

Evaluation of Bio-chemical Productivity in Cyanobacterium *Spirulina platensis*-S5 under Heavy Metal Stress-Part II

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Effect of heavy metals namely cadmium, cobalt, nickel and manganese was examined on chlorophyll, carotenoid, phycobiliprotein, carbohydrate and protein contents of *Spirulina platensis*-S5 in this part of the study. All the heavy metals tested in the study showed acute toxicity over a sub-lethal concentration-gradient of 0.05–0.20 mg/L with respect to chlorophyll, carotenoid and phycobiliprotein contents, the order of toxicity being Cd > Co > Ni > Mn. Carbohydrates and proteins, however, resisted heavy metal toxicity suggesting their role in metal chelation.

Key Words : Heavy metals, Concentration gradient, Phytotoxicity, Metal chelation, *Spirulina platensis*-S5.

INTRODUCTION

The unrestricted developmental activities carried out by human beings during the past few years, to cope up with the needs of ever increasing population, have given rise to a serious problem of environmental contamination. The environmental contaminants do exert toxic effects on different types of organisms and affect biological processes at all levels of life. A general increase in the level of heavy metals, for that matter, poses a pervasive threat to the natural ecosystem.

Although many heavy metals, in traces, are essential for various metabolic processes because they form co-factors and activators of different enzymes¹, yet, at higher concentrations, they become toxic due to creation of physiological stress conditions. The metal toxicity is expectedly caused due to chemical reactivity of metals with cellular structural proteins, enzymes and membranes. The most problematic metals, for that matter, are Cr, Cd, V, Zn, As, Ni, Hg and Pb.

Pollution by heavy metals can be a much more serious problem than that caused by organic substances because they cannot be degraded by natural processes and persist in the sediments and from there are gradually released into water. These are then taken up by the primary producers of aquatic systems leading to bio-accumulation² and biomagnification with increasing trophic levels.

The toxicity of heavy metals towards eukaryotic algae has been widely investigated and reviewed^{3–7}; however, reports on heavy metal toxicity concern-

ing blue-green algae (cyanobacteria), in general, and *Spirulina*, in particular, are scanty⁸⁻¹⁰. The phytotoxic effect of commonly occurring heavy metals such as Cd, Co, Ni and Mn on the cell constituents like chlorophylls, carotenoids, phycobiliproteins, carbohydrates and proteins of commercially important blue-green alga (cyanobacterium) *Spirulina platensis* was examined on its S5 species in the present study.

EXPERIMENTAL

Spirulina platensis-S5 was procured from National Centre for Utilization and Conservation of Blue-Green Algae, IARI, New Delhi, and raised in modified Zarrouk's medium under aseptic conditions¹¹. The stock and test cultures were maintained at $30 \pm 1^\circ\text{C}$ in a BOD illuminated with 20 W fluorescent tubes providing a light intensity of 2000 ± 200 lux around the culture vessels following a 16 : 8 h light/dark regime. A pH of 9.0 was maintained for the appropriate growth of test cyanobacterium. All the glasswares were washed with 20% HCl and rinsed thoroughly with distilled water, prior to use, so as to prevent the binding of metals to the walls of the wares. For examining heavy metal toxicity on the biochemical productivity of *Spirulina platensis*-SS, cadmium as CdCl_2 , cobalt as $\text{Co}(\text{NO}_3)_2$, nickel as NiCl_2 and manganese as MnCl_2 were separately added to the fresh medium in calculated amounts to obtain final concentrations of 0.05, 0.10, 0.15 and 0.20 mg/L. The stock solutions of different test metals were prepared in double distilled water. For 'control', *Spirulina platensis*-S5 biomass was grown under identical culture conditions without adding any heavy metal. The cultures were raised in bulk and the cells harvested by filtration through sterile fine nylon cloth, washed twice with distilled water and oven dried (70°C , 24 h).

Biochemical analysis of the 11 days old harvested biomass was carried out in triplicate for determining chlorophyll, carotenoid, phycobiliprotein, carbohydrate and protein contents following the standard protocols^{12, 13}. Each experiment was repeated twice for authenticity of the results.

RESULTS AND DISCUSSION

Usually, changes in growth, photosynthesis and membrane permeability are used as parameters for examining metal toxicity. However, in the present investigation, we have observed the toxic effect of heavy metals on cell constituents rather than on cellular processes. On this accord, the biochemical analysis of *Spirulina platensis*-S5 biomass grown separately in presence of different concentrations of Cd as CdCl_2 , Co as $\text{Co}(\text{NO}_3)_2$, Ni as NiCl_2 and Mn as MnCl_2 for evaluating chlorophyll, carotenoid and phycobiliprotein contents exhibited a sharp decline (Table-1, Figs. 1-3) indicating that these metals exert a toxic effect on the light harvesting pigments. The order of metal toxicity for chlorophyll, carotenoids and phycobiliproteins in our study was found to be

$$\text{Cd} > \text{Co} > \text{Ni} > \text{Mn}.$$

TABLE-1
LEVELS OF CELLULAR CONSTITUENTS IN *SPIRULINA PLATENSIS*-S5 UNDER CONTROL AND HEAVY METAL STRESS CONDITIONS

Sample	Cellular Constituent (mg/g)				
	Chloro- phyll	Carote- noid	Phycobili- protein	Carbo- hydrate	Protein
Control(No stress)	5.18	1.55	93.50	123.50	441.00
0.05 mg/L Cd ²⁺ ion stress	4.08	0.70	28.68	137.80	441.50
0.10 mg/L Cd ²⁺ ion stress	3.59	0.69	22.90	139.20	457.50
0.15 mg/L Cd ²⁺ ion stress	2.48	0.66	20.40	182.70	474.50
0.20 mg/L Cd ²⁺ ion stress	1.84	0.63	18.05	195.70	522.50
0.05 mg/L Co ²⁺ ion stress	4.23	0.91	34.10	161.70	446.50
0.10 mg/L Co ²⁺ ion stress	4.01	0.77	30.20	174.20	459.50
0.15 mg/L Co ²⁺ ion stress	2.52	0.73	29.90	186.50	482.00
0.20 mg/L Co ²⁺ ion stress	1.87	0.65	26.30	200.00	529.00
0.05 mg/L Ni ²⁺ ion stress	4.50	1.14	54.80	166.45	454.50
0.10 mg/L Ni ²⁺ ion stress	4.28	1.04	46.20	182.50	464.50
0.15 mg/L Ni ²⁺ ion stress	3.10	1.03	47.60	197.50	493.00
0.20 mg/L Ni ²⁺ ion stress	1.91	0.83	43.40	215.00	554.50
0.05 mg/L Mn ²⁺ ion stress	4.78	1.19	55.70	170.00	466.00
0.10 mg/L Mn ²⁺ ion stress	4.41	1.12	52.50	196.40	509.00
0.15 mg/L Mn ²⁺ ion stress	3.57	1.08	43.80	198.30	535.50
0.20 mg/L Mn ²⁺ ion stress	2.43	1.02	42.80	217.30	551.50

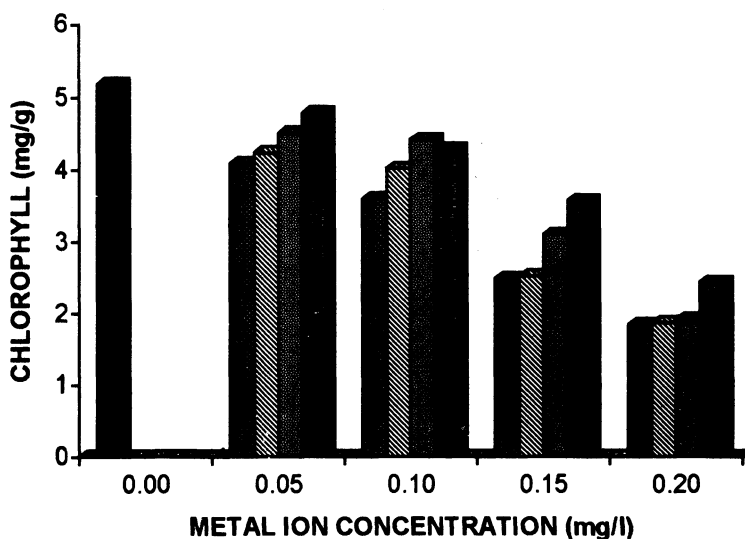


Fig. 1. Effect of heavy metal stress on chlorophyll content in *Spirulina platensis*-S5

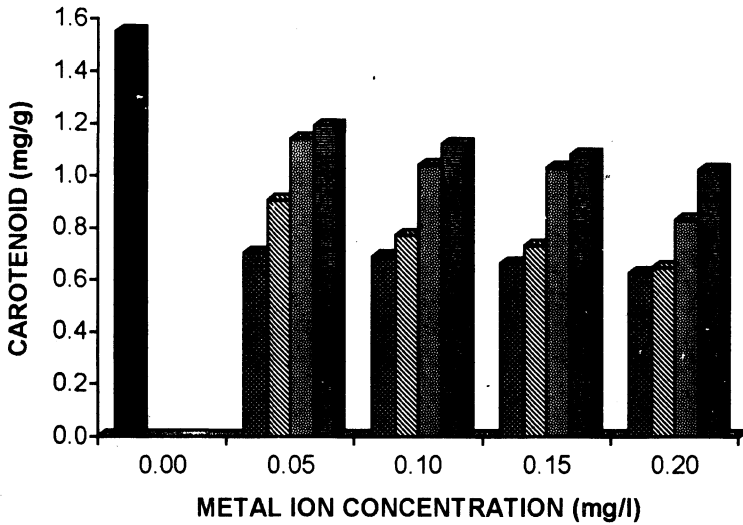


Fig. 2. Effect of heavy metal stress on carotenoid content in *Spirulina platensis*-S5

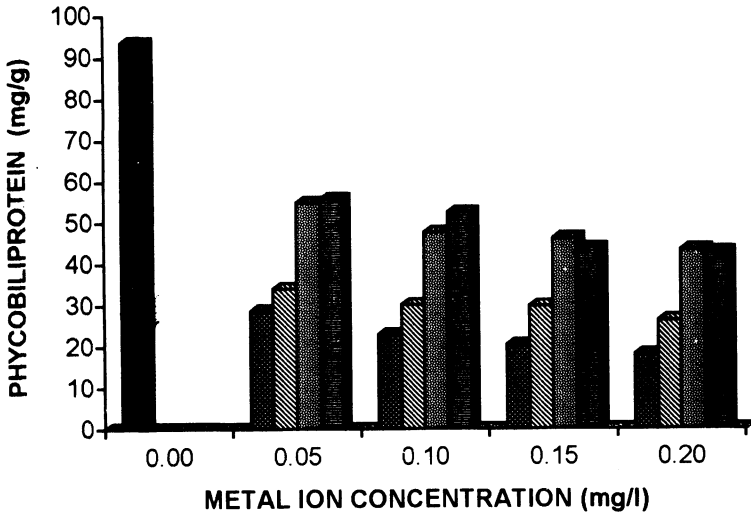


Fig. 3. Effect of heavy metal stress on phycobiliprotein content in *Spirulina platensis*-S5

In the present investigation, the per cent decrease in the amount of chlorophyll was found to be 212, 18.3, 13.13 and 7.72% (at 0.05 mg/L concentration); 30.7, 22.58, 17.37 and 14.86% (at 0.10 mg/L concentration); 52.10, 51.35, 40.15 and 31.08% (at 0.15 mg/L concentration) and 64.47, 63.89, 63.12 and 53.08% (at 0.20 mg/L concentration) for Cd^{2+} , Co^{2+} , Ni^{2+} and Mn^{2+} ions respectively, with respect to the control value of 5.18 mg/g (Table-1). This may either be due to the increased *Chlorophyllase* activity, displacement of magnesium ion or the produc-

tion of free radicals¹⁴. The decrease may as well be because of reduced synthesis or accelerated degradation of pigments¹⁵. The decline in pigment content may be due to the lysis of cell- wall and disruption of the thylakoid membrane as reported for *Anabaena flos-aquae*¹⁶. Thylakoid being the photosynthetic lamella of the cells containing most of the cellular chlorophylls and carotenoids, any reduction in their surface area on exposure to heavy metals will lead to loss of photosynthetic potential of the cells¹⁶. Reduction in the level of carotenoids in the study was found to be 54.58, 41.61, 26.45 and 23.22% (at 0.05 mg/L concentration); 55.48, 50.19, 32.90 and 27.74% (at 0.10 mg/L concentration); 57.16, 52.86, 33.55 and 30.32% (at 0.15 mg/L concentration) and 59.61, 58.19, 46.45 and 34.19 (at 0.20 mg/L concentration) for Cd²⁺, Co²⁺, Ni²⁺ and Mn²⁺ ion respectively, with respect to the control value of 1.55 mg/g (Table-1). Decrease in chlorophyll and carotenoid contents on heavy metal exposure on eukaryotic green algae and cyanobacteria has also been reported earlier^{4, 17, 18}.

Like chlorophyll and carotenoids, phycobiliproteins also showed a per cent decrease of 69.32, 63.53, 41.39 and 40.42% (at 0.05 mg/L concentration); 75.50, 67.70, 49.09 and 43.85% (at 0.10 mg/L concentration); 78.18, 68.02, 50.59 and 53.16% (at 0.15 mg/L concentration) and 80.21, 71.87, 53.58 and 54.22% (at 0.20 mg/L concentration) for Cd²⁺, Co²⁺, Ni²⁺ and Mn²⁺ ion respectively, with respect to the control value of 93.5 mg/g (Table-1). Depletion of phycobiliproteins has been reported earlier under iron, sulphur, phosphorus and nitrogen stresses with massive accumulation of glycoprotein^{19, 20}. Decrease in phycobiliprotein content has been reported earlier from this laboratory in response to copper stress in *Anacystis nidulans* and *Phormidium*²¹.

The above results also evidenced that decline in the pigment content under metal stress is a function of metal ion concentration—more is the metal ion concentration, greater is the decline in pigment content.

The behaviour of test metals towards carbohydrate and protein contents was, however, strikingly different from that observed in case of light harvesting pigments. Unlike pigments, the levels of carbohydrate and protein contents in the test blue-green alga *Spirulina platenis*-S5 showed an increase under heavy metal stress condition as compared to the respective control values (Table-1, Figs. 4 and 5). The decreasing order of (increased) carbohydrate and protein contents among stressed samples was found to be Cd > Co > Ni > Mn (*i.e.*, the maximum decrease with Cd and the minimum with Mn).

In our study, the per cent increase in carbohydrate content was found to be 11.58, 30.93, 34.78 and 37.65% (at 0.05 mg/L concentration); 12.71, 41.05, 47.78 and 59.02% (at 0.10 mg/L concentration); 47.94, 51.01, 59.92 and 60.57% (at 0.15 mg/L concentration) and 58.46, 61.94, 74.09 and 76.36% (at 0.20 mg/L concentration) for Cd²⁺, Co²⁺, Ni²⁺ and Mn²⁺ ions respectively, with respect to the control value of 123.5 mg/g (Table-1). A similar increase has been reported earlier in algae and higher plants under drought, salinity and pollution stress²². The increase in carbohydrate content may be due to accumulation of glycoprotein at the expense of phycobilisomes. The results depicting no marked decline up to the maximum concentration of 0.20 mg/L of metal ion as compared to the control

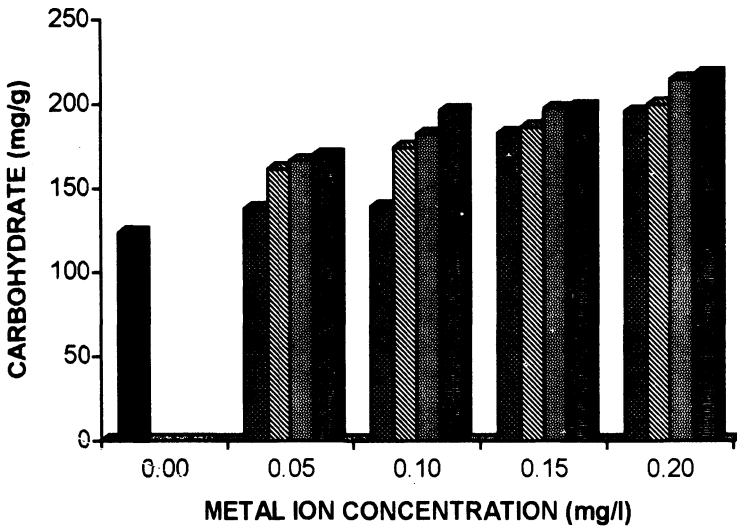


Fig. 4. Effect of heavy metal stress on carbohydrate content in *Spirulina platensis*-S5

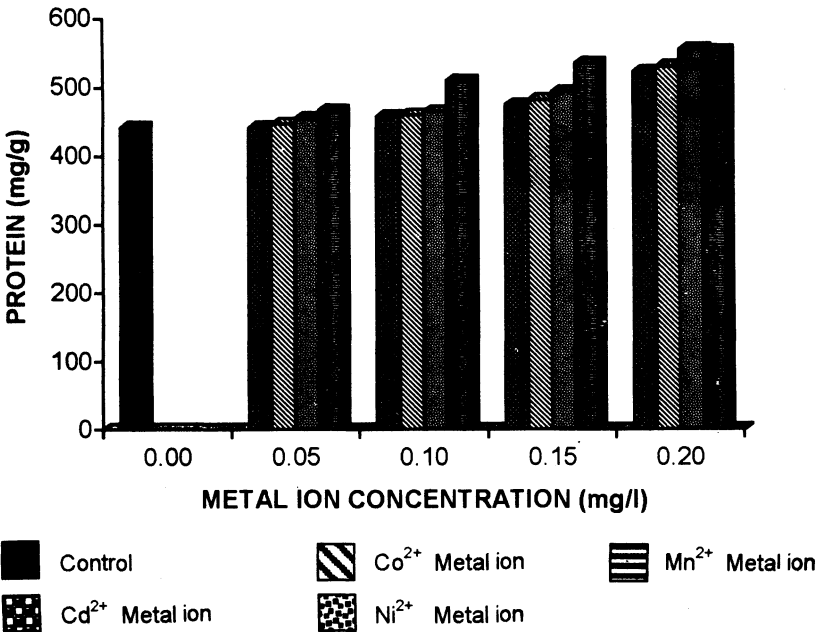


Fig. 5. Effect of heavy metal stress on protein content in *Spirulina platensis*-S5

value suggest that carbohydrates are largely skeletal components which are not prone to degradation²¹.

The per cent increase in protein content in the study was found to be 0.11, 1.25, 3.07 and 5.67% (at 0.05 mg/L concentration); 3.74, 4.20, 5.33 and 15.42% (at 0.10

mg/L concentration); 5.33, 9.29, 11.79 and 21.43% (at 0.15 mg/L concentration) and 18.48, 19.95, 25.74 and 25.05% (at 0.20 mg/L concentration) for Cd²⁺, Co²⁺, Ni²⁺ and Mn²⁺, respectively, with respect to the control value of 441 mg/g (Table-1). Considering the protein molecules as primary metal binding sites of the cells of the organism, the possible increase in the protein content might be due to the sequestering of heavy metals and hence reduced metal toxicity. This may also be due to the synthesis of metallothionein²³ or metallothionein/phytochelatin like proteins²⁴ or other binding peptides which temporarily sequester heavy metals and lower free metal ion concentration in the cells and, thus, reduce their toxicity

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