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Biopigments and Rubisco expression under Heavy metal stress in *Spirulina platensis*

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ABSTRACT

Blue-green algae can absorb and remove heavy metals and other contaminants from environment which are produced mainly from use of heavy metals containing compounds in industrial, domestic and agricultural activities. Commercially effect of heavy metals on algal biopigments is of importance as algal pigments are used as natural dyes for food and cosmetics, they also have application in pharmaceuticals and as fluorescent markers in biomedical research. Extensive presence and the ability of cyanobacteria to grow and concentrate heavy metals ensures their suitability for practical uses to bioremediate waste water. For research work freshwater Cyanobacterium *Spirulina platensis* was isolated from Mansagar Lake of Jaipur and pure cultures were subjected different concentrations of heavy metal Nickel and effects were studied on biomass and biopigments such as chlorophyll-a, carotenoids, phycocyanin, allophycocyanin, and phycoerythrin. Effect on Rubisco protein was also studied. Significant changes in growth and biopigments' production and Rubisco expression was reported due to heavy metal stress.

Key words *: Blue-green algae, Spirulina platensis, Heavy metal, Biopigments, Nickel, Cyanobacteria, Rubisco*

Introduction

Heavy metals are found in the earth's crust, activities such as mining, rapid industrial development, intensive agriculture, transportation, and urbanization and use of heavymetals and metal-containing compounds results in environmental contamination and exposure from them (Shallari *et al*., 1998). Heavy metals such as lead, mercury, and cadmium are widely used in industry and can find their way into aquaticfood webs, where they are highly toxic.

Heavy metals may change chemical forms, but are not subjected to chemical or biological destruction, consequently they are persisting pollutants after release into the ecosystem. Usage by humans also facilitated the distribution of heavy metals by extracting contaminants and transportation to other areas of the world from localized mine depots.

Heavy metals are extremely reactive and harmful at low concentrations and pose significant threat to human health and the environment (Ensley, 2000; Wuana and Okieimen, 2011). Heavy metals cannot be destroyed unlike their organic contaminants counterparts. Consequently, the ecosystem has persisted with heavy metals even after the elimination of point sources of emissions. The loss of biodiversity due to increased bioaccumulation and amplification of food chain toxicants has contributed to the destruction of marine ecosystems caused by emissions of heavy metals from industrial and home resources. Blue green algae, due to their cosmopolitan nature, primitive origin and basic bacterial architecture with physiology ancestral to higher plants, are one of the best prokaryotic phyla to study stress biology. They use heavy metals in various enzymes (Waldron and Robinson, 2009).

Nickel is an essential micronutrient for proper functioning of many metallo enzymes (Brown *et al*., 1987; Sakamoto and Bryant, 2001) The following are major enzymes that require Ni for catalysis, either in lower or higher plants: urease, superoxide dismutase, Ni Fehydrogenases, methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme-A synthase, hydrogenases, and RNase-A (Brown, 2006; Küpper and Kroneck, 2007; Ragsdale, 2009; Ahmad and Ashraf, 2012).

Spirulina is a multicellular, filamentous cyanobacterium (blue-green alga) composed of cylindrical, nonheterocystous cells arranged in an unbranched helicoidaltrichome. Due to its economic and nutritional significance, the cyanobacterium *Spirulina platensis* has been the focus of significant biotechnological research. Its name derives from its filaments' spiral or helical nature. This cyanobacterium is recognizable by the genus' main morphological feature, i.e. the arrangement of multicellular cylindrical trichomes along the entire length of the filaments in an open left helix (Vonshak, 1997). Different *Spirulina sp*. have been studied under heavy metal stress to study effects on biopigments (Choudhary *et al*., 2007; Gong *et al*., 2005; Babu *et al*., 2010)

Cyanobacterial pigments are also used in biomedical science not only as food additives and natural dyes, but also as pharmaceuticals and fluorescent markers (Venugopal *et al*., 2005).

Each specific heavy metals have different effects on same genus or different genera of Blue green algae. Different *Spirulina sp*. have been studied under heavy metal stress to study effects on biopigments (Choudhary *et al*., 2007; Gong *et al*., 2005; Babu *et al*., 2010)

The aim of this study was to evaluate the effect of Nickel (heavy metal) on growth, biopigments and Rubisco protein of Spirulinaplatensis.

Materials and Methods

Organism and culture condition

The isolation of *Spirulinaplatensis* was done from Mansagar Lake, Jaipur. Dilution and plating technique was used for isolation and purification in distilled water at 26 + 2 °C with a 12h / day photoo period and cultivated in a modified Zarrouk's medium (Zarrouk, 1966). The three sets of 500 mL conical flasks containing 250 mL sterilized medium of Zarrouk's were used to transfer three days old prepared inoculums of unialgal cultures. The flasks were tightly packed with cotton wool and covered with laboratory sealing films. Two times a day, every culture had been shaken to prevent the clumping of the cells.

Determination of Growth

Optical density with a photochemical colorimeter at 650 nm was reported every seventh day for one month, gor calculating growth as suggested by Wetherell (1961).

Algal cell counting

Using the Haemocytometer Chamber, cell number was identified. Haemocytometer 0.1 mm deep,with Naubauer ruling was used. A drop of the algal suspension was pipetted, coated and left for algal consolidation on slide for two minutes. Three replicate mean counts were considered and cell /mL algal suspension data were given.

Biopigments Analysis

The chlorophyll-a is primarily the light harvesting pigment for blue green algae. The quantity of chlorophyll in the known quantity of algal sample was calculated in accordance with the method and equation proposed by Parsons and Strickland (1965), Carotenoids by Jensen (1978) andphycobiliproteins by Bennett and Bogorad (1971).

Rubisco Analysis

Extraction and qualitative assay for Ribulose-1-5-bisphosphate carboxylase (Rubisco) from *Spirulina platensis* was done using method suggested by Keen *et al*. (1988).

Treatment

A standard initial inoculum of the isolated algae was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile nutrient medium (Zarrouk's medium). The culture flasks were supplied with various concentrations of Nickel chloride hexahydrate ranging from 1ppm to 5 ppm and control were used.

Results and Discussion

The findings indicate variation in growth and expression of biopigments and Rubisco in *Spirulina platensis* at various concentrations of heavy metals.

Effect on Growth: Analysis of growth of *nickel* exhibits various development rates

in specific heavy metal concentrations. The best growth of *S. platensis* compared to other heavy metal levels was at 2 ppm (Fig. 1).

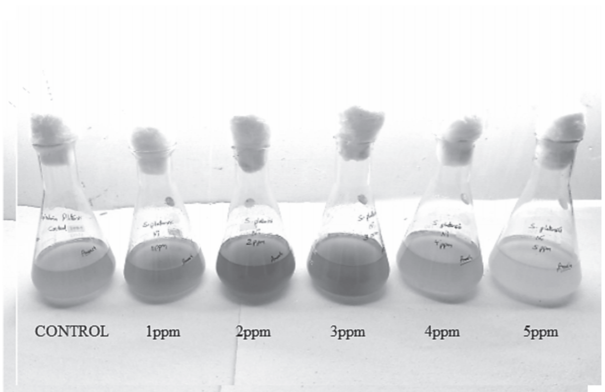
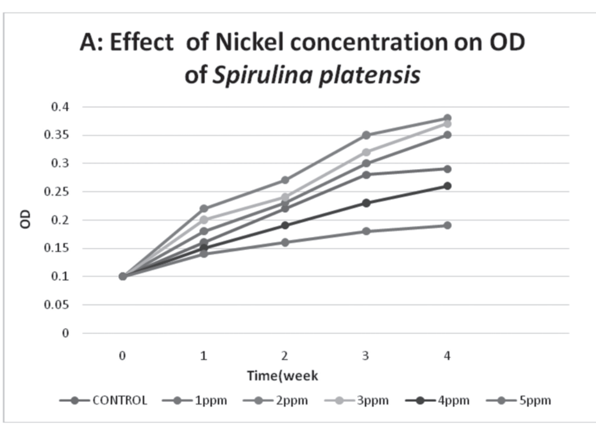


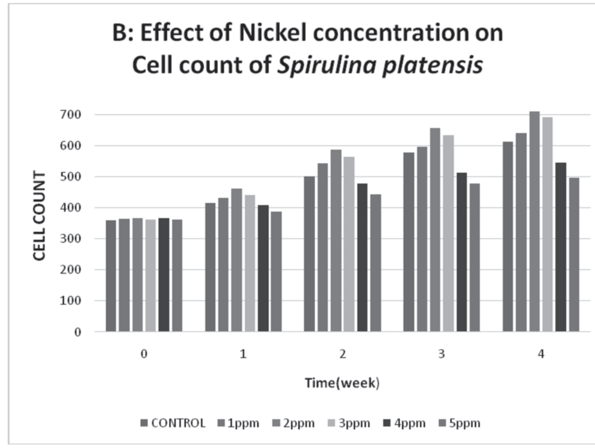
Fig. 1. *S. platensis* grown under different nickel concentrations showing various response after 4 week of exposure

The optical density was exponentially increased at 2 ppm until the end of this experiment. The magnitude rose by 3.8 times more than the initial one and the number of cells also aided optical density (Graph: a, b).

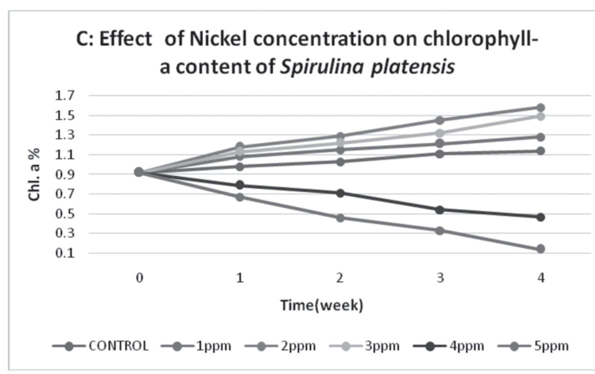
In terms of algae growth, the concentration of 3 ppm was next to 2 ppm. The recorded optical density and number of cells of the algal sample was increased, optical density was around 3.7 times and cell count 1.91 times their initial figures were observed.

Relative to all the concentrations examined, 5ppm was found least effective in promoting the growth of *S.platensis*. At the conclusion of the experiment, the effects of the optical intensity and the cell count were estimated to 1.9 times and 1.37 times as the original values respectively. (Graph: A, B)

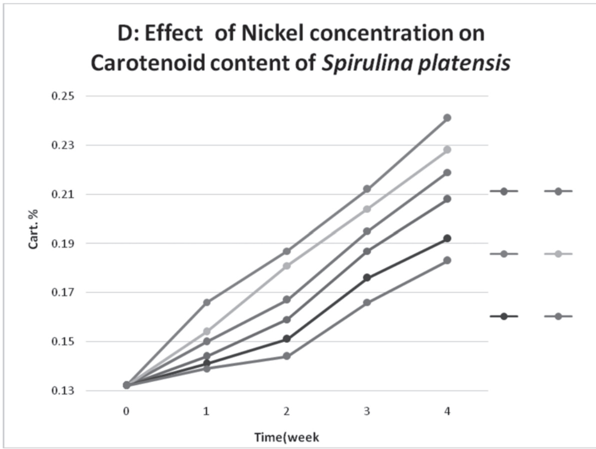




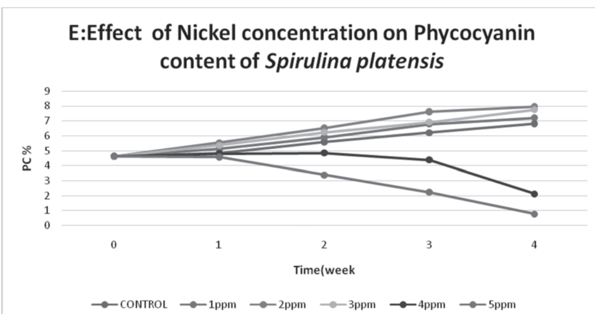
Effect on Biopigment Content: The Biopigment productionin algae correlates with the growth of *S. platensis*. The highest Chlorophyll a content was found at 2 ppm value followed by 3 ppm, 1 ppm and control. Least chlorophyll a content was recorded at 5 ppm. At 4 ppm and 5 ppm concentration, less chlorophyll a content was recorded than initial value. (Graph C) While total Carotenoids content was observed highest value at 2 ppm followed by 3 ppm and least carotenoids was found at 5 ppm. Overall all concentrations with control showed upward trend in content (Graph D).

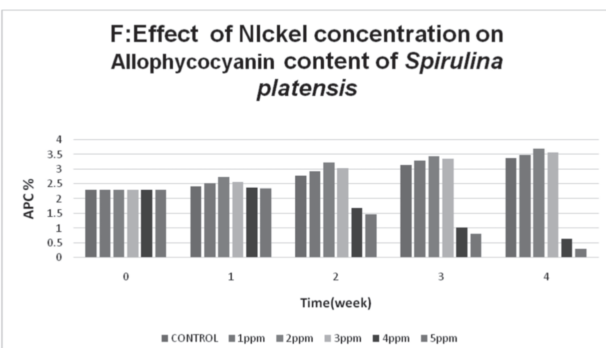


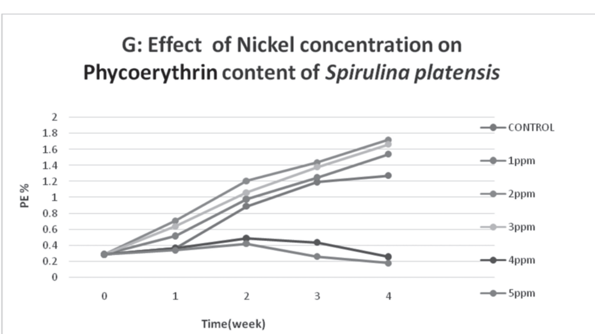
Phycobiliproteins (phycocyanin, allophycocyanin, and phycoerythrin) content showed similar trend like Chlorophyll a with maximum content found at 2 ppm followed by 3 ppm and 1 ppm concentration. Least content was found in 5 ppm concentration followed by 4 ppm concentration of Nickel at end of experiment. At the end of four weeks, 2 ppm concentration reported 1.72



times increase in Phycocyanin content, 1.59 times increase in Allophycocyanin content and 5.93 times increase in Phycoerythrin content (Graph. E, F, G).







Effect on Ribulose-1-5- bisphosphate carboxylase (Rubisco) : Qualitative analysis of Rubisco extracted from *S. platensis* cultures subjected under heavy metal stress by SDS- PAGE showed that three polypeptides of 55 kDa, 42 kDa and 15 kDa were mainly expressed (Fig. 2). 55 kDa Polypetide represents Rubisco large subunit (RbcL) and 15 kDa polypeptide represents Rubisco small subunit (RbcS). RbcL was highest expressed at 2 ppm followed by 3 ppm compared to control condition. RbcS was also highest expressed at 2 ppm followed by 3 ppm. At 5 ppm stress concentration of Nickel both RbcL and RbcSand 42 kDa polypeptide were least expressed.

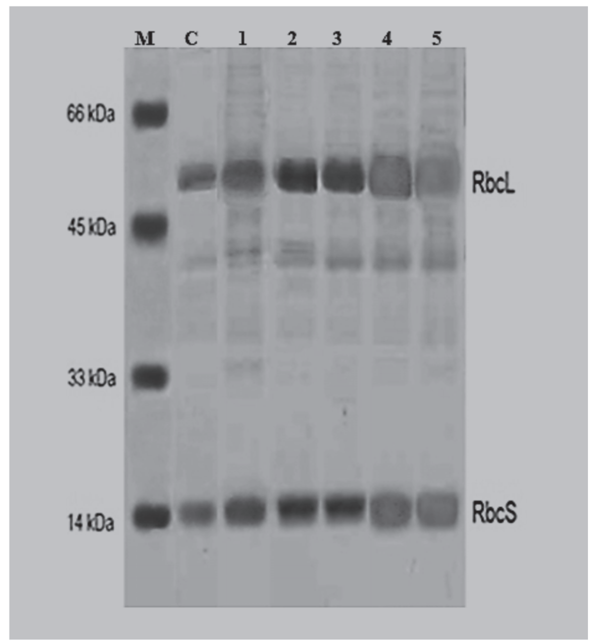


Fig. 2. Coomassie blue – stained SDS-polyacrylamide gel showing Rubisco protein banding pattern of *Spirulina platensis* in Nickel stress condition of 1,2,3,4,5 ppm (lane 1,2,3,4,5 respectively). M is Molecular weight MARKER LANE AND C is CONTROL Lane. Position of Rubisco large subunit (RbcL) and Rubisco small subunit (RbcS) is indicated on Right.

Conclusion

The impact of heavy metals on species depend on their concentration. Various species, though, have various sensitivities due to the same metal and the same species can be more or less hindered by specific metals.

The results show that the photosynthesis is adversely affected under higher stress conditions, so the content of chlorophyll a and phycobiliproteins (Phycocyanin, allophycocyanin and phycoerythrin) has preliminary increase, and then reduced expression with an increase in the heavy metal content (Ahuja *et al*., 2001).

Carotenoids are secondary metabolites that the cell generates under unfavorable circumstances, such as stress. The production of carotenoids is increased by high heavy metal concentrations which act like stress. It was observed that the ratio of carotenoids to chlorophyll raised steadily with increased concentration of Nickel, and as a result the alga modified its appearance from green to yellow-green (Priyadarshani and Rath, 2012). On the groundwork of our findings we concluded that the maximum growth with high biopigments content in *Spirulina platensis* was observed in cultures grown at 2 ppm Nickel concentration.

Qualitative analysis of Rubisco by SDS PAGE showed two main polypeptides of 55 kDa and 15 kDa corresponding to large and small subunit of Rubisco (Spreitzer and Salvucci, 2002). Both subunits showed maximum expression at 2ppm and minimum expression at 5 ppm showing that in stress conditions Rubisco expression is adversely affected in higher concentrations. A 42 kDa polypeptide also showed decrease in expression with increase in stress levels.

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Conflict of Interest

The authors declare that they have no conflict of interest regarding this manuscript.

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