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Evaluation of Bio-Chemical Productivity in Cyanobacterium *Spirulina platensis*-S5 under Heavy Metal Stress

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Effect of heavy metals, namely, lead, chromium, copper and zinc was studied on chlorophyll, carotenoid, phycobiliprotein, carbohydrate and protein contents of *Spirulina platensis*. All the heavy metals tested 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 Cr > Pb > Cu > Zn. Carbohydrates and proteins, however, posed resistance to heavy metal toxicity suggesting their role in metal chelation.

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

INTRODUCTION

The improvement of basic standard of living of the world's population demands sustainable development without unnecessary depletion of the finite natural resources and further degradation of the environment in which we dwell. However, rapid industrialization, urbanization, mining activities and use of modern agricultural practices in the recent past, to cope up with the needs of increased population, have accelerated the pace of environmental degradation besides improving the quality of life of the people. The unrestricted developmental activities carried out by human beings during the past few years have given rise to a serious problem of environmental contamination. The environmental contaminants do exert toxic effect 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.

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 system leading to bio-accumulation and bio-magnification with increasing trophic levels.

Although many heavy metals, in traces, are essential for various metabolic processes because they form co-factors and activators of different enzymes yet at higher concentrations, they become toxic due to creation of physiological stress conditions[1].

The metal toxicity is 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.

The toxicity of heavy metals towards eukaryotic algae has been widely investigated and reviewed[2–6]; however, information on heavy metal toxicity concerning blue-green algae in general, and *Spirulina* in particular, is scanty[7, 8]. The phytotoxic effect of commonly occurring heavy metals such as Pb, Cr, Cu and Zn on the cell constituents like chlorophylls, carotenoids, phycobiliproteins, carbohydrates and proteins of the commercially important blue-green alga *Spirulina platensis* was studied on its S5 species in the present investigation.

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[9]. The stock and test cultures were maintained at 30 ± 1°C in a BOD illuminated with 20 W fluorescent tubes providing a light intensity of 2000 ± 200 lux around the culture vessels for 16 : 8 h light/dark regime. The glass wares 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 evaluating heavy metal toxicity, lead as $Pb(NO_3)_2$, chromium as K_2CrO_4, copper as $CuSO_4$ and zinc as $ZnSO_4$ 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 bio-mass was carried out in triplicate for chlorophyll, carotenoid, phycobiliprotein, carbohydrate and protein contents following the standard protocols and repeating the experiments twice.

RESULTS AND DISCUSSION

Usually, changes in growth, photosynthesis and membrane permeability are used as parameters for toxicity measurement. However, in the present study, 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 Pb as $Pb(NO_3)_2$, Cr as K_2CrO_4, Cu as $CuSO_4$ and Zn as $ZnSO_4$ for evaluating chlorophyll, carotenoid and phycobiliprotein contents exhibited a sharp decline (Table-1) indicating that these metals exert toxic effect on the light harvesting pigments. The order of metal toxicity for chlorophyll, carotenoids and phycobiliproteins in our study was found to be Cr > Pb > Cu > Zn.

Decrease in chlorophyll and carotenoid contents on heavy metal exposure on eukaryotic green algae and cyanobacteria was also observed earlier[4, 11, 12]. In the present investigation, per cent decrease in the amount of chlorophyll was found to be 33.7, 35.8, 10.6 and 3.9 (at 0.05 mg/L conc.); 39.5, 41.8, 15.3 and 13.0 (at

0.10 mg/L conc.); 58.1, 59.3, 30.6 and 17.4 (at 0.15 mg/L conc.) and 63.2, 66.5, 33.7 and 24.1 (at 0.20 mg/L conc.) for Pb^{2+}, CrO_4^{2-}, Cu^{2+} and Zn^{2+} ions respectively, with respect to the control value of 8.6 mg/g (Table-1). This may either be due to the increased chlorophyllase activity, displacement of magnesium ion or the production of free radicals[13]. The decrease may as well be either because of reduced synthesis or accelerated degradation of pigments[14]. 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*[15]. Since thylakoid is 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 cells[15]. The per cent reduction in the level of carotenoids was found to be 9.4, 43.9, 6.7 and 6.0 (at 0.05 mg/L conc.); 37.5, 49.3, 30.4 and 13.5 (at 0.10 mg/L conc.) ; 54.7, 65.5, 44.5 and 22.9 (at 0.15 mg/L conc.) and 62.1, 70.9, 47.2 and 33.7 (at 0.20 mg/L conc.) for Pb^{2+}, CrO_4^{2-}, Cu^{2+} and Zn^{2+} ion respectively, with respect to the control value of 1.48 mg/g (Table-1). Like chlorophyll and carotenoids, phycobiliproteins also showed a per cent decrease of 38.56, 44.61, 17.11 and 14.09 (at 0.05 mg/L conc.); 46.32, 50.65, 26.08 and 18.52 (at 0.10 mg/L conc.); 62.22, 64.75, 60.02 and 55.48 (at 0.15 mg/L conc.) and 62.94, 71.80, 61.53 and 60.02 (at 0.20 mg/L conc.) for Pb^{2+}, CrO_4^{2-}, Cu^{2+} and Zn^{2+} ion respectively, with respect to the control value of 99.30 mg/g (Table-1). These results also reveal that more is the metal ion concentration, greater is the decline in pigment content. Depletion of phycobiliproteins has been reported earlier under iron, sulfur, phosphorus and nitrogen stresses with massive accumulation of glycoprotein[16, 17]. Decrease in phycobiliprotein content has been reported earlier from this laboratory in response to copper stress in *Anacystis nidulans* and *Phormidium*[18].

However, the behaviour of test metals towards carbohydrate and protein contents was strikingly different from that observed in case of light harvesting pigments. The decreasing order of (increased) carbohydrate and protein contents among stressed samples was found to be Cr > Pb > Cu > Zn (*i.e.*, the maximum decrease with Cr and the minimum with Zn).

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 value (Table-1). The per cent increase in carbohydrate content was found to be 7.8, 5.8, 10.7 and 15.0 (at 0.05 mg/L conc.); 27.7, 9.0, 30.7 and 38.5 (at 0.10 mg/L conc.); 39.7, 35.4, 46.8 and 52.5 (at 0.15 mg/L conc.) and 47.8, 37.6, 53.5 and 58.3 (at 0.20 mg/L conc.) for Pb^{2+}, CrO_4^{2-}, Cu^{2+} and Zn^{2+} metal ions respectively, with respect to the control value of 126.5 mg/g (Table-1). A similar increase has been reported earlier in algae and higher plants under drought, salinity and pollution stress[19, 20].

The increase in carbohydrate content may be due to accumulation of glycoporotein at the expense of phcobilisomes. The results depicting no marked decline up to the maximum concentration of 0.20 mg/L of metal ion as compared to the control value suggest that carbohydrates are largely skeletal components which are not prone to degradation[18]

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

Sample	Cellular constituents (mg/g)				
	Chloro-phyll	Carote-noid	Phycobili-protein	Carbo-hydrate	Protein
Control	8.60	1.48	99.30	126.50	450.00
0.05 mg/L Pb^{2+} ion stress	5.70	1.34	61.00	136.37	472.00
0.10 mg/L Pb^{2+} ion stress	5.20	0.93	53.30	161.62	488.00
0.15 mg/L Pb^{2+} ion stress	3.60	0.67	38.50	176.75	530.00
0.20 mg/L Pb^{2+} ion stress	3.16	0.56	36.80	187.00	549.00
0.05 mg/L CrO_4^{2-} ion stress	5.52	0.80	55.30	131.37	453.00
0.10 mg/L CrO_4^{2-} ion stress	5.00	0.75	49.00	138.00	463.00
0.15 mg/L CrO_4^{2-} ion stress	3.50	0.51	35.00	171.37	527.00
0.20 mg/L CrO_4^{2-} ion stress	2.88	0.43	28.00	179.12	546.00
0.05 mg/L Cu^{2+} ion stress	7.68	1.38	82.30	140.12	510.00
0.10 mg/L Cu^{2+} ion stress	7.28	1.03	73.40	165.37	570.00
0.15 mg/L Cu^{2+} ion stress	5.96	0.82	39.70	185.75	601.00
0.20 mg/L Cu^{2+} ion stress	5.70	0.78	38.20	194.25	617.00
0.05 mg/L Zn^{2+} ion stress	8.26	1.39	85.30	145.60	524.00
0.10 mg/L Zn^{2+} ion stress	7.48	1.28	80.90	175.30	549.00
0.15 mg/L Zn^{2+} ion stress	7.10	1.14	44.20	193.00	588.00
0.20 mg/L Zn^{2+} ion stress	6.52	0.98	39.70	200.30	624.00

The per cent increase in protein content was found to be 4.8, 0.66, 13.3 and 16.9 (at 0.05 mg/L conc.); 8.4, 2.8, 26.6 and 22.0 (at 0.10 mg/L conc.); 17.7, 17.1, 33.5 and 30.6 (at 0.15 mg/L conc.) and 22.0, 21.3, 37.1 and 38.6 (at 0.20 mg/L conc.) for Pb^{2+}, CrO_4^{2-}, Cu^{2+} and Zn^{2+} respectively, with respect to the control value of 450 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 reducing metal toxicity. This may also be due to the synthesis of metallothionein[21] or metallothionein/phytochelatin like proteins[22] 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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