acceleration of uptake to be due to the active binding groups of the cell wall. Rangsayatorn *et al.* (2004) and Horikoshi *et al.* (1979) reported that cadmium was rapidly adsorbed by *S. platensis* during the first 5 min and by *C. regularis* within 6 min, respectively. Such rapid uptake of heavy metals by living cells is very significant when the cells are used in bioremediation process. Jayant Doke *et al.* (2004) reported that Ni was removed up to 57% and Pb up to 97% the total bacterial count was reduced up to 75% in the growth period of eight days by using *Spirulina* sp.

Immobilized cells of *Spirulina* showed a maximum uptake of 47.89 mg/g dry weight of biomass was obtained at 1.6 mg/L (Fig.5). Immobilized cells of the cyanobacteria have shown an effective and increased uptake of cadmium than the free living cells. This may be attributed to the increased biomass loading and the increased surface volume ratio of contact and a higher period of retention. Utilization of immobilized biomass in a packed bed reactor is an economically feasible technology for the removal of metal ion from aqueous solution as they can protect the biomass and the separation of the cells is more practicable. The results obtained suggest the use of the cyanobacterium in aerated lagooning process a polishing tertiary treatment to be more efficient and practicable. Vecchio *et al.* (1998) reported the similar result using immobilized algal biomass.

## CONCLUSION

*Spirulina platensis* play an important role in the environmental fate of toxic metals and metalloids with physico-chemical and biological mechanisms effecting transformations between soluble and insoluble phases. Such mechanisms are important components of natural processes being of potential application to the treatment of contaminated materials. This study led to the conclusion that *Spirulina platensis* rapid cadmium adsorption rate and made them well suited for the removal of cadmium in wastewater. In addition, living cells

of *Spirulina platensis* were found to have high tolerance to cadmium and can be regarded as an attractive adsorbate option for the biosorption of heavy metal contaminant. Thus *Spirulina platensis* biomass is suitable for fast remediation of industrial waste waters contain cadmium by the way of biosorption.

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