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Study on Chl A Synthesis by Laser Induced Fluorescence and UV- VIS Spectrophotometer in the presence of Heavy Metals

Iffat Zareen Ahmad and Shanthy Sundaram
Centre for Biotechnology, Nehru Science Centre, University of Allahabad

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Abstract: The effect of heavy metals was seen on different cyanobacterial strains by using helium-neon lamp and uv-vis spectrophotometer. The cyanobacterial strains used for the present study included unicellular, non-heterocystous strain like *Synechococcus sp. PCC 7942* and filamentous, heterocystous strain like *Nostoc muscorum*. These strains were grown in the presence of heavy metals, namely, nickel, copper, cadmium and mercury. Final concentration of the heavy metal solution used in the culture were 0.1, 0.4 and 1uM. 5ml aliquots of the cyanobacterial culture in the exponential phase were withdrawn and analyzed in terms of absorption at 670nm using UV-vis spectrophotometer (Ultrospec 4000) which works on the principal of Lambart- Beer's Law and intensity using helium-neon lamp at 632.8nm in a quartz cuvette. Some metals are known to inhibit the synthesis of different pigments due to substitution of the Mg2+ ion from the pyrrole ring which are found in cyanobacteria. After observing the overall result carefully, it can be said that the different metals have inhibited the synthesis of chlorophyll but in some cases the result was opposite, that is, the highest metal concentration (1uM) was seen to induce pigment synthesis. The heavy metal concentration of 0.1uM and 0.4uM were seen to induce chlorophyll synthesis in some cases while higher concentration of 1uM inhibits the synthesis. The order of toxicity shown by different metals was Hg>Cu>Ni>Cd.

1. Introduction

Cyanobacteria are Gram –ve prokaryotes and are the first organisms to evolve on the earth. They perform oxygenic phoyosynthesis like highr plants. They possess pigments like chl a, carotenoids, fucoxanthin, xeaxantin and phycobiliproteins – phycocyanin C and phycoerythrin C. Some cyanobacteria possess heterocysts which are physiologically modified cells containing ability to fix atmospheric nitrogen. Therefore, cyanobacteria are used as biofertilizers. They are important in increasing the fertility of paddy and sugarcane fields.

Heavy metal pollution is a global concern for the environmentalists. Virtually all metals, essential or non-essential can exhibit toxicity above threshold concentrations. Toxicity mechanisms include the blocking of functional groups of important molecules

like enzymes, polynucleotides, transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes and disruption of cell and organellar membrane integrity in a biochemical context. Every index of living cells integrity may be affected under conditions of metal pollution (Nagalakshmi and Prasad[1,2]; Prasad[3]; Reddy and Prasad; Prasad *et al*[4]). Cyanobacterial response to heavy metals renders them to be a suitable model to study such responses that are comparable to higher plants.

2. Materials and Methods

Collection and maintenance of cyanobacterial cultures: Cyanobacterial strains were collected from natural sources like soil and water. These are then purified and identified in Algal Laboratory, Department of Botany, University of Allahabad. They are maintained in the culture room at a temperatute of 25 + 1°C, illuminated by white cool fluorescent tubes to receive a light intensity of 4000 – 5000 lux. The cultures were kept in 14 hours light period and 10 hours dark period.

The strains used for the present study were *Synechococcus sp. PCC 7942* which is unicellular and non-heterocystous and *Nostoc muscorum* which is filamentous and heterocystous.

The medium used for growing the cyanobacterial cultures was BG–11 (given by Hughes *et. al.*[5] and modified by Stanier *et. al.*[6]). Non-heterocystous cyanobacterial cultures were grown by external nitrogen source, 100mM KNO_3 into the BG–11 medium. The medium was buffered to pH 7.5 with 10mM NaOH. All the manipulations involving the transfer of cultures in the liquid media were carried out under aseptic conditions.

Preparation of heavy metal solution: 50mM stock solutions of four metal chlorides ($HgCl_2$, $CuCl_2$, $CdCl_2$ & $NiCl_2$) were prepared. The stocks were diluted 10 times to make it 5mM solution. Final concentration of the heavy metal solutions used in the cultures were 0.1. 0.4 & 1 μM.

5ml aliquots of the grown cyanobacterial cultures in exponential phase were withdrawn and analyzed in terms of absorption at 670nm using UV-Vis spectrophotometer and intensity using helium-neon lamp at 632.8nm in a quartz cuvette.

3. Results and Discussion

It was seen from the spectra that under metal stress the synthesis of chlorophyll-a was inhibited in case of nickel and mercury stress but was enhanced in case of copper and cadmium stress in both *N. muscorum* and *Synechococcus* sp. PCC 7942. Some metal ions act as cofactors of some enzymes, which could be the reason for the enhancement of the pigment synthesis.

Both the UV-Visible and laser induced fluorescence spectra showed the inhibition

of chl a synthesis under nickel stress. In case of copper, the results were different, Cu enhanced the synthesis of chl a on increasing the concentration in both the strains. Under cadmium stress, the synthesis of chl a was inhibited in *N. muscorum* but was enhanced in *Synechococcus sp. PCC 7942*. Mercury was seen to be highly toxic as it completely inhibited the synthesis of chl a in both strains.

4. Conclusion

Some metals were known to inhibit the synthesis of different pigments due to substitution of the Mg2+ ion from the pyrrole ring which are found in cyanobacteria (Kupper *et. al.*, 1998). The aim of present study was to see the effect of different toxic metals on the synthesis or inhibition of chlorophyll. The study was conducted in two parts. Firstly, the laser induced chlorophyll fluorescence was recorded by Atomic fluorescence spectrophotometer using the helium–neon lamp. Secondly, the absorbance of the samples was recorded by UV- Vis spectrophotometer (Ultrospec 4000). After observing the overall result, it can be said that the different metals taken into account (Ni & Hg) have inhibited the synthesis of chl a but in case of some metals (Cu & Cd) the synthesis of chl a was enhanced.

The heavy metal concentration of 0.1uM and 0.4uM was seen to induce chl a synthesis in some cases while higher concentration of 1uM inhibits the synthesis. This may be because of the tolerance range of different strains. Some cyanobacteria are known to metabolize these metals by a process known as Bioremediation.

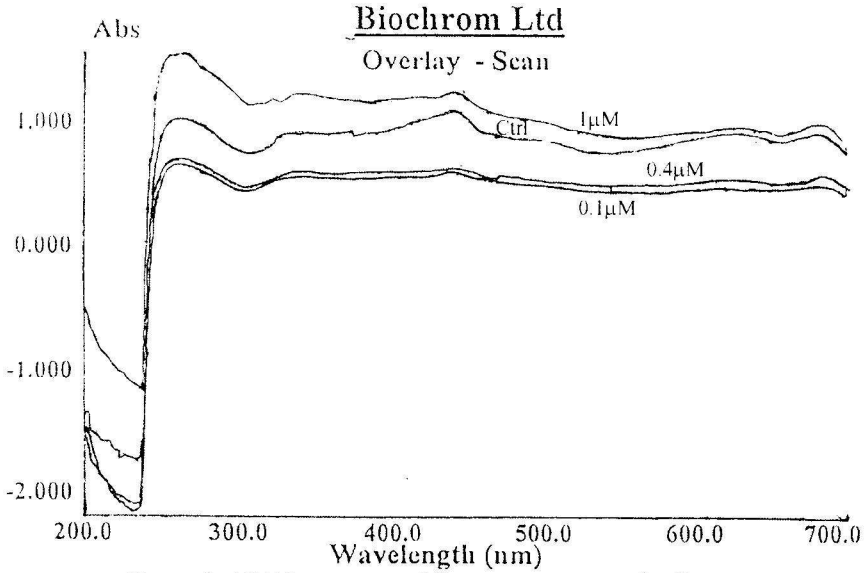

Figure 1 : UV-Vis spectrum of *Nostoc muscorum* under Cu stress.

After the long time exposure with metals, these strains become resistant to metals and are known as Tolerant strains or Resistant strains. We can also see in the graphs some new peaks (200 – 400nm) in heavy metal treated strains, which are not present in the control. These peaks may be of some proteins called Heavy Metal Stress proteins which cyanobacteria starts synthesizing to combat the stress.

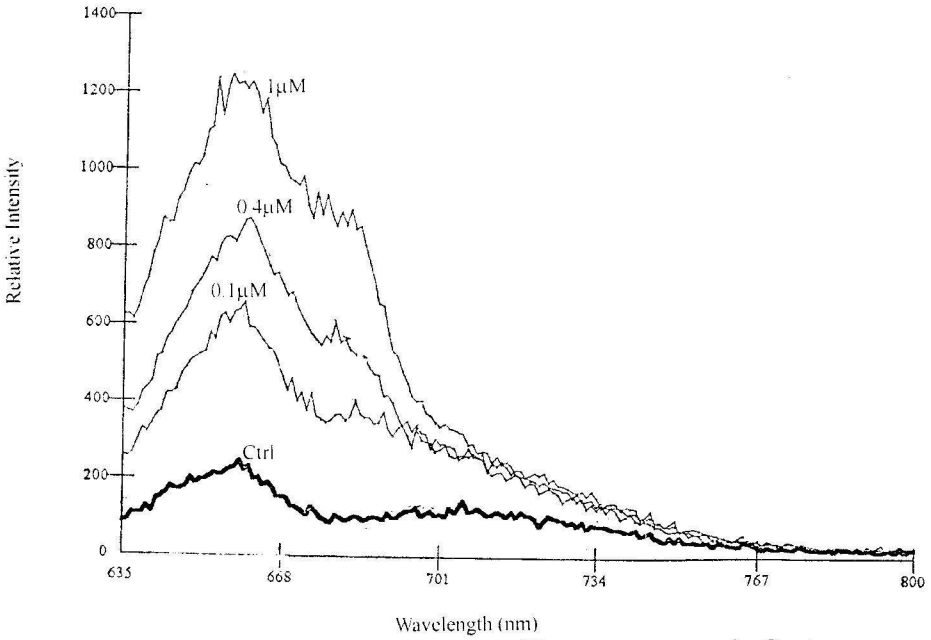

Figure 2 : Atomic Fluorescence spectrum of *Nostoc muscorum* under Cu stress.

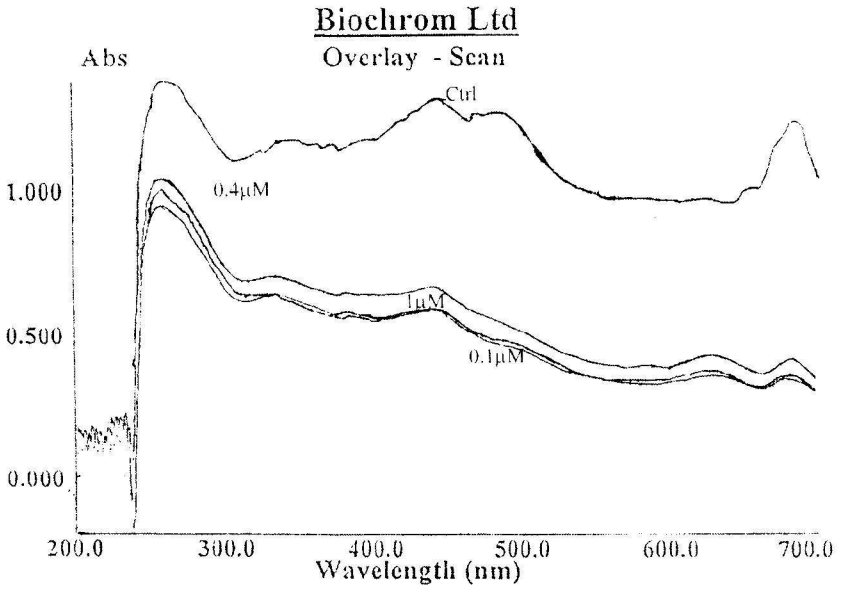

Figure 3 : UV-Vis spectrum of *Synechococcus sp. PCC 7942* under Cu stress.

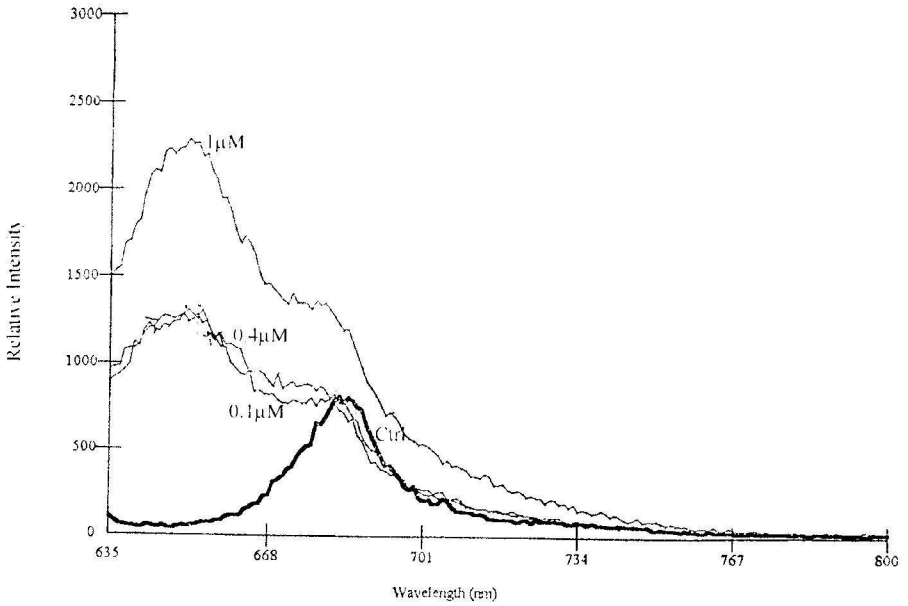

Figure 4 : Atomic Fluorescence spectrum of *Synechococcus sp. PCC 7942* under Cu stress.

5. References

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