Phytotoxicity of Heavy Metals on Spirulina Platensis

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ABSTRACT:

Effect of heavy metals namely cadmium, nickel, chromium and lead was studied on chlorophyll, carotenoids, phycobiliproteins, carbohydrates and lipids of *Spirulina platensis*. All the heavy metals tested showed acute toxicity at sub-lethal concentration (15 ppm) with respect to chlorophyll, carotenoids and phycobiliproteins. The order of toxicity being Cr>Ni>Cd>Pb. Protein, carbohydrates and lipids imparted resistance to heavy metal toxicity indicating their role in metal chelation.

Key Words: Heavy metals, pigments, phytotoxicity, *Spirulina platensis*.

INTRODUCTION:

Contamination of the environment with heavy metals is widespread as a consequence of rapid industrialization, urbanization, mining activities and modern agricultural practices. A general increase in the level of these heavy metals threatens the existence of terrestrial and aquatic organism. Pollution by heavy metals can be a much more serious problem than by organic substances because they cannot be degraded by natural process and persist in sediments from where they are released gradually into water. These heavy metals are taken up by the primary producers of aquatic systems leading to biomagnifications along the increasing trophic levels.

Many heavy metals in traces are essential for various metabolic processes because they from cofactors and activators of different enzymes (Van Assche and clijsters, 1990) but at higher concentrations they become toxic due to creation of physiological stress conditions. The toxicity of heavy metals towards eukaryotic algae has been widely investigated and reviewed (stokes, 1983; whitton, 1984; Rai and aggarwal, 1995; Say and Whitton, 1997; Gipps and Biro, 1978; Rai and Kumar, 1980; Rai *et al.*, 1981a; Rai *et al*., 1998; Venkataraman *et al*., 1992), however, information in relation to heavy metals concerning blue green algae in general and *Spirulina* in particular has been scanty (Ahluwalia and Kaur,1989; Ahluwalia and Kochar, 1992; Angadi *et al.*, Rai and Raizada,1985; Raizada and Rai, 1988; Singh and Singh,1987; Singh and Verma 1988; Stratton and Corke,1979). The phytotoxic effect of commonly occurring heavy metals such as Ni, Cd, Cr and Pb on cell constituents like chlorophyll, carotenoids, phycobiliproteins, protein carbohydrates and lipids of commercially important blue green alga, *Spirulina platensis* was investigated in the present study.

MATERIAL AND METHODS:

Spirulina platensis was obtained from Central food Technology Research Institute, Mysore, India and grown in modified Zarrouk’s medium (Zarrouk, 1966). Stock and test cultures were maintained at 34±1ºC in an orbital shaker at 100rpm, illuminated with fluorescent lamps providing a light intensity of 1800 lux around the culture vessels for 16:8 h light/dark regime. The glassware was washed with 20% HCL and rinsed thoroughly with distilled water prior to use in order to prevent the binding of metals to the walls of the flasks.

For toxicity assay, cadmium as $CdCl_2.H_2O$, nickel as $NiCl_2.6H_2O$, chromium as K_2CrO_4 and lead as $Pb(NO_3)_2$ were separately added to the fresh medium at the sub lethal concentration of 15ppm. Stocks of different test metals were prepared in distilled water. For control, *Spirulina platensis* biomass was grown under identical culture conditions without any heavy metal. The cultures were grown in bulk and the cells harvested by filtration through a fine polyester cloth, washed twice with distilled water and dried. Biochemical analysis of the 10 days old harvested biomass was done in triplicate for chlorophyll, carotenoids, phycobiliproteins, proteins, carbohydrates and lipids following standard methods (Anonymous., 1993; Hellebust and Craige, 1978) and repeated twice.

RESULT AND DISCUSSION:

Change in growth, photosynthesis and membrane permeability is usually used as toxicity measurement parameters. But in present study we had observed effect of heavy metals on cell constituents rather than on cellular processes. Biochemical analysis of the *Spirulina platensis* biomass grown separately in presence of cadmium, nickel, chromium and lead for chlorophyll, carotenoids and phycobiliproteins revealed a sharp decline (Table 1) indicating that these metals had toxic effects. The order of toxicity for chlorophyll, carotenoids as well as phycobiliproteins was the Cr>Ni>Cd>Pb.

Heavy metals have been shown to cause reduction in the amount of light harvesting pigments (Tripathy *et al.,1981*). Loss of chlorophyll and carotenoids as a consequence of heavy metal exposure in eukaryotic green algae and cyanobacteria was also observed earlier (Rosko and Rachlin, 1975; Rai *et al.*, 1981b, 1990, 1991; Leena and Fatma, 1998). In the present study per cent decrease in the amount of chlorophyll was found to be 79.05, 65.79, 56.80, and 46.60 for Cr, Ni, Cd and Pb respectively, which may be either due to the increased chlorophyllase activity, displacement of magnesium ion or the production of free radicals (Vos De and Schat, 1991). The decrease may as well be either because of reduced synthesis or accelerated degradation of pigments (Nag *et al.*,1981). The decline of pigment content may be due to the lysis of cell wall and disruption of thylakoid membrane as reported for *Anabaena flos-aquae* (Rai *et al*., 1990a). Since thylakoids are the photosynthetic lamellae of cells containing most of the cellular chlorophylls and carotenoids, any reduction in their surface area due to exposure to heavy metals will lead to loss of photosynthetic potential of the cells (Rai *et al*., 1990a). Reduction in the level of carotenoids was 73.30% for Cr, 59.68% For Ni, 46.77% for Cd and 45.65% for Pb.

Like chlorophyll and carotenoids, phycobiliproteins also showed 72.80, 56.45, 41.51 and 38.61 per cent decrease for Cr, Ni, Cd and Pb respectively. Depletion of phycobiliproteins has been reported under iron, sulfur, phosphorus and nitrogen stress with massive accumulation of glycoprotein (Sherman and Sherman, 1983; Stevens *et al*., Warner *et al*., 1986).

Table 1: Effect of heavy metals (15ppm) on the cell constituents of *Spirulina platensis*

Biochemical Components	Control	Lead	Cadmium	Nickel	Chromium
Chlorophyll	8.48±0.16	4.52±0.16	3.66±0.07	2.90±0.16	1.77±0.12
Carotenoids	1.96±0.09	1.06±0.13	1.04±0.17	0.79±0.13	0.52±0.16
Phycobiliproteins	129.11±0.88	79.26±0.13	75.51±0.37	56.22±0.11	35.11±0.08
Proteins	434.66±1.66	753.33±1.58	713.33±1.30	622.66±1.66	494.66±1.42
Carbohydrates	84.80±0.03	118.80±0.26	108.66±0.26	104.53±0.53	92.94±0.57
Lipids	12.00±0.09	21.60±0.014	19.44±0.019	18.00±0.02	13.68±0.018

However, the behaviour of the all heavy metals towards protein, carbohydrates and lipids was strikingly different. Levels of protein, carbohydrate and lipids showed an increase under metal stress condition (Table 1). The increase was 13.80% for Cr, 43.25% for Ni, 64.11% for Cd and 73.30% for Pb in protein content. Since proteins are generally considered as the primary metal binding sites, the increase in protein content might be a response to sequester the heavy metal effect. It may also be due to the synthesis of metallothionein (Olfson *et al*., 1988; Rauser, 1993) or metallothionein/phytochelatins (Mallick *et al*., 1994) like proteins which are not reported in *Spirulina* to the best of our knowledge.

The per cent increase in carbohydrate content was 10.11, 23.85, 28.77 and 40.75 in presence of Cr, Ni, Cd and Pb. Similar increase has also been observed in higher plants (Hodge and Lorio, 1969) and algae (Saxena, 1968) under drought, salinity and pollution stress. In presence of heavy metals total lipid content of *Spirulina platensis* had shown an increasing trend 14% for Cr, 50% for Ni, 64% for Cd and 80% for Pb. Increased production of lipid under metal stress condition had also been reported in Chlorell vulgaris in order to increase the membrane permeability and efflux of Na^+ and K^+ (Rai et al ., 1991).

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