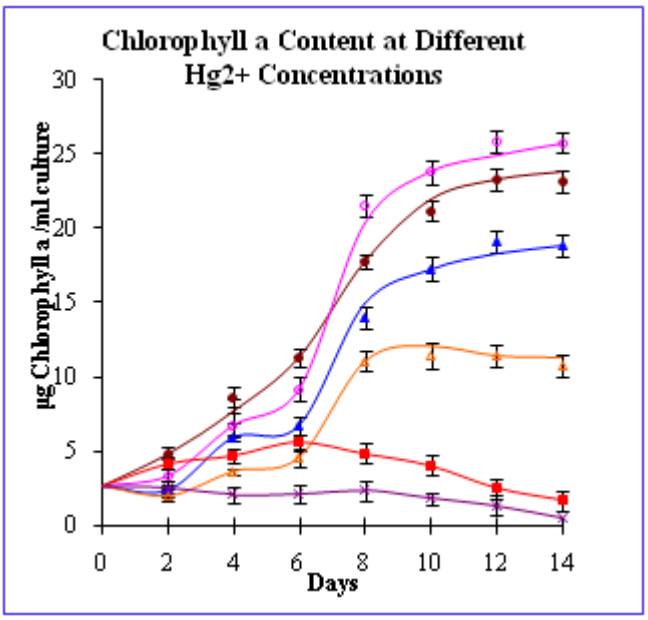

Figure 4.1: Variations in pigment Chlorophyll *a* content of *Anabaena variabilis* under the influence of Hg^{2+}-less control cultures (●), 0.045 µ mol Hg^{2+}/ml culture (o), 0.096 µ mol Hg^{2+}/ml culture (▲), 0.184 µ mol Hg^{2+}/ml culture (Δ), 0.435 µ mol Hg^{2+}/ml culture (■) and 0.531 µ mol Hg^{2+}/ml culture (x). Values are mean ±SE. ($F_{Hg^{2+}6,48}$= 9.12, p<0.01; $F_{days8,48}$= 7.63, p<0.05; r= 0.78, p< 0.025).

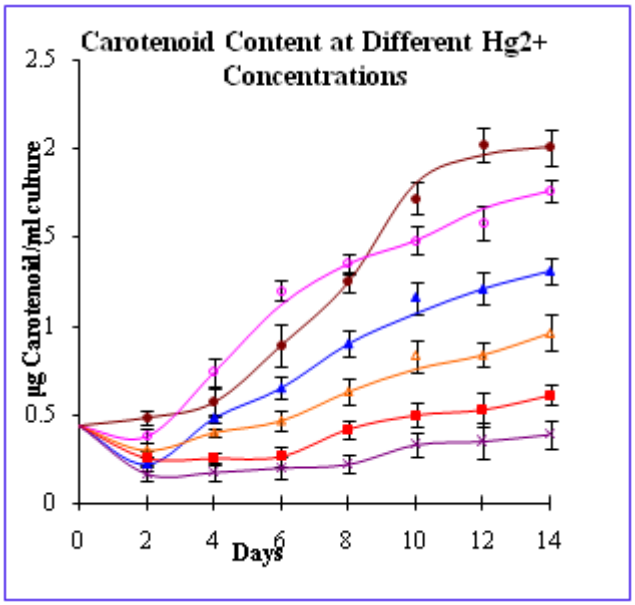

Figure 4.2: Variations in pigment Carotenoid content of *Anabaena variabilis* under the influence of Hg^{2+}-less control cultures (●), 0.045 µ mol Hg^{2+}/ml culture (o), 0.096 µ mol Hg^{2+}/ml culture (▲), 0.184 µ mol Hg^{2+}/ml culture (Δ), 0.435 µ mol Hg^{2+}/ml culture (■) and 0.531 µ mol Hg^{2+}/ml culture (x). Values are mean ±SE. $F_{Hg^{2+}6,48}$= 15.33, p<0.025; $F_{days8,48}$= 12.5, p<0.025; r= 0.82, p< 0.025.

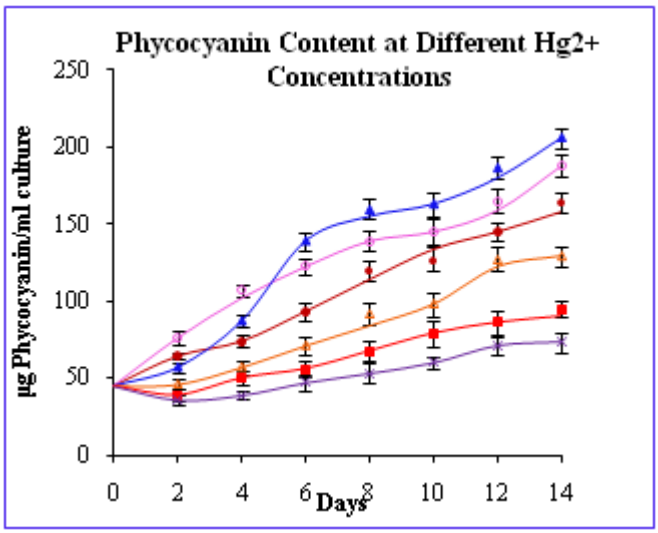

Figure 4.3: Variations in pigment Phycocyanin content of *Anabaena variabilis* under the influence of Hg^{2+}-less control cultures (●), 0.045 µ mol Hg^{2+}/ml culture (o), 0.096 µ mol Hg^{2+}/ml culture (▲), 0.184 µ mol Hg^{2+}/ml culture (Δ), 0.435 µ mol Hg^{2+}/ml culture (■) and 0.531 µ mol Hg^{2+}/ml culture (x). Values are mean ±SE. $F_{Hg^{2+}6,48}$= 89.58, p<0.01; $F_{days8,48}$= 0.475, p<0.025; r= 0.61, p< 0.025.

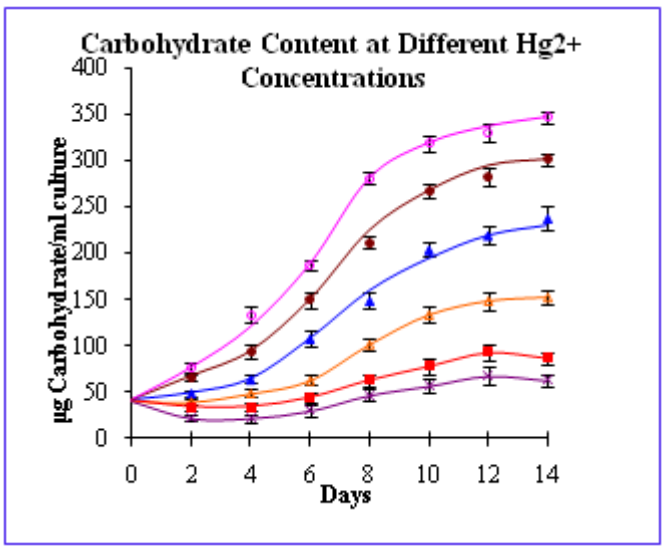

Figure 5: Impact of intracellular Hg^{2+} on Carbohydrate content of *Anabaena variabilis* cells during 0-14 days exposure to: Hg^{2+}-less control cultures (●), 0.045 µ mol Hg^{2+}/ml culture (o), 0.096 µ mol Hg^{2+}/ml culture (▲), 0.184 µ mol Hg^{2+}/ml culture (Δ), 0.435 µ mol Hg^{2+}/ml culture (■) and 0.531 µ mol Hg^{2+}/ml culture (x). Values are mean ±SE. $F_{Hg^{2+}6,48}$= 23.35, p<0.01; $F_{days8,48}$= 17.01, p<0.05; r= 0.84, p< 0.05.

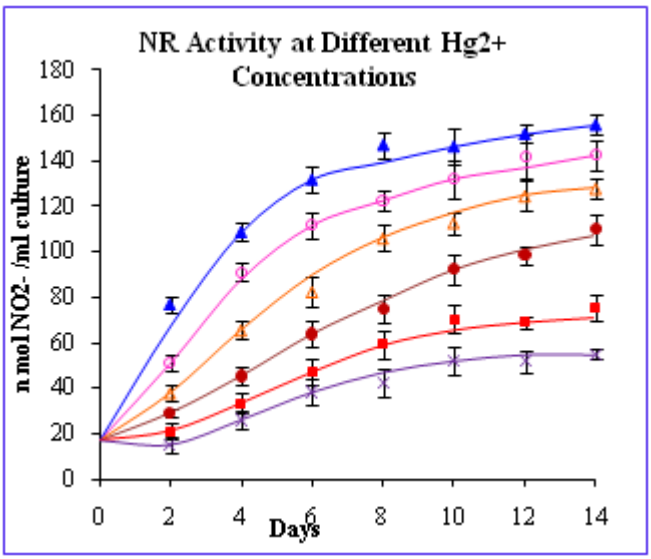

Figure 6.1: Impact of intracellular Hg^{2+} on Nitrogenase Reductase Activity of *Anabaena variabilis* cells during 0-14 days exposure to: Hg^{2+}-less control cultures (●), 0.045 µ mol Hg^{2+}/ml culture (o), 0.096 µ mol Hg^{2+}/ml culture (▲), 0.184 µ mol Hg^{2+}/ml culture (Δ), 0.435 µ mol Hg^{2+}/ml culture (■) and 0.531 µ mol Hg^{2+}/ml culture (x). Values are mean ±SE. $F_{Hg^{2+}6,48}$= 45.48, p<0.01; $F_{days8,48}$= 25.07, p<0.01; r= 0.96, p< 0.05.

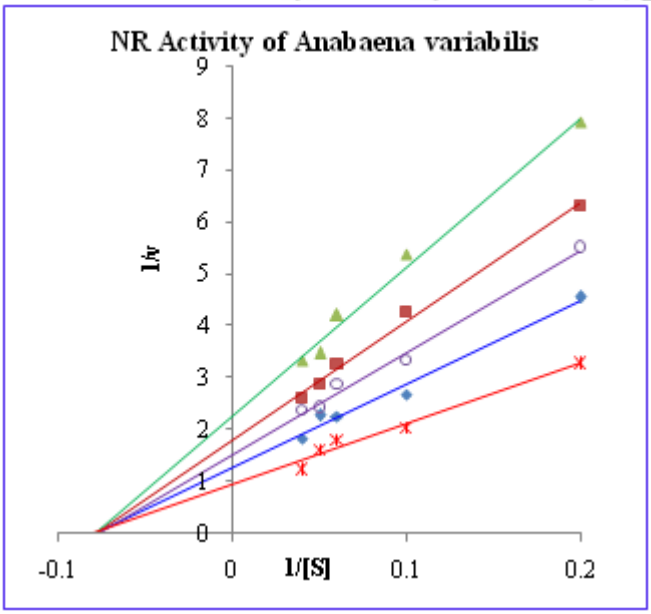

Figure 6.2: Double reciprocal plot for Nitrate Reductase Activity by *Anabaena variabilis* cells, interacting with some other heavy metals (0.5 µM, Extracellular, each) : Hg^{2+}-less control (●), Hg^{2+} -alone (■), Hg^{2+}+ Cu^{2+} (▲), Hg^{2+}+ Cd^{2+} (o), Hg^{2+}+ Zn^{2+} (x).

4. Discussion

Uptake of metals and metalloids can be harmLess (i.e. no effects), toxic or even beneficial (Awasthi, 2005). The bioassay studies on the cyanobacterium, *Anabaena variabilis* has clearly indicated that the organism to be very sensitive to very low concentrations of mercury (Murugesan and Ruby, 2005). The growth inhibitory effects of Hg^{2+} on plants have been directly linked to ultrastructural damage. The effect of the metal ion is dependent on the varying degree of metal concentration in the cell and also the amount of ion that can transfer the cell membrane (Sinkiss, 1979). Biosorption studies on the Hg^{2+} uptake on a short term exposure showed its dependence on the metal concentration in the ambient medium. Hg^{2+} treatment reduced the amount of chlorophyll and resulted in breakdown of thylakoids. Furthermore, Hg^{2+}-stress inhibited the activity of NADPH:prochlorophyllide oxidoreductase (POR), which is responsible for the biosynthesis of chlorophyll (Lenti *et al.*, 2002). The metals were found to cause disruption of the thylakoid membranes in *Anabaena flos-aquae*, resulting in the degradation of the light harvesting pigment and thus decreasing their contents of the cells (Rai and Dubey, 1989).

The metal ions ability to enter a chemical reaction as a positively charged ion and their capacity to bind to the enzyme prosthetic group is an important reason behind the mechanism of enzyme inhibition. Metals like Zn^{2+}, Ni^{2+} and Cd^{2+} had interacted negatively with the NR activity of blue green algae, *Anacystis nidulans* and a green algae, *Chlorella vulgaris* in both free and immobilized states (Awasthi, 2005). The displacement of an essential metal ion forming the central and functional part of the enzyme protein may be one of the reasons for inhibition of NR by heavy metals, and secondly, interference with sulphydryl (-SH) group which often determine the secondary and tertiary structures of proteins. Besides, a reduced energy supply due to inhibition of photosynthetic electron transport and an indirect inhibition of uptake of substrate (NO_3^-) of energy may be other important reason. The sensitive histochemical based detection of Hg-triggered accumulation of ROS and oxidative injury can be used as biomarkers to indicate the toxicity in plants (Chen and Yang, 2012).

In plants, nitrate reductase (NR) and nitric oxide synthase (NOS) have been proposed as two enzymatic systems responsible for NO production. NO is involved in alleviation of heavy metals induced toxicity by directly regulating accumulation and translocation of heavy metals in plants. NO have been demonstrated to involve the regulation of Hg-induced oxidative stress and plant tolerance to algae. NO is shown to depress the generation of H2O2 and alleviate phytotoxicity by enhancing antioxidative capability (Wang and Yang, 2005). Heavy metals can regulate the generation of endogenous NO which is closely associated with the intrinsically physiological processes in plants.

Any stimulation in the ATP pool and availability of NADPH may stimulate ATP dependent processes like NR activity. Thus, enhanced photosynthetic activity indicates towards increase of the enzymatic activities.

5. Conclusion

Mercury inhibited synthesis of photosynthetic pigments and macromolecules. The blue green alga, *Anabaena variabilis* may be used as a bioindicator of Hg^{2+}-pollution in aquatic ecosystems, exploring its various physiological parameters (particularly the phycocyanin pigment content and nitrate Reductase activity). Being a tolerant species, *A. variabilis* can also be explored as a tool for bioremediation of mercury.

6. Acknowledgements

The author acknowledges the host institution for providing necessary infrastructure facility for the present work. She is also grateful to CSIR, India for providing financial support in the form of SRF.

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