Molybdenum Stress And Its Effect On Growth And Biopigment Profile Of Blue Green Alga Oscillatoria Agardhii

Ameesh Dev Singh, Gajendra Pal Singh

Abstract: Heavy metals are naturally occurring elements that are found in the earth's crust but their environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial, domestic and agricultural use of metals and metal-containing compounds. Blue green algae have high metal absorption capacities which help in removal of heavy metals and other nutrients from the waste streams. Effect of heavy metal stress on biopigments is commercially important as algal pigments are used as natural dyes for food and cosmetics, as pharmaceuticals and also as fluorescent markers in biomedical research. Wide-spread occurrence of Blue green algae along with their ability to grow and concentrate heavy metals, ascertains their suitability in practical applications of waste-water bioremediation. For present research work freshwater filamentous Blue green algas Oscillatoria agardhii was isolated from Mansagar Lake of Jaipur and was subjected to heavy metal stress. Research work shows the effects of different concentrations of heavy metal Molybdenum in growth media of Oscillatoria agardhii on growth and biopigments such as chlorophyll-a, carotenoids, phycocyanin, allophycocyanin, and phycoerythrin. Pure cultures of Oscillatoria agardhii were grown on Zarrouk's medium containing different concentrations (1-5ppm) of Molybdenum trioxide. Significant changes in growth and biopigments' production was reported due to heavy metal stress.

Keywords: Oscillatoria agardhii, Heavy Metal, Biopigments, Molybdenum, Cyanobacteria

INTRODUCTION

Polluting vast areas worldwide, heavy metals are highly reactive and toxic at low concentrations, posing severe risks to human and ecosystem health (Ensley, 2000; Wuana and Okieimen, 2011). Unlike their organic pollutant counterparts, heavy metals cannot be degraded. As a result, heavy metals persist in the environment for years, well after point sources of pollution have been removed. The disturbance of aquatic ecosystems resulted by heavy metals pollution from industrial and domestic sources, has resulted in the loss of biological diversity and also increased bioaccumulation & magnification of toxicants in the food chain (Pena-Castro et al., 2004). Blue green algae are one of the best prokaryotic phyla to study stress biology due to their widespread occurrence, primitive origin and simple bacterial architecture with a physiology ancient to higher plants. They use heavy metal Molybdenum in sulfite oxidases, nitrogenases and nitrate reductases; Nickel in hydrogenase and urease (Waldron and Robinson, 2009). Molybdenum is an essential microelement that is a component of wide range of metalloenzymes in bacteria, fungi, algae, plants and animal (Mendel, 2005) and acts a co-factor in several physiological processes. Molybdenum is present in the active center of bacterial nitrogenases which fix atmospheric nitrogen in the nodules of the legumes. The Molybdenum is required for the synthesis of leghemoglobin—the protein which transports Oxygen in the nodules. As an activating metal, molybdenum is required for amination and transamination reactions, for the inclusion of amino acids in the peptide chain, for other enzyme activity. (Duca, 2015). It is an important micronutrient in both animal and plant nutrition (Deosthale, 1990). Oscillatoria is a genus of filamentous cyanobacterium which is named for the oscillation in its movement. Use of Cyanobacterial pigments as ingredients and natural dyes for food and cosmetics is common in recent times, they are also being used as pharmaceuticals and fluorescent markers in biomedical research (Venugopal et al., 2005). Each specific heavy metals have different effects on same genus or different genera of Blue green algae. Different Oscillatoria sp. have been studied under heavy metal stress to study effects on biopigments (Jayshree et al., 2012; Anusha et al., 2017; Begum et al., 2016) The aim of this study was to evaluate the effect of Molybdenum (heavy metal) on growth and biopigments of Oscillatoria agardhi .

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MATERIALS AND METHODS

Organism and culture condition:
Oscillatoria agardhii was isolated from Mansagar Lake, Jaipur. Isolation and purification was made by dilution and plating technique, grown in modified Zarrouk's medium (Zarrouk 1966) in distilled water at 26+2°C with a photoperiod 12h/ day.Three day old prepared inoculums of unialgal culture were added to the three sets of 500ml conical flask containing 250ml sterilized Zarrouk's medium. The flasks were covered perfectly by cotton wools and sealed with laboratory sealing film. All cultures were shaken twice daily to prevent cells from clumping.

Determination of Growth:
Growth was recorded through optical density with the help of a photochem colorimeter at 650 nm every 7th day, over a period of one month recommended by Wetherell (1961).

Algal cell counting:
Cell number was determined using a Haemocytometer Chamber. Haemocytometer 0.1 mm deep, having improved Naubauer ruling was used. One drop of the algal suspension was pipetted on the slide, covered and left for

two minutes for algal settling. The mean counts of three replicates were taken into consideration and the data were given as cell mL-1 algal suspension.

Biopigments Analysis:
Basically blue green algae have chlorophyll-a as the light harvesting pigment. The quantity of the chlorophyll present in the known amount of algal sample was determined by procedure and equation suggested by Parsons and Strickland (1965), Carotenoids by Jensen (1978) and phycobiliproteins by Bennett and Bogorad (1971)

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 Molybdenum trioxide ranging from 1ppm to 5 ppm and control were used.

RESULTS AND DISCUSSION

The results yielded from this research show variability in growth and biopigment production in different heavy metal concentrations in Oscillatoria agardhii.

Effect on Growth: Growth analysis of O. agardhii in various heavy metal concentrations shows different growth patterns. As compared to the other heavy metal concentrations, the best growth of O. agardhii was calculated at 3 ppm (**Fig: 1).** At 3ppm, the optical density increased exponentially up to the end of the experiment. It increased 3.45 times more than the initial value and the cell count also supported optical density, it increased considerably than initial values. (**Graph: a, b**).

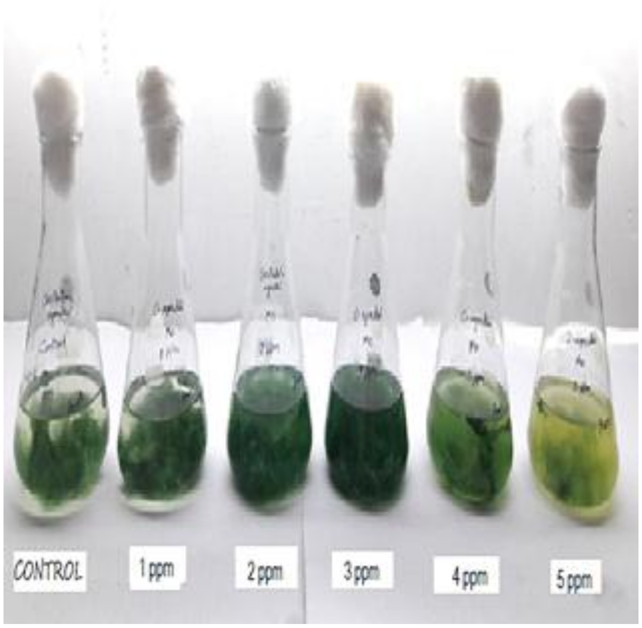
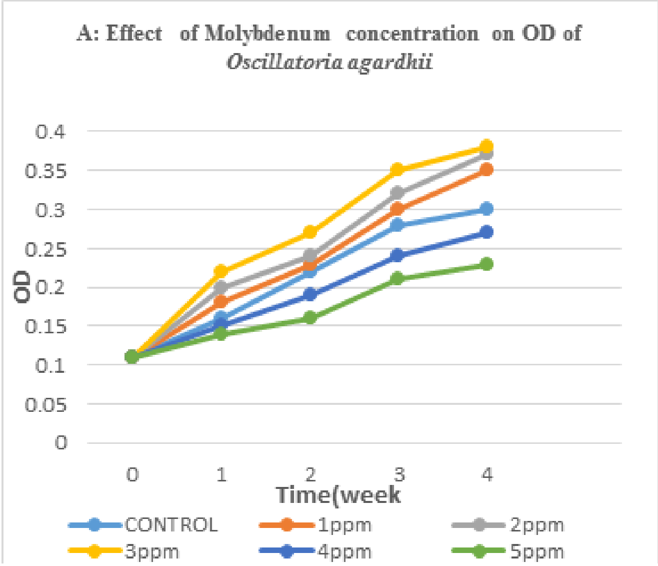
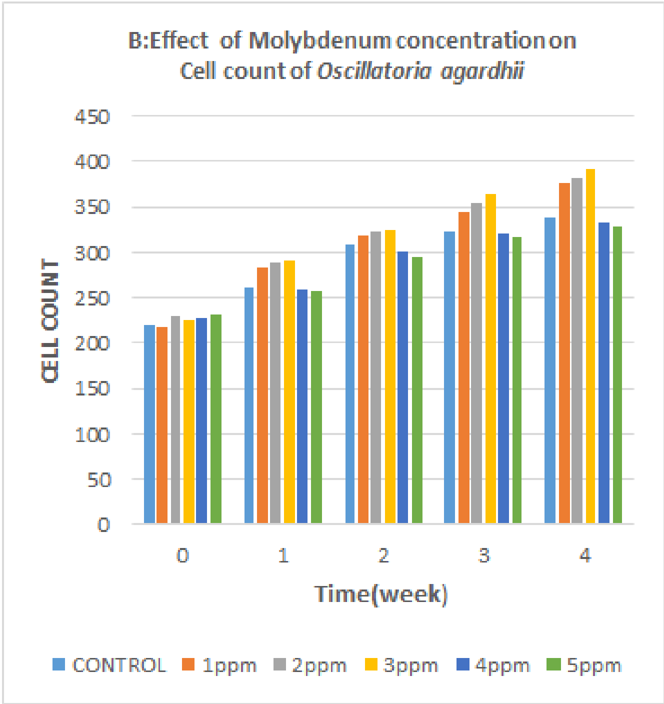


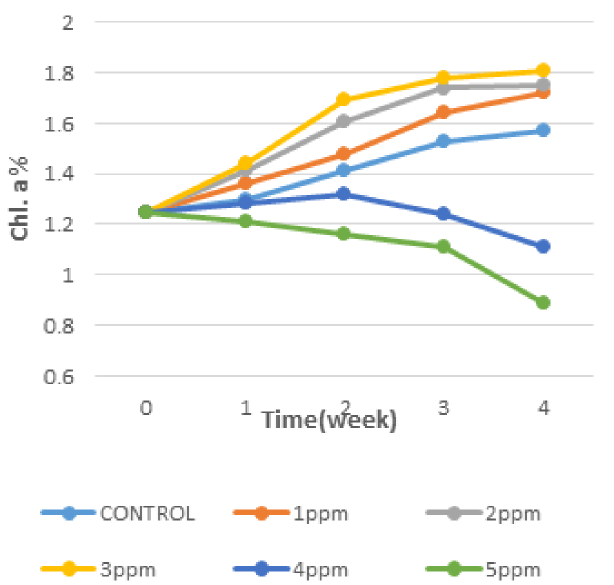
FIG. 1: O.agardhii GROWN UNDER DIFFERENT MOLYBDENUM CONCENTRATIONS SHOWING VARIOUS RESPONSE AFTER IV WEEK OF EXPOSURE



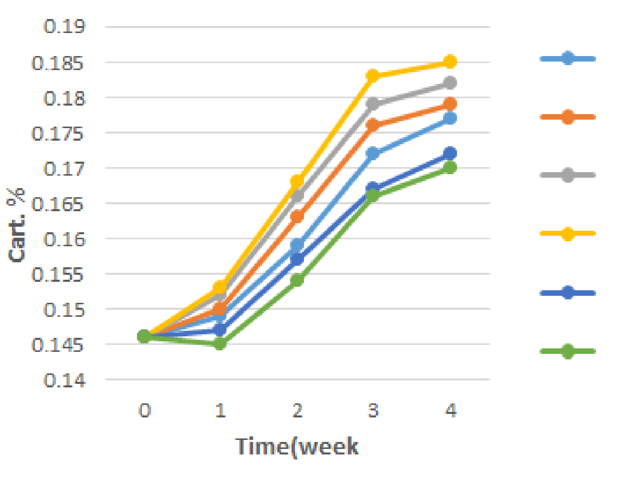


2 ppm concentration was next to 3 ppm, in terms of growth of the alga. The optical density and cell count recorded of algal sample were increased, optical density was about 3.36 times and cell count was found 1.66 times than their initial values. In comparison to all the concentrations tested, 5ppm was found to be least effective in promoting the growth of O.agardhii. At 5ppm optical density and cell count results were calculated to be 1.65 times and 1.18 times respectively lower than 3ppm concentration, at the end of experiment. (**Graph: A, B**)

C:Effect of Molybdenum concentration on chlorophyll-a content of *Oscillatoria agardhii*



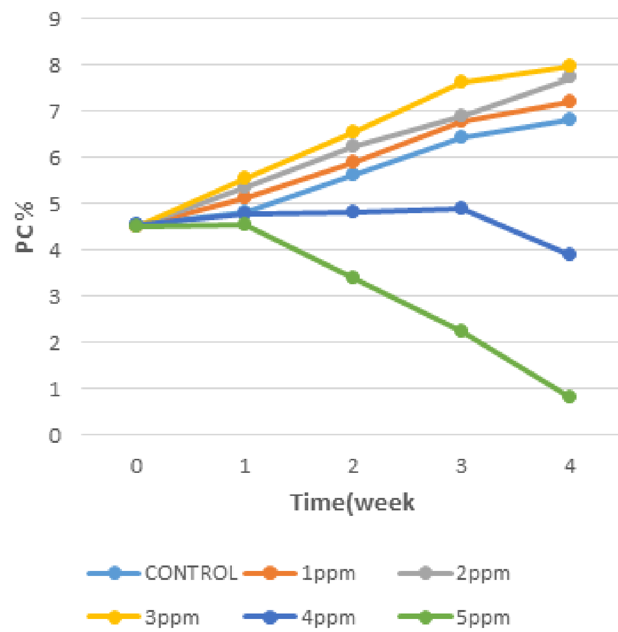
D: Effect of Molybdenum concentration on Total Carotenoid content of *Oscillatoria agardhii*



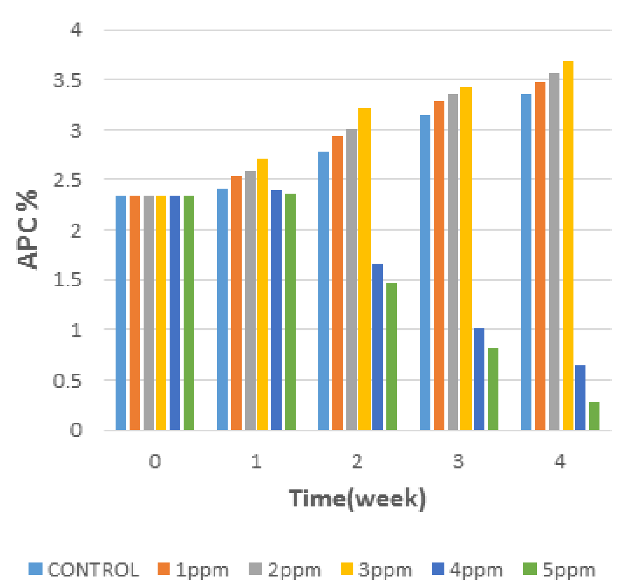
Effect on Biopigment Content: The Biopigment composition of algae correlates with the growth of O. agardhii. The maximum Chlorophyll a content was found at 3ppm value followed by 2ppm, 1ppm and control. Least chlorophyll content was recorded at 5 ppm. At 4 ppm and 5 ppm concentration less chlorophyll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

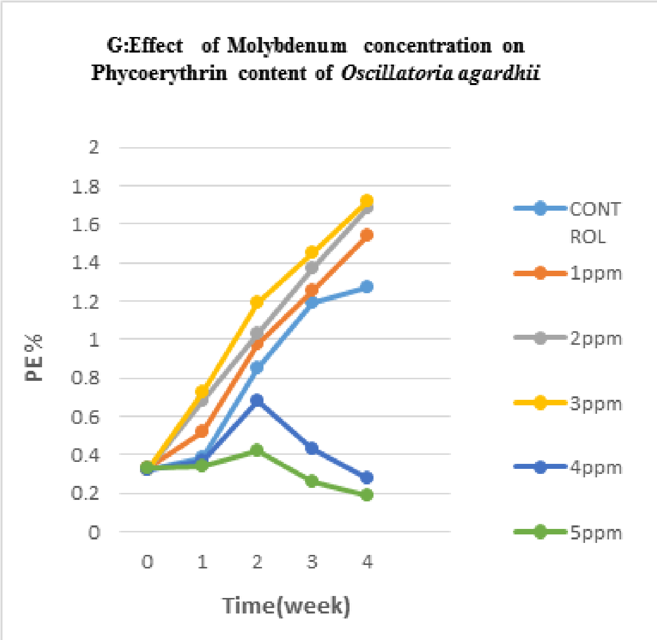
Phycobiliproteins (phycocyanin, allophycocyanin, and phycoerythrin) content showed similar trend like Chlorophyll a with maximum content found at 3ppm followed by 2ppm and 1ppm concentration. Least content was found in 5ppm concentration followed by 4ppm concentration of Molybdenum at end of experiment. At the end of four weeks, 3ppm concentration reported 1.76 times increase in Phycocyanin content, 1.57 times increase in Allophycocyanin content and 5.37 times increase in Phycoerythrin content (**GRAPH : E,F,G**).

E: Effect of Molybdenum concentration on Phycocyanin content of *Oscillatoria agardhii*



F:Effect of Molybdenum concentration on Allophycocyanin content of *Oscillatoria agardhii*





CONCLUSION

The inhibitory and invigorating effects of stress depend on concentration of heavy metals. Different organisms have unique sensitivities to different heavy metals which can show resemblances in encounters of organisms and heavy metals, related or unrelated to each other. The results demonstrate that at high stress conditions, photosynthetic activity is negatively affected, hence the chlorophyll a and Phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) content showed initial increase and then decrease in production with increase in heavy metal concentration (Ahuja et al.,2001). Carotenoids are secondary metabolites, produced by the organism in stress condition as cell protecting process. When heavy metal concentration is increased it acts as stress that enhances carotenoids production. It was found that the carotenoids to chlorophyll ratio gradually increased with increases in Molybdenum concentration and as a result the alga changed its appearance from light green to yellow-green (Priyadarshani and Rath, 2012). On the basis of our findings we concluded that the maximum growth with high biopigments content in Oscillatoria agardhii was observed in cultures grown at 3 ppm Molybdenum concentration.

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CONFLICT OF INTEREST:

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

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